IFST Advances in Food Science

Nutraceutical and Functional Food Processing Technology

Edited by

Joyce Irene Boye

Institute of Food Science + Technology ifst

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IFST Advances in Food Science is a series of books dedicated to the most important and popular topics in food science and technology, highlighting major developments across all sectors of the global food industry. Each volume is a detailed and in-depth edited work, featuring contributions by recognized international experts, and which focuses on new developments in the field. Taken together, the series forms a comprehensive library of the latest food science research and practice, and provides valuable insights into the food processing techniques that are essential to the understanding and development of this rapidly evolving industry.

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Current and Emerging Trends in the Formulation and Manufacture of Nutraceuticals and Functional Food Products

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1.1 Introduction

In the last few decades, emphases on the role of foods have shifted from substances consumed merely to quell hunger or to provide needed nutrients for normal cellular function to substances that can potentially promote health and wellness and, particularly, reduce risk of disease. These foods are frequently referred to as *nutraceuticals* and/or *functional foods* with various reported bioactive functions (e.g., immunomodulators, antihypertensives, osteoprotectives, hypocholesterolemics, antioxidatives, and antimicrobials). Nutraceuticals and/or functional foods are a fast-growing, multi-billion-dollar global industry that has been expanding annually. Strong market growths of these foods confirm their perceived nutritional benefits and, in some cases, provide a surrogate substantiation of their health claims. It also provides evidence of increasing product innovations, consumer acceptance of healthy-living lifestyles through nutrition, and a growing shift from pharmaceutically derived supplements. Consumers are interested in preventing

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and/or slowing the progression of illness and disability before they become irreversible and costly to quality of life. In response to this demand, food companies are developing technologies for processing health and wellness products that will improve the efficacy of these products, maximize the potential benefits to consumers, and be cost-effective for the industry's survival in a competitive marketplace.

1.2 Overview, Classification, and Benefits of Nutraceuticals and Functional Foods

There is no universal definition of nutraceuticals and/or functional foods as it varies across countries and markets. All foods are generally functional because they provide nutrients and energy to sustain growth and support vital cellular processes. Functional foods, however, are generally considered to go beyond the provision of basic nutrients to potentially offer additional benefits such as reducing the risk of disease and/or promoting optimal health to the consumer (Hasler 2002). A study presented at the annual meeting of the American Institute for Cancer Research, in Bethesda (Maryland, United States) on November 7, 2013, showed a correlation between poor diets (high in sugar and saturated fats) and the risk of early death caused by inflammation-related health conditions (gastrointestinal [GI] tract cancers – i.e., cancers of the esophagus, stomach, colon, and rectum). The study sample included 10,500 people who were followed from 1987 through 2003 (The Weekly 2013). Of the 259 participants that had died at the end of the study period, 30 had died from GI tract cancers. The study showed that the participants who lived on poor diets were four times as likely to die from GI tract cancers as a result of poor diets that cause inflammation than those participants who consumed plant-based diets purported to be anti-inflammatory to GI tracts.

According to Health Canada (1998), the governmental authority that oversees the approval of food health claims in Canada, a functional food "is similar in appearance to, or may be, a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions, i.e. they contain bioactive compounds." The Institute of Medicine's Food and Nutrition Board defines functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains." Other definitions of functional food are listed in Table 1.1. Health Canada (1998) further defines a nutraceutical as a "product isolated or purified from foods that is generally sold in medicinal forms not usually associated with foods. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease." Zeisel (1999) deduced the definition of nutraceuticals from the description of dietary supplements ("ingredients extracted from foods,

Table 1.1 Some definitions of functional foods

Organization	Definition
Academy of Nutrition and Dietetics	"Whole foods along with fortified, enriched, or enhanced foods that have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis at effective levels."
International Food Information Council	"Foods or dietary components that may provide a health benefit beyond basic nutrition and may play a role in reducing or minimizing the risk of certain diseases and other health conditions."
Institute of Food Technologists	"Foods and food components that provide a health benefit beyond basic nutrition (for the intended population)."
International Life Sciences Institute	"Foods that by virtue of the presence of physiologically active food components provide health benefits beyond basic nutrition."
European Commission	"A food that beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. It is part of a normal food pattern. It is not a pill, a capsule or any form of dietary supplement."
Japanese Ministry of Health, Labour, and Welfare	"FOSHU [Food for specified health uses] refers to foods containing ingredient with functions for health and officially approved to claim its physiological effects on the human body. FOSHU is intended to be consumed for the maintenance/promotion of health or special health uses by people who wish to control health conditions, including blood pressure or blood cholesterol."

Source: Academy of Nutrition and Dietetics 2013. Reproduced with permission of Elsevier.

herbs, and plants that are taken, without further modification outside of foods, for their presumed health-enhancing benefits intended to supplement the diet, that bears or contains one or more of the following dietary ingredients: a vitamin, mineral, amino acid, herb, or other botanical in the form of a capsule, powder, softgel, or gelcap, and not represented as a conventional food or as a sole item of a meal or the diet") as a "diet supplement that delivers a concentrated form of a biologically active component of food in a non-food matrix in order to enhance health."

As Table 1.1 indicates, the definition of a functional food depends on the demography and the designated regulatory authority involved. The vast array of different ingredients used in the formulation of functional foods helps to explain the endless options and combinations available in the marketplace. A casual observation in any supermarket will confirm the multitude of different categories of products available in this sub-sector including solid foods, beverages, and supplements, which continue to expand on a daily basis. Over 5,500 new types of these products have been introduced to the Japanese market since 1990, the birthplace of functional foods (Siró et al. 2008), and

537 products valued at US$6.3 billion have been granted FOSHU (Foods for Specific Health Use) status since 2005 (Hartmann and Meisel 2007).

The American Dietetic Association expands the definition by categorizing functional foods into four groups. These are conventional, modified, medical, and foods for special dietary use. Conventional foods include whole foods such as garlic, nuts, whole grains, oily fish, and tomatoes, which contain bioactive chemicals and polyunsaturated fatty acids (PUFAs). For instance, oatmeal is considered a functional food because it naturally contains soluble fiber that can help lower cholesterol levels. Modified foods are those that have been enriched, enhanced, or fortified to have or increase health benefits by adding bioactive substances such as phytochemicals or other antioxidants. Such foods include omega-3 (or ω-3) enriched eggs, yoghurts with live beneficial bacterial cultures, calcium-fortified orange juice, folate-enriched bread, and energy bars. Medical foods are those that serve specific medical purposes and those for dietary use, including products such as lactose-free milk and gluten-free breads. Some of these distinctions provide another basis for classifying functional foods, as shown in Table 1.2.

With increasing incidence of cardiovascular disease (CVD) – for example, coronary heart disease (CHD), which can result in heart attacks; and cerebrovascular disease, which can result in stroke and high blood pressure (hypertension) – it is estimated that 23.6 million people worldwide could die from heart disease and stroke by 2030 (WHO 2013). A growing body of literature on the role of diet on health shows that risk factors cumulating from unhealthy dietary lifestyle, obesity, high blood pressure, diabetes, and raised lipids can lead to high incidence of CVD. Similarly, oxidative stress and inflammation have been linked to the initiation and propagation of many diseases including hypertension and CVD. Despite the popularity of pharmacological interventions to disease and ill health, some drugs may have serious side effects, and some treatments may be unsuccessful. As a result, many consumers have turned to functional foods with bioactive components such as lycopene, conjugated linoleic acid (CLA), omega-3 fatty acids (FAs), and fiber, which are reported to play a role in the treatment and prevention of chronic and metabolic diseases such as obesity, diabetes, cancer, arthritis, and CVD (Paiva and Russell 1999; Gibson 2004; Krinsky and Johnson 2005; Spence 2006; Boots et al. 2008; Siró et al. 2008; Patisaul and Jefferson 2010; Plaza et al. 2010; Escobar et al. 2012; Karppi et al. 2012; Harms-Ringdahl et al. 2012; Xaplanteris et al. 2012; Houston 2013; Jacques et al. 2013). Indeed, the use of functional foods may in some instances offer safe and effective alternatives to prevent, mitigate, and/or treat some of these conditions. Tables 1.3 and 1.4 provide a list of some sources and components of foods and food ingredients reported to have potential health benefits.

Whereas there are no specific regulations regarding functional foods in most countries, standards have been set in other jurisdictions (e.g., the

Table 1.2 Categories of functional foods

Categories	Definition	Examples
Basic/whole/ unaltered products	Foods naturally containing increased content of nutrients or components	• Carrots (containing the natural level of the antioxidant β-carotene)
Fortified products	Foods with higher contents of existing nutrients through the addition of extra quantities of those nutrients	• Fruit juices with vitamin C
Enriched or supplemented products	Foods with added new nutrients or components not normally found in a particular food	• Margarine with plant sterol ester, probiotics, prebiotics • Yogurts with probiotics • Calcium-enriched fruit juice • Muffins with β-glucan • Drinks with herb blends
Altered products	Foods from which a deleterious component has been removed, reduced, or replaced with another substance with beneficial effects	• Fibers as fat releasers in meat or ice cream products
Enhanced products	Foods that have been enhanced to have more of a functional component (via traditional breeding, special livestock feeding or genetic engineering)	• Tomatoes with higher levels of lycopene • Oat bran with higher levels of beta glucan • Eggs with increased ω-3 achieved by altered chicken feed
Processed foods	Foods that have been processed to contain their natural levels of functional components	• Oat bran cereal (containing the natural level of β-glucan)

Source: Spence 2006. Reproduced with permission of Elsevier.

United States – Food and Drug Administration [FDA]; the European Union – European Food Safety Authority [EFSA]; and Canada – Health Canada) on how a product can be marketed (e.g., as a food additive, conventional food, or dietary supplement) and on the types of nutrient or health claims that can be made. The processes leading to accepting the evidence of health claims can be complex and rigorous due to the stringent rules and regulations set out by these bodies to protect consumers from false claims and especially to ascertain the safe use of these products. Marketers may use permitted labeling to highlight and communicate the beneficial

Table 1.3 Benefits of nutraceuticals and functional foods

Component	Source	Potential benefits
Carotenoids		
Alpha-carotene/ β-carotene	Carrots, fruits, vegetables	Neutralizes free radicals, which may cause damage to cells
Lutein	Green vegetables	Reduces the risk of macular degeneration
Lycopene	Tomato products (ketchup, sauces)	Reduces the risk of prostate cancer
Dietary Fiber		
Insoluble fiber	Wheat bran	Reduces risk of breast or colon cancer
Beta-glucan	Oats, barley	Reduces risk of cardiovascular disease; protects against heart disease and some cancers; lowers LDL and total cholesterol
Soluble fiber	Psyllium	Reduces risk of cardiovascular disease; protects against heart disease and some cancers; lowers LDL and total cholesterol
Fatty Acids		
Long-chain omega-3 FAs-DHA/EPA	Salmon and other fish oils	Reduces risk of cardiovascular disease; improves mental and visual functions
Conjugated linoleic acid (CLA)	Cheese, meat products	Improves body composition; decreases risk of certain cancers
Phenolics		
Anthocyanidins	Fruits	Neutralizes free radicals; reduces risk of cancer
Catechins	Tea	Neutralizes free radicals; reduces risk of cancer
Flavonones	Citrus	Neutralizes free radicals; reduces risk of cancer
Flavones	Fruits, vegetables	Neutralizes free radicals; reduces risk of cancer
Lignans	Flax, rye, vegetables	Prevention of cancer, renal failure
Tannins (proantho-cyanidins)	Cranberries, cranberry products, cocoa, chocolate	Improves urinary tract health; reduces risk of CVD
Plant Sterols		
Stanol esters	Corn, soy, wheat, wood oils	Lowers blood cholesterol levels by inhibiting cholesterol absorption
Prebiotics/Probiotics		
Fructo-oligosaccharides (FOS)	Jerusalem artichokes, shallots, onion powder	Improves quality of intestinal microflora and GI health
Lactobacillus	Yogurt, other dairy	Improves quality of intestinal microflora and GI health
Soy Phytoestrogens		
Isoflavones: Daidzein Genistein	Soybeans and soy-based foods	Helps alleviate menopausal symptoms such as hot flashes; protects against heart disease and some cancers; lowers LDL and total cholesterol

Source: AAFC 2012. What are functional foods and nutraceuticals? http://www.agr.gc.ca/eng/industry-markets-and-trade/statistics-and-market-information/by-product-sector/functional-foods-and-natural-health-products/functional-foods-and-nutraceuticals-canadian-industry/what-are-functional-foods-and-nutraceuticals-/?id=1171305207040.

Table 1.4 Sources of nutraceuticals and functional foods

Categories	Examples
Products extracted or purified from plants	• Beta-glucan (e.g., from oats) • Antioxidants (e.g., from blueberries) • Isoflavones (e.g., from soy) • Carotenoids (e.g., from carrots) • Lutein (e.g., from wheat) • Sterols (e.g., from wood pulp) • Essential FAs (e.g., from vegetable oil such as flax oil) • Soluble fiber (e.g., from fenugreek)
Products ground, dried, powdered, and pressed from plant materials	• Echinacea • Fenugreek • Valerian • Ginseng
Products produced, extracted, or purified from animals and microorganisms	• Omega-3 from fish oils • Essential FAs • Enzymes • Carotenoids (accumulated from the diet) • Probiotics
Products produced from marine sources	• Glucosamine • Chitosan • Fish oils

Source: AAFC 2012. What are functional foods and nutraceuticals? http://www.agr.gc.ca/eng/industry-markets-and-trade/statistics-and-market-information/by-product-sector/functional-foods-and-natural-health-products/functional-foods-and-nutraceuticals-canadian-industry/what-are-functional-foods-and-nutraceuticals-/?id=1171305207040.

health properties of their products by relying on consumer awareness and understanding of such claims.

Bioactive components in functional food and nutraceutical products are naturally found in plants, animals, bacteria, fungi, and microalgae, and their primary and secondary metabolites (Tables 1.3 and 1.4). When health benefits are proven, these natural food sources could serve as natural substitutes for synthetic pharmaceutical products for intervention purposes and to prevent potential adverse effects from the use of some pharmaceutical drugs.

Primary metabolites, which include amino acids, nucleic acids, and FAs, are required for normal healthy growth and development, while secondary metabolites, such as carotenoids, terpenoids, and alkaloids, are synthesized in specialized cell types under specific conditions. Apart from their role when ingested live in diary and non-dairy products to improve the quality of intestinal microflora and GI health (probiotic effect), some generally recognized as safe (GRAS) microorganisms may be indirect sources of high-yielding nutraceutical and functional ingredients (e.g., CLA, bioactive peptides, and vitamins liberated during fermentation). Probiotic microorganisms may further provide useful beneficial effects such as the prevention of food intolerance and/or sensitivity, and they may further decrease food allergies by degrading and decreasing allergenic epitopes required to elicit an inflammatory response (Gibson 2004; Champagne et al. 2005; Di Criscio et al. 2010; Vasudha and Mishra 2013).

In addition to the potential health benefits of nutraceuticals and functional foods, their production may also support economic development, as well as offer a way for some producers to diversify their agricultural and marine-based product offerings (Siró et al. 2008). The global nutraceuticals market is predicted to reach nearly US$207 billion by 2016, with a projected compound annual growth rate (CAGR) of 6.5% between 2011 and 2016 (BCC Research, 2011a). The functional beverages market sub-sector is experiencing the highest growth and is expected to reach approximately US$87 billion by 2016, followed by US$67 billion from food and around US$51 billion from the supplement sectors at CAGRs of 8.8%, 6.4%, and 4.8%, respectively, during the same 5-year period (i.e., 2011–2016).

1.2.1 Characteristics and Properties of Selected Bioactive Ingredients

Bioactive proteins and peptides, PUFAs, fibers, phenolics, probiotics, and prebiotics are some of the main active ingredients (Tables 1.3 and 1.4) contained in functional food and nutraceutical formulations. These compounds purportedly confer diverse health benefits and are believed to interfere with the pathogenesis of several diseases, including but not limited to GI inflammation, carcinogenesis, hypertension, CVD, developmental disorders, brain and cognitive disabilities, and aging (Gibson 2004; Phelan et al. 2009; Patisaul and Jefferson 2010; Jacques et al. 2013; Théolier et al. 2013). Most studies to date on these active ingredients are complex, confusing, controversial, and offer no clear consensus on the helpfulness or harmfulness (if any) of some of these ingredients, or if the potential benefits might be contraindicated for some groups of individuals based on age, sex, health status, and even the presence or absence of risk factors (Setchell et al. 2003; Bar-El and Reifen 2010; Patisaul and Jefferson 2010; Cederroth et al. 2012). In addition to the main active ingredient in a particular functional food or nutraceutical, synergistic interactions with other bioactive compounds present may contribute to their health effects (Spence 2006; Kris-Etherton et al. 2008; Kay et al. 2010; Ros 2010; Bao et al. 2013). As an example, a recent report from two prospective cohort studies involving nearly 120,000 people over 30 years (76,464 women in the Nurses' Health Study [1980–2010] and 42,498 men in the Health Professionals Follow-up Study [1986–2010]) confirmed the beneficial effects of consuming nuts. The report showed inverse associations between nut consumption and the risk of major chronic diseases, including CVD, type-2 diabetes, weight gain, and total and cause-specific mortality (Bao et al. 2013). The results were similar for all nuts, that is, nuts that grow underneath the earth, such as peanuts (groundnuts, a legume), and nuts that grow on trees, such as walnuts, hazelnuts, almonds, Brazil nuts, cashews, macadamias, pecans, pistachios, and pine nuts. In addition to high amounts of fats, mostly unsaturated FAs, nuts are also good sources of fiber (4–11 g/100 g), protein (7.9–38.1 g/100 g), PUFAs (1.5–47.2 g/100 g), phenolic compounds, and phytosterols (72–220 μg/100 g), and they contain traces of vitamins, minerals, as well as other bioactive substances (Table 1.5). In view of the wide-ranging nutrients, phytochemicals, and salutary health effects, most nuts hold an FDA-qualified health claim, such as follows: "eating 43 g (1.5 oz) per day of most nuts [such as name of specific nut] as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease" (FDA 2003).

Bioavailability, which refers to the body's ability to fully or partially absorb ingested bioactives, is crucial to the ability to exert beneficial effects. The bioavailability and efficacy of active ingredients in nutraceuticals and functional foods are important considerations in their formulation (Charalampopoulos et al. 2002; Havrlentová et al. 2011). For instance, the bioavailability of active ingredients may be altered depending on the specific compound or isomer formed during formulation (Kurzer and Xu 1997; Rao et al. 1998; Benakmoum et al. 2008; Xaplanteri et al. 2012). Additionally, the fate, characteristics, and behavior of bioactive components subjected to varying conditions of processing and storage (e.g., high or low temperature) and their inherent properties (e.g., high heat stability or lability, pH tolerance, shear stress tolerance) and the possible alterations that could occur following ingestion, digestion, and absorption may variously affect their potential health benefits. Knowledge of these properties and susceptibilities is important to mitigate any adverse effects during processing and storage. Other factors that need to be considered include appropriate dosage (i.e., acute or large single exposures vs. continuous small exposures), mode of delivery (e.g., oral or topical), possible interactions, toxicology, fate of carrier materials, and short- and long-term side effects based on age, sex, and health status (Paiva and Russell 1999; Setchell et al. 2003; Patisaul and Jefferson 2010; Grooms et al. 2013).

Functional foods and nutraceuticals may also contain inert components or excipients as part of the formulation. While the active ingredients are the components that confer the actual benefit, the inert components are primarily the carriers that help deliver the active ingredients to the target organ (Brownlie 2007; Hébrard et al. 2010; Kuang et al. 2010; Wichchukit et al. 2013). These inert ingredients may enhance the utility of the product or provide benefits such as disguising a bad taste or flavor (e.g., tablets coated with

Table 1.5 Nutrient composition of some raw nuts (per 100 g)

Nuts	Energy (KJ)	Fats (g)	SFA (g)	MUFA (g)	PUFA (g)	LA (g)	ALA (g)	Protein (g)	Fiber (g)	Folate (μg)	PS (mg)	Ca (mg)	Mg (mg)	K (mg)	Na (mg)
Almonds	2418	50.6	3.9	32.2	12.2	12.2	0.00	21.3	8.8	29	120	248	275	728	1
Brazil nuts (dried)	2743	66.4	15.1	24.5	20.6	20.5	0.05	14.3	7.5	22	NR	160	376	659	3
Cashews	2314	46.4	9.2	27.3	7.8	7.7	0.15	18.2	5.9	25	158	37	292	660	12
Hazelnuts	2629	60.8	4.5	45.7	7.9	7.8	0.09	15.0	10.4	113	96	114	163	680	0
Macadamia nuts	3004	75.8	12.1	58.9	1.5	1.3	0.21	7.9	6.0	11	11	85	130	368	5
Peanuts	2220	49.2	6.8	24.4	15.6	15.6	0.00	25.8	8.5	145	220	92	168	705	18
Pecans	2889	72.0	6.2	40.8	21.6	20.6	1.00	9.2	8.4	22	102	70	121	410	0
Pine nuts (dried)	2816	68.4	4.9	18.8	34.1	33.2	0.16	13.7	3.7	34	141	16	251	597	2
Pistachios	2332	44.4	5.4	23.3	13.5	13.2	0.25	20.6	9.0	51	214	107	121	1025	1
Walnuts, English	2738	65.2	6.1	8.9	47.2	38.1	9.08	15.2	6.4	98	72	98	158	441	2

Source: Ros 2010. Reproduced under a Creative Commons License; http://creativecommons.org/licenses/by/3.0/

sugar or wax) or making the tablet resistant to gastric acid such that it only disintegrates at the appropriate site as a result of enzyme action or alkaline pH (Gaudette and Pickering 2013; Jantzen et al. 2013; Nesterenko et al. 2013). Other examples of inert materials used in food formulation include surfactants, stabilizers (gums), emulsifiers, and colorants.

Brief reviews on specific bioactive components are provided in the following text. For more detailed information on the structure, distribution, metabolism, bioavailability, possible mechanism, and potential health benefits of various bioactive components, readers are referred to the following publications: bioactive proteins and peptides (Duranti 2006; Möller et al. 2008; Chatterton et al. 2013; Théolier et al. 2013), fiber (Gibson 2004; Havrlentová et al. 2011), PUFAs (Simopoulos 2002a, 2002b; Strobel et al. 2012; Ammann et al. 2013; AHA 2013; Brasky et al. 2013; Janczyk et al. 2013; van den Elsen et al. 2013), phytochemicals (De Pascual-Teresa et al. 2010; Patisaul and Jefferson 2010; Xaplanteris et al. 2012; Cederroth et al. 2012; Jacques et al. 2013; Vitale et al. 2013), and prebiotics and probiotics (FAO 2001; Gibson 2004; Champagne et al. 2005; Di Criscio et al. 2010; Hébrard et al. 2010; Śliżewska et al. 2012; Al-Sheraji et al. 2013).

1.2.2 Bioactive Proteins and Peptides

In addition to the dispensable and indispensable amino acids that proteins provide for structural and biological functions to sustain life, their potential health benefits beyond basic nutrition have been reported (Duranti 2006; Möller et al. 2008; Phelan et al. 2009; Mochida et al. 2010; Barbana and Boye 2010; Chou et al. 2012; Rui et al. 2012; Chatterton et al. 2013; Théolier et al. 2013). Plants (e.g., soybean, wheat, and other cereal grains and legumes) and animals (e.g., milk, eggs, other dairy products, meat, and fish) are important food sources of protein with encrypted biological activities (Lam and Lumen 2003; Hartmann and Meisel 2007; Phelan et al. 2009; Barbana and Boye 2010). Table 1.6 shows some plant protein sources and their estimated protein content, which can vary markedly.

Many food proteins have been used as precursors of bioactive peptides, which may be released upon hydrolysis during GI digestion by digestive or microbial enzymes, or by fermentation or ripening during food processing with isolated or microbial enzymes. These bioactive peptides may exert a wide variety of beneficial biological functions in the body (Table 1.7; Phelan et al. 2009), including, for example, regulating serum cholesterol and hypocholesterolemic effect through binding of bile acids (which are synthesized from cholesterol in the liver) (Kahlon and Woodruff 2002; Barbana et al. 2011). Eliminating bile acids may increase cholesterol metabolism and help reduce cholesterol levels in the blood. Bioactive hydrolysates and peptides may also produce inhibitory effects against angiotensin-I-converting enzyme

Table 1.6 Protein content of common edible legumes

Source	Protein (%)
Soybean	34.3
Peanut	27.6
Pea	24.5
Cowpea	22.0
Chickpea	19.5
Pigeon pea	19.5
Fava bean	24.8
Lupin	39.7
Winged bean	32.8

Source: Adapted from Lam and de Lumen 2003. Reproduced with permission of Elsevier.

Table 1.7 Immunomodulatory, antihypertensive, and osteoprotective proteins and peptides

Protein/Peptide	Effect	Model
Immunomodulatory		
Caseins (and digests)	T-lymphocyte proliferation ↑	Cell culture
	Immunoglobulin secretion ↑	Cell culture
Whey	Lymphocyte blastogenesis ↓	Cell culture
Proline-rich polypeptides (and derivates) from ovine colostrum	B-lymphocyte growth, differentiation ↑, antibody secretion ↑	Cell culture
		Animal culture
Fish protein	IgA-, IL-4-, IL-6-, IL-10-positive cells ↑	Animal culture
Antihypertensive		
α_{s1}- and β-casein	ACE ↓	In vitro
	Hypertension ↓	Animal
γ-Zein	ACE ↓	In vitro
Wheat germ	ACE ↓	In vitro
Hordein (barley)	ACE ↓	In vitro
Bonito	ACE ↓	In vitro
Osteoprotective		
Casein	Absorption of intestinal calcium ↑	Animal
Whey protein	Absorption of intestinal calcium ↑	Animal
Milk basic protein	Bone mineral density ↑	Human
Antilipemic		
Fish protein hydrolysate	mRNA of desaturases ↓, HDL-cholesterol/total cholesterol ↑	Animal
Lupin protein isolate	Total cholesterol ↓, LDL-cholesterol ↓	Animal
α′ subunits of soybean	Plasma cholesterol ↓, triglycerides ↓, VLDL receptor binding ↑	Animal

Source: Möller et al. 2008. Reproduced with permission of Springer.

(ACE) (by inhibiting the conversion of angiotensin I [decapeptide] to the more potent vasoconstrictor angiotensin II [octapeptide] by ACE) with possible blood-pressure-lowering effects (Vermeirssen et al. 2005; Barbana and Boye 2010; Rui et al. 2012). Bioactive hydrolysates and peptides may further possess antimicrobial activity and antioxidant properties that can enhance the body's defense mechanisms. Other bioactive proteins and peptides may produce immunomodulating, opioid, and anti-thrombotic activities, as well as provide positive influence on calcium absorption and dental health by inhibiting plaque-forming bacteria and tooth enamel demineralization (Table 1.6; Möller et al. 2008; Phelan et al. 2009; Chou et al. 2012; Nam et al. 2012; Théolier et al. 2013).

Peptide bioactivity can be affected by the source of protein, chemical composition, degree of hydrolysis, and the type of proteolytic enzyme used (Möller et al. 2008; Phelan et al. 2009; Nam et al. 2012; Théolier et al. 2013). Hydrolyzed proteins show higher digestibility and absorption compared to intact proteins and thus create new sources of functional foods. As sources of free amino acids, these bioactive hydrolysates have been used to potently increase the bioavailability of the building blocks of proteins for synthesis of contractile proteins, managing CVD and diabetes (Blomstrand et al. 2006; Schimomura et al. 2006; Greenfield et al. 2008; Mochida et al. 2010; Clemmensen et al. 2013; Higuchi et al. 2013; Nogiec and Kasif 2013). In sports nutrition, where performance and faster recovery following strenuous exercise are very important, hydrolyzed or predigested protein fractions are highly sought after. Amino acids acting alone or in conjunction with other amino acids have been demonstrated to be more effective in the synthesis of proteins that build muscle mass than intact proteins, as they promote better glucose uptake and synthesis of muscle glycogen, which promotes muscle restoration and recovery before, during, and after exercise (Nogiec and Kasif 2013). Branched-chain amino acids (BCAA) (i.e., leucine, valine, and isoleucine) are particularly useful in protein synthesis, especially after exercise (Blomstrand et al. 2006; Schimomura et al. 2006; Nogiec and Kasif 2013). It has recently been reported that BCAA may provide an immediate energy source needed for protein synthesis due to their preferential oxidization over glucose and FAs (Nogiec and Kasif 2013). Glutamine, however, has been shown not to be as effective in increasing protein synthesis and muscle mass as originally reported (Gleeson 2008; Greenfield et al. 2008); rather, it stimulates the release of glucagon-like peptide 1 (GLP-1), required to augment insulin secretion in obese- and type 2 diabetic individuals, and thereby improve glucose tolerance and clearance (Clemmensen et al. 2013). Amino acid L-arginine is a precursor of the endogenous vasodilator, nitric oxide, and may also play a role in promoting healthy blood pressure levels and vascular function, and in decreasing the risk of various diseases associated with vascular dysfunction (Clemmensen et al. 2013). Proteins are important sources of enzymes (e.g., protease inhibitors that inhibit protein degradation by selectively protecting proteins of interest or blocking the activity of endogenous proteolytic enzymes by reversibly or irreversibly binding to that protease). This may be important in the management of pathogens such as human immunodeficiency viruses, which break up large proteins into smaller peptides, which become precursors for assembling new viral particles (Liu et al. 2012; Koistinen et al. 2014). Although the virus can still replicate in the presence of protease inhibitors, the resulting virions are less able to infect new cells. Examples of proteases found in fruits include bromelain in pineapple (thiol proteinase, EC 3.4.22.4), papain in papaya (cysteine protease, EC 3.4.22.2), and actinidin in kiwi (sulfhydryl proteases, EC 3.4.22.14). These enzymes improve overall health by acting as digestive aids that may also reduce intestinal inflammation (Rutherfurd et al. 2011; Ha et al. 2012; Kaur and Boland 2013).

The high cost of traditional protein sources is leading to more innovation in identifying new protein ingredients. Plant-sourced proteins from legumes, as an example, are attractive alternatives to animal-derived proteins, due to their relatively lower cost, inherent and unique nutritional profile, anti-allergenic properties, and increasingly greater consumer acceptance (Barbana and Boye 2010; Rui et al. 2012). Soybean is an example of a protein source with official FDA acknowledgement of beneficial health effects (Duranti 2006; FDA 2013a). Foods that contain soy protein can carry the approved health claim stating that “25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.”

1.2.3 PUFAs

The role of specific FAs in human health is still highly debated (Gebauer et al. 2011; Tan et al. 2012; AHA 2013; Ammann et al. 2013; Brasky et al. 2013; Janczyk et al. 2013; Roncaglioni et al. 2013; Zheng et al. 2013). The basic functions of fats in structural, membrane, metabolism, and gene expression are widely known. The American Heart Association (AHA 2013) recommends that 25–35% of daily total calories be obtained as fats from oils and fats in foods. PUFAs are FAs with more than a single carbon–carbon double bond. These are an interesting group of FAs, well-studied and extensively investigated for their health benefits (Simopoulos 2002a, 2002b; Strobel et al. 2012; Ammann et al. 2013; Brasky et al. 2013; Janczyk et al. 2013). Examples of their reported beneficial effects include anti-inflammatory, immunomodulatory, cardioprotective, and antiatherosclerotic effects. The low incidence of CVD among Greenland Eskimos has long been known and attributed to a high-fish diet.

Well-known examples of PUFAs are the long-chain ω-3 FAs, which are considered to be essential because they cannot be effectively synthesized by the body due to the low activity of the rate-limiting enzyme

Δ6-desaturase. Mammals also have limited ability and efficiency to convert the shorter-chained ω-3 FAs, such as α-linolenic acid (ALA, 18:3), to the more important long-chain ω-3 PUFAs (LC ω-3 PUFA), eicosapentaenoic acid (EPA; 20:5), and docosahexaenoic acid (DHA, 22:6), and this is also further impaired with aging. Functional foods may compensate for these insufficient endogenous essential FAs needed to cover metabolic requirements. Omega-3 FAs (i.e., ALA, EPA, and DHA), stearidonic acid (STA; 18:4), and ω-6 FAs (i.e., gamma-linolenic acid [GLA] and arachidonic acid [ARA]), as well as conjugated linoleic acid (CLA, 18:2), an isomer of ω-6, have all been identified as functional lipids (Simopoulos 2002a; Strobel et al. 2012). While long-chain ω-3 PUFAs may help reduce inflammation, ω-6 FAs such as GLA and ARA tend to promote inflammation (Simopoulos 2002a, 2002b; Strobel et al. 2012). A lower ratio of ω-3/ω-6 FAs is more desirable, since it reduces the risk and pathogenesis of many diseases, whereas the reverse exerts suppressive effects. Formulated foods containing a mixture of ω-3 and ω-6 FAs are also preferred over a dominance of either one.

Different types of fish – including anchovies, salmon, mackerel, herring, sardines, tuna, and trout, and marine mammals are uniquely rich sources of PUFAs (Table 1.8). The long-chain ω-3 PUFA and total fat content of fish and fish products vary greatly depending on fish species, feeding conditions (wild or farmed), and processing and preparation methods (e.g., fillet, breaded, pre-fried fishes, etc.) (Gebauer et al. 2006; Strobel et al. 2012; Raatz et al. 2013), which is why it is advisable to consume a variety of different fish species and fish products. Regular ingestion of fried fish has been associated with a 32% increased risk for prostate cancer; environmental chemicals such as polychlorinated biphenyls (PCBs), heavy metals, and other toxic chemicals may affect the quality of fish or fish oil and also contribute to prostate cancer (Mullins and Loeb 2012).

Other natural sources of PUFAs include human milk and cultivated marine algae. Omega-6 FAs such as GLA are found in plant-based oils such as evening primrose oil, blackcurrant seed oil, and borage seed oil. Other known sources of PUFAs are avocados, peanut butter, many nuts and seeds (e.g., flaxseeds, chia seeds, walnuts, pumpkin seeds), and the oils of canola (rapeseed), corn, olive, flaxseed, sesame, soybean, and sunflower. Table 1.9 shows the ALA content of selected oils, seeds, and nuts, and the amounts needed to obtain the adequate daily intake levels for men and women.

Several physiological processes affected by PUFAs may account for their perceived benefits (Simopoulos 2002a; Furuhjelm et al. 2009; Janczyk et al. 2013; Roncaglioni et al. 2013). For instance, some beneficial effects on cellular physiology have been attributed to the presence of long-chain ω-3 PUFAs in cardiac and brain membrane phospholipids (especially DHA). PUFAs also serve as precursors for prostaglandins, leukotrienes, and eicosanoids such as resolvins and protectins, which are known for their anti-inflammatory and neuroprotective activities. Other beneficial effects of long-chain PUFAs

Table 1.8 Omega-3 content of fish and seafood (g/100 g)

Fish	Total ω-3	EPA	DPA	DHA
Farmed				
Salmon, Atlantic	2.359	0.862	0.393	1.104
Trout, rainbow	0.824	0.217	0.091	0.516
Catfish, channel	0.089	0.017	0.015	0.057
Wild				
Herring, Pacific	1.830	0.969	0.172	0.689
Salmon, Atlantic	1.723	0.321	0.287	1.115
Herring, Atlantic	1.626	0.709	0.055	0.862
Sardine, Pacific, canned in tomato sauce	1.457	0.532	0.061	0.864
Whitefish, mixed species	1.421	0.317	0.163	0.941
Mackerel, canned	1.334	0.434	0.104	0.796
Salmon, pink, canned	1.166	0.334	0.089	0.743
Sardine, Atlantic, canned in oil	0.982	0.473	0.000	0.509
Tuna, white (Albacore), canned in water	0.880	0.233	0.018	0.629
Bass, striped	0.754	0.169	0.000	0.585
Mollusks, oyster, Pacific	0.708	0.438	0.020	0.250
Trout, rainbow	0.693	0.167	0.106	0.420
Sea bass, mixed species	0.671	0.161	0.076	0.434
Salmon, Chinook, smoked (lox), regular	0.523	0.183	0.073	0.267
Catfish, channel	0.464	0.130	0.100	0.234
Mollusks, mussel, blue	0.463	0.188	0.022	0.253
Cisco	0.405	0.095	0.053	0.257
Pike, walleye	0.349	0.086	0.038	0.225
Crustaceans, crab, blue	0.320	0.170	0.000	0.150
Croaker, Atlantic	0.306	0.123	0.086	0.097
Flatfish (Flounder/Sole)	0.273	0.137	0.028	0.108
Crustaceans, crab, Dungeness	0.237	0.219	0.010	0.008
Tuna, light, canned in water	0.228	0.028	0.004	0.196
Halibut, Atlantic and Pacific	0.210	0.066	0.016	0.128
Cod, Atlantic	0.194	0.064	0.010	0.120
Crustaceans, lobster, northern	0.176	0.102	0.006	0.068
Pollock, Alaska	0.169	0.049	0.004	0.116
Tilapia	0.134	0.005	0.043	0.086
Haddock	0.136	0.042	0.005	0.089
Cod, Pacific	0.134	0.034	0.004	0.096
Mollusks, clams, mixed species	0.114	0.043	0.007	0.064
Mollusks, scallop, mixed species	0.106	0.042	0.003	0.061
Crustaceans, shrimp, mixed species	0.064	0.030	0.003	0.031

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Table 1.9 α-Linolenic acid (ALA) content of selected oils, seeds, and nuts and the amounts needed to obtain recommended adequate daily intake (RDI)

Source of ALA	ALA (g/tbsp)	Amount needed by men to meet RDI of 1.6 g ALA/d (tbsp)	Amount needed by women to meet RDI of 1.1 g ALA/d (tbsp)
Pumpkin seeds	0.051	31.4	21.6
Olive oil	0.103	15.5	10.7
Walnuts, black	0.156	10.3	7.05
Soybean oil	1.231	1.3	0.89
Rapeseed oil	1.302	1.2	0.84
Walnut oil	1.414	1.1	0.78
Flaxseeds	2.350	0.68	0.47
Walnuts, English	2.574	0.62	0.43
Flaxseed oil	7.249	0.22	0.15

Source: Gebauer et al. 2006. Reproduced with permission of American Society for Nutrition.

include lowering plasma triglyceride concentration, improving plasma lipoprotein profile, supporting fetal brain and eye development, cognitive health and maintenance, better performance or preservation of cognitive function in aging persons, improved cardiovascular health, and reduced risk of metabolic-syndrome-related conditions such as obesity and insulin resistance syndrome. Dietary supplementation with long-chain ω-3 PUFAs during pregnancy and in early stages of life may play a critical role in reducing allergic sensitization in children (Furuhjelm et al. 2009; Kremmyda et al. 2011; Noakes et al. 2012; van den Elsen et al. 2013). The role of PUFAs in promotion of the synthesis of inflammatory cytokines and autoimmune diseases such as rheumatoid arthritis and certain cancers has been described (Simopoulos 2002b).

Dietary intake of fish is the most desirable way to increase marine ω-3 PUFA intake, owing to the higher amount of long-chain ω-3 PUFAs in circulation and tissue stores after fish intake compared to fish oil supplements. This suggests a larger uptake from fish than from fish oil supplements, which may be due to differences in physiochemical structure of the lipids and better digestion and absorption of the former. Based on these perceived benefits, various professional groups and health organizations worldwide have made dietary recommendations for EPA and DHA and fish intake to primarily lower triglyceride, and to reduce risk of and treat existing CVD (Gebauer et al. 2006; Lucas et al. 2009). Recommendations also have been made for DHA intake for pregnant women and infants (Table 1.10). Table 1.11 shows the PUFA content of some commonly eaten fish and shellfish in the United States.

Table 1.10 Recommendations for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake

Organization	Year	Recommendations
UK Committee on Medical Aspects of Food Policy	1994	100–200 mg/d EPA and DHA
Eurodiet	2000	200 mg/d
Apports Nutritionnels Conseillés (France)	2001	450 mg/d (DHA, 110–120)
Health Council of the Netherlands	2001	200 mg/d
American Heart Association/American Heart Association Nutrition Committee (United States)	2002, 2006	Two servings of fatty fish per week for general health (∼430–570 mg/d) 1,000 mg/d of ω-3 EPA and DHA for patients with CHD 2,000–4,000 mg/d of ω-3 EPA/DHA for patients with high triglycerides
Food and Nutrition Board of the Institute of Medicine of the National Academies of Science	2002	130–270 mg/d (EPA and DHA can contribute up to 10% of total ω-3 intake and, therefore, up to this percentage can contribute toward the adequate intake of α-LA (1.3–2.7 g/d)
European Society of Cardiology	2003	1,000 mg/d of ω-3 EPA/DHA for patients with CHD
WHO/FAO	2003	400–1,000 (1–2 fish meals/week)
International Society for the Study of Fatty Acids and Lipids Workshop	2004	≥500 mg/d
UK Scientific Advisory Committee on Nutrition	2004	Minimum two fish meals/week (one fatty fish) ∼450 mg
National Health and Medical Research Council (Australia)	2005	430 mg/d EPA, DHA, DPA for women 610 mg/d EPA, DHA, DPA for men
Dietitians of Canada	2007	Two fish meals/week (fatty fish), 8 oz cooked fish ∼500 mg
European Food Safety Authority	2010	250 mg/d EPA and DHA for adults 250 EPA and DHA mg/d plus 100–200 mg DHA for pregnant/lactating women

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Table 1.11 Total eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) (and amount needed to get 500 mg), and mercury content cost of commonly eaten fish and shellfish in the United States

Fish	EPA + DHA (mg/serving)	Amount needed to get 500 mg EPA + DHA/d (serving)	Amount needed to get 500 mg EPA + DHA/d (serving/week)	Mean Mercury Concentration (ppm)
Cod	134	307	25.9	0.11
Catfish	151	3.3	23.1	0.05
Haddock	203	2.5	17.5	0.03
Clams	241	2.1	14.7	ND
Shrimp	267	1.9	13.3	ND
Flounder	426	1.2	804	0.05
Pollock	460	1.1	7.7	0.06
Flatfish	498	1	7	0.05
Tuna, canned	733	0.68	4.8	0.12 (light); 0.35 (Albacore)
Salmon	1825	0.27	1.9	0.01

Source: Gebauer et al. 2006. Reproduced with permission of American Society for Nutrition.

EPA and DHA were recently approved for three heart health claims by the EU/ESFA (EFSA 2012; Eur-Lex 2013). The permitted health claims state that "DHA contributes to the maintenance of normal blood triglyceride levels," "DHA and EPA contribute to the maintenance of normal blood pressure," and "DHA and EPA contribute to the maintenance of normal blood triglyceride levels." The main difference between the second and the third claims is a daily intake of 3 g of DHA–EPA in the former and 2 g in the latter. The claim may be used only for food that provides a daily intake of 2 g of DHA in combination with EPA, and also should inform consumers not to exceed a supplemental daily intake of 5 g of EPA and DHA combined per day. All three claims must contain these conditions.

Whereas the overwhelming conclusion is that of a plausible association of long-chain PUFA intake and several health benefits, there are some inconsistencies in reported literature. Previous and recent studies using various analytical assessments, including prospective or retrospective cohort studies, nested case-control, case-cohort assessment, and randomized controlled trials of the perceived effects of PUFAs, have been mixed (Tan et al. 2012; Ammann et al. 2013; Brasky et al. 2013; Galet et al. 2013; Janczyk et al. 2013; Roncaglioni et al. 2013; Zheng et al. 2013). A recent study involving 834 men found increased prostate cancer risk among men with high blood concentrations of long-chain ω-3 PUFAs (Brasky et al. 2013). However, previous studies reported the opposite. For instance, in a study involving 6,272 Swedish men who were followed for 30 years, an association between fish consumption and decreased risk of prostate cancer was reported (Terry et al. 2001). Men who ate no fish had a twofold to threefold increase in the risk of developing prostate cancer compared with those who consumed moderate to large amounts of fish in their diet. Similar studies with American men also suggested the association of ω-3 FAs from fish intakes with lower risk of prostate cancer. In another study carried out by the Harvard School of Public Health for over 12 years involving 47,882 men, eating fish more than three times a week reduced the risk of prostate cancer. The study also showed the greater impact of the consumption of these fats on the risk of metastatic prostate cancer. For instance, for each additional 500 mg of marine fat consumed, the risk of metastatic disease decreased by 24% (Augustsson et al. 2003). A recent report on a follow-up study supported the previous findings that daily fish oil supplementation in conjunction with a low-fat diet slows the growth of cancer cells in men with prostate cancer (i.e., lower amounts of pro-inflammatory substances in their blood and a lower cell cycle progression score, a measure that correlates prostate cancer aggression and likelihood of recurrence) (Galet et al. 2013). In another study involving 1,575 older people (average age 67 years) who were free of dementia, Tan et al. (2012) reported an association between lower red blood cells, DHA levels, and smaller brain volumes, and a vascular pattern of cognitive impairment even in persons free of clinical dementia. However, recent studies involving women aged 65 years and older did not find any difference in memory and thinking test scores based on levels of ω-3 FAs in the blood (Ammann et al. 2013).

Other studies have investigated the role of dietary ω-3 PUFAs in health. In a large general-practice cohort of 12,513 patients with multiple cardiovascular risk factors but no history of myocardial infarction, daily treatment with ω-3 FAs did not reduce cardiovascular mortality and morbidity (Roncaglioni et al. 2013). In a study involving 76 patients, aged 5–19 years, long-chain ω-3 PUFAs were found to improve the lipid profile by lowering triglycerides and decreasing insulin resistance and cytokine synthesis, thereby tackling the mechanisms involved in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) (Janczyk et al. 2013). In a meta-analysis involving 883,585 women, the intake of marine ω-3 PUFAs were inversely associated with risk of breast cancer. Women with the highest intake of marine-sourced ω-3 PUFAs were found to have a 14% reduction in their risk of developing breast cancer compared with women with the lowest intake (Zheng et al. 2013).

CLAs have also been a focus of several studies due to their potential health benefits (Hasler 2002; Schmid et al. 2006; Kelley et al. 2007; Larsson et al. 2009; Gebauer et al. 2011). CLA is a collective term for a group of octadecadienoic acids that are geometric-, positional-, and stereo-isomers of LA with a conjugated double bond. Dietary sources of CLA are predominant in ruminant-derived foods such as meat and milk and their products due

Table 1.12 Amounts of total FAs and conjugated linoleic acid in commonly consumed ruminant products

Food	Total fat (g/100 g)	TFA (g/100 g)	TFA (% total fat)	TFA (g/serving)	CLA (mg/g fat)
Dairy products					
Cheese, cheddar (28 g, 1 oz)	36.4	0.87	2.39	0.24	3.6 (93)
Milk, whole (244 g, 1 cup)	3.10	0.09	2.90	0.21	5.5 (92)
Yogurt, plain, low-fat (255 g, 1 cup)	1.16	0.03	2.59	0.06	4.4 (86)
Meat					
Meat, beef, ground, 20.8% fat, raw (115 g, 4 oz)	21	0.79	3.76	0.91	4.3 (85)
Meat, beef, ground, 22.1% fat, raw (115 g, 4 oz)	22.1	0.93	4.21	1.07	4.3 (85)

Source: Gebauer et al. 2011. Reproduced with permission of American Society for Nutrition.

Table 1.13 Conjugated linoleic acid (CLA) concentration of raw meat

Source	CLA (mg/g fat)
Lamb	4.32–19.0
Beef	1.2–10.0
Veal	2.7
Pork	0.6–0.7
Chicken	0.7–1.5
Turkey	2.0–2.5

Source: Schmid et al. 2006. Reproduced with permission of Elsevier.

to the action of rumen microorganisms in PUFA bio-hydrogenation and/or isomerization. Cheese, beef, yogurt, and milk, respectively, contain ~3.6, 4.3, 4.4, and 5.5 mg of CLA per gram of fat (Table 1.12). The concentration of CLA also varies substantially among raw meat of commonly consumed animals (Table 1.13). CLA contents in these sources were not negatively altered with cooking and storing (Schmid et al. 2006).

In CLA-rich sources such as beef and dairy products, the most abundant (>90%) isomer is *cis*-9, *trans*-11 (c9, t11–18:2). Other positional and geometric isomers such as c9, t10, c12, t9, and t11 have been identified. Physiological properties of CLA include anticarcinogenic, antiatherosclerotic, and antiatherogenic effects, as well as beneficial influence on body composition.

The mechanisms by which mixtures of CLA isomers may inhibit tumor growth may include inhibiting the initiation, promotion, progression, and metastasis of malignant tumors (Kelley et al. 2007). These were attributed to the role of CLA in altering lipid peroxidation, tissue FA composition, eicosanoid metabolism, gene expression, cell cycle regulation, and proliferation and apoptosis. As previously discussed for long-chain PUFAs, reports of the beneficial effects of CLA intake on health have been inconsistent, with some recording an inverse relationship, no association, or simply inconclusive results (Gebauer et al. 2011). For instance, in a cohort study of 61,433 cancer-free Swedish women over a period of 3 years (1987–1990), no evidence of the protective effect of dietary intake of CLA on the risk of breast cancer was observed (Larsson et al. 2009).

Partial hydrogenation has been used industrially to produce *trans* FAs (TFAs), an analog of CLA, primarily to increase shelf life of foods and as an alternative to animal fats (lard, tallow, and butter) from vegetable oil. The c9, t11-CLA is essentially a TFA derived from ruminants. Several studies have linked vegetable-oil-derived TFA to CHD, certain risk factors of CVD, and various cancers (Gebauer et al. 2011), recently prompting the FDA (2013b) to issue a preliminary determination that *trans fats* are not GRAS. This could eventually lead to the classification of *trans fats* as a food additive and subject them to premarket regulatory approval. Health Canada (2007) has adapted the Trans Fat Task Force recommendation of limiting *trans* fats to 2% of total fat content in vegetable oils and margarine, and 5% in all other foods.

1.2.4 Phytochemicals and Phytoestrogens

Phytochemicals are a group of plant secondary metabolites valued for their health benefits (e.g., as radical scavengers and quenchers of singlet oxygen) (Di Majo et al. 2005; Fanga and Bhandaria 2010; De Pascual-Teresa et al. 2010). Reactive oxygen species (ROS) are continuously produced in cells and can damage cell membranes, tamper with DNA, and even cause cell death. These reactive species are considered to be the cause of oxidative stress and inflammation involved in several diseases. Inflammation normally occurs in response to tissue damage caused by either physical or ischemic injuries, infections, and toxins. The body's response may cause cellular changes and immune responses at the site of injury. In certain cancers, inflammation may promote the spreading and mutation of cancer cells, angiogenesis, and an alteration of signaling pathways (De Pascual-Teresa et al. 2010; Patisaul and Jefferson 2010). Chronic inflammation can increase the risk of disease by creating a conducive environment for its development, bolster treatment resistance, and exacerbate the problem. The biological effects of phytochemicals may thus extend well beyond their antioxidant properties. Whereas there are many aspects to consider when treating inflammation, such as slowing the progression of a disease, it seems logical to use nutraceuticals as non-pharmacological interventions to prevent and manage inflammation, especially considering their role in quenching singlet oxygen (De Pascual-Teresa et al. 2010; Patisaul and Jefferson 2010; Cederroth et al. 2012).

Phytochemicals possess a broad spectrum of health benefits, and have been shown to safely suppress pro-inflammatory pathways, reduce low-density lipoprotein (LDL) cholesterol, protect against damage caused by LDL cholesterol, and reduce the risk of atherosclerosis or plaque build-up in arteries that can lead to heart attack or stroke and contribute to CHD (Adolphe et al. 2010). Their antimicrobial properties may prevent the reversible and epigenetic changes in the body's cells often linked to diseases. Phytochemicals include flavonoids (flavanols, flavones, flavanones, isoflavones, catechins, anthocyanidins, and proanthocyanidins), carotenoids, as well as other polyphenols (Scalbert et al. 2005; Boots et al. 2008; De Pascual-Teresa et al. 2010; Vitale et al. 2013), and are found in a variety of foods (Tables 1.14 and 1.15).

Flavonoid is the collective name given to a group of compounds characterized by two aromatic rings linked by a three-carbon bridge. Flavanols, flavones, and flavanones are the main flavonoids that occur in citrus. Several studies have suggested that the high concentrations of flavanols found in cocoa-rich products and dark chocolates increase the formation of endothelial nitric oxide, which promotes vasodilation and therefore may reduce blood pressure and risk of CVD (Taubert et al. 2007; Ried et al. 2010; 2012; Shrime et al. 2011; Hooper et al. 2012). Their anti-cancer mechanisms include immunomodulating properties by interfering in the initiation, enhancement, and progression of cancer as a result of regulating different enzymes and receptors in signal transduction pathways related to cellular proliferation, differentiation, apoptosis, inflammation, angiogenesis, metastasis, and reversal of multi-drug resistance (Adolphe et al. 2010; De Pascual-Teresa et al. 2010). The role of polyphenols in general in the prevention of degenerative diseases such as CVD and cancers is based on their antioxidant properties and modulation of oxidative stress (De Pascual-Teresa et al. 2010). The properties of the various flavonoids are dependent on the chemical structure, structural class, degree of hydroxylation, substitutions and conjugations, and degree of polymerization (Di Majo et al. 2005; Park et al. 2006; Christensen 2009). The spatial arrangements of the groups may have greater influences on the antioxidant property than the flavan backbone by determining the stability of the radicals (Di Majo et al. 2005).

Phytoestrogens are plant-derived phenolic compounds that are structurally and/or functionally similar to mammalian estrogens (Kurzer and Xu 1997; Patisaul and Jefferson 2010). They include isoflavones, coumestans, and lignans, which are most notably found in soybeans, clover and alfalfa sprouts, and oilseeds (such as flaxseed), respectively. Soybean is

Table 1.14 Sources and properties of polyphenols

Polyphenol	Forms	Sources	Properties
Anthocyanidins	Cyanidin, pelargonidin, peonidin, petunidin, delphinidin, malvidin, and their glycosides	Red, blue, and purple berries, flowers	Natural pigments; highly sensitive to temperature, oxidation, pH, and lights; water soluble
Catechins	Catechin, epicatechin, epigallocatechin, gallocatechin, and epigallocatechin gallate	Tea	Sensitive to oxidation, lights, and pH; astringent and bitter; slightly soluble in water
Flavanones	Hesperetin, homoeriodictyol, hesperidin, naringenin, naringin	Citrus	Sensitive to oxidation, lights and pH; aglycones are insoluble in water but glycosides are soluble in aqueous solutions
Flavones	Apigenin, luteolin, tangeritin	Thyme, oregano, parsley, rosemary, and some fruits and vegetables	Natural pigments; sensitive to oxidation and pH; aglycones slightly soluble but glycosides soluble in water
Flavonols	Kaempferol, myricetin, quercetin, and their glycosides	Onions and citrus fruits and vegetables	Sensitive to oxidation, lights, and pH; aglycones slightly soluble but glycosides soluble in water
Isoflavones	Daidzein, genistein, glycitein	Soybeans, peanuts	Sensitive to alkaline pH; astringent and bitter; soy smell; water soluble
Hydroxybenzoic acids	Gallic acid, ρ-hydroxybenzoic, vanillic acid	Berries, tea, wheat	Sensitive to temperature, oxidation, pH, and light; most soluble in water
Hydroxycinnamic acids	Caffeic acid, ferulic acid, ρ-coumaric acid, sinapic acid	Fruit, oats, rice	Sensitive to oxidation and pH; most slightly soluble in water
Lignans	Pinoresinol, podophyllotoxin, steganacin	Flax, sesame, vegetables	Relatively stable under normal conditions; unpleasant flavor; water soluble.
Tannins (proanthocyanidins)	Castalin, pentagalloyl glucose, procyanidins	Tea, wines, cocoa, berries, grape	Sensitive to high temperature and oxidation; astringent and bitter; water soluble

Source: Boots et al. 2008. Reproduced with permission of Elsevier.

Table 1.15 Total polyphenolic content of some plant foods and beverages

Food	Total Polyphenols	Food/Beverages	Total Polyphenols
Cereals (mg/100 g dm)		**Vegetables (mg/100 g fm)**	
Barley	1,200–1,500	Black currant	140–1,200
Corn	30.9	Blueberry	135–280
Millet	591–1,060	Cherry	60–90
Oats	8.7	Apple	27–298
Rice	8.6	Cranberry	77–247
Sorghum	170–10,260	Gooseberry	22–75
Wheat	22–40	Grape	50–490
Legumes (mg/100 g dm)		Grape fruit	50
Black gram	540–1,200	Orange	50–100
Chickpeas	78–230	Pear	2–25
Cowpea	175–590	Plum	4–225
Common beans	34–280	Raspberry	37–429
Green gram	440–800	Red currant	17–20
Pigeon peas	380–1,710	Strawberry	38–218
		Tomato	85–130
Nuts (% dm)		**Fruit Juices (mg/L)**	
Betel nuts	26–33	Apple juice	2–16
Cashew nuts	33.7	Orange juice	370–7,100
Peanuts	0.04		
Pecan nuts	8–14		
Vegetables (mg/100 g fm)		**Beverages**	
Brussels sprouts	6–15	Tea (mg/200 mL)	150–210
Cabbage	25	Coffee (mg/150 mL)	200–550
Leek	20–40	White wine (mg/L)	200–300
Onion	100–2,025	Red wine (mg/L)	1,000–4,000
Parsley	55–180	Beer (mg/L)	60–100
Celery	94		

Source: Sivam, 2002. Reproduced with permission of Taylor & Francis Group (dm, dry matter; fm, fresh matter).

uniquely rich in the isoflavones genistein (4′,5,7-trihydroxyisoflavone) and daidzein (4′,7-trihydroxyisoflavone), and, to a small extent, glycitein. Isoflavones may exist in various isomeric forms: unconjugated (aglycones: daidzein and genistein), glucoside (daidzin, genistin, and glycitin), acetyl-glucoside (6′′-O-acetyldaidzin, 6′′-O-acetylgenistin, 6′′-O-acetylglycitin), and malonylglucoside (6′′-O-malonyldaidzin, 6′′-O-malonylgenistin, 6′′-O-malonylglycitin). The 4′-methyl ethers isomers (daidzein: formononetin, and genistein: biochanin) are found in clover. Flaxseeds contain the lignan secoisolariciresinol diglucoside, matairesinol, pinoresinol, and lariciresinol (Adolphe et al. 2010).

Phytoestrogens may play an important role in obesity and diabetes by improving glucose control and insulin resistance via pancreatic insulin secretion mechanisms. Phytoestrogens are also of particular importance in women's health, and their role includes reducing the risk of osteoporosis, heart disease, breast cancer, and menopausal symptoms, among others (Kurzer and Xu 1997; Setchell et al. 2001; 2003; Christensen 2009; Adolphe et al. 2010; Patisaul and Jefferson 2010; Cederroth et al. 2012). It is well established that estrogens promote breast tumorigenesis. Under certain conditions, isoflavones (genistein and daidzein) bind to both estrogen receptors (ER-alpha and ER-beta) but preferentially bind to and activate ER-beta and exert estrogen-like effects by stimulating the growth of estrogen-sensitive tumors (Kurzer and Xu 1997; Christensen 2009; Vitale et al. 2013). It is therefore recommended that women with serious risk factors for breast cancer or a family history of breast cancer be cautious about incorporating soy in their diets (Patisaul and Jefferson 2010). Phytoestrogens have also been identified as endocrine disruptors with adverse effects on numerous molecular and cellular targets that can impact reproductive development and endocrine systems (Bar-El and Reifen 2010; Cederroth et al. 2012). Consequently, there has been some concern about the safety of soy isoflavone phytoestrogens genistein and daidzein in infant formula and the threat that these compounds may pose to infant development (Bhatia and Greer 2008; Patisaul and Jefferson 2010). Advisories on the use of soy in formulas have so far been confusing (Patisaul and Jefferson 2010). The removal of the outer fiber layer of some grains, legumes, and vegetables during processing tends to reduce lignan content (Bhathena and Velasquez 2002), and judicious selection of processing conditions may also help to reduce the level of isoflavone in soy products.

Carotenoids are natural pigments synthesized by plants and are responsible for the bright colors of various fruits and vegetables (Paiva and Russell 1999; Krinsky and Johnson 2005). They are made up of two main classes: xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). Examples of carotenes are α- and β-carotene and lycopene, whereas xanthophylls include lutein and zeaxanthin. In addition to the red anthocyanin and yellow xanthophyll pigments, carotenes are responsible for the bright red to pink lycopene and orange pigments in fruits and vegetables. According to a report from BCC research (2011b), the market value of commercially used carotenoids is expected to reach US$1.4 billion in 2018 with a CAGR of 2.3%; β-carotene is predicted to reach almost US$334 million by 2018 at a CAGR of 3.1% a year; and lutein is expected to reach US$309 million (CAGR of 3.6%) by 2018. Carotenoids are valued for their antioxidant properties and their ability to reduce the risk of certain cancers and eye disease. Dietary levels of carotenoids (including β-carotene) were found to promote health in certain sub-populations (Paiva and Russell 1999). Epidemiologic studies have also shown an inverse relationship between dietary carotenoids or blood carotenoid levels and various cancers. However, intervention trials with high dose of β-carotene did not show protective effects against cancer or CVD, while the high-risk population commonly comprising of smokers and asbestos workers in the trials showed an increase in cancer and angina cases (Paiva and Russell 1999). Alpha- and β-carotene are involved in cellular metabolism. Beta-carotene can be converted to vitamin A, unlike lycopene (because of the absence of a terminal β-ionone ring). Lutein and zeaxanthin help to filter and absorb the damaging blue light that enters the eye. Tomato and tomato products are the most significant dietary sources of lycopene, whereas egg yolk and dark-green leafy vegetables (spinach and kale) are highly bioavailable sources of lutein and zeaxanthin (Ma and Lin 2010). Other sources of lycopene are pink grapefruit, watermelon, papaya, guava, and other fruits.

Increased lycopene intake has been reported to reduce the incidence of CVD and coronary heart disease by 17% and 26%, respectively, and improve the functioning of blood vessels compared to effects observed at lower intake levels (Xaplanteris et al. 2012; Jacques et al. 2013). Tomato juice was recently shown to reduce biomarkers of oxidative stress and damage, after exhaustive exercise or in patients with diabetes, cardiovascular diseases, and inflammation (Harms-Ringdahl et al. 2012). The authors suggested that the antioxidant effect of tomato juice was primarily due to lycopene (the most abundant and stable carotenoid in industrial-processed tomato juice) and not the other components of tomatoes (e.g., vitamin C, tocopherols, and polyphenols), which tend to be easily destroyed during processing. Increased intake of tomatoes and tomato-based products, and therefore high serum concentrations of lycopene, was also found to decrease the risk of stroke including ischemic stroke in men (Karppi et al. 2012). Medium-term (14 d) daily consumption of 70 g of tomato paste (containing 33.3 mg of lycopene) improved the function of blood vessels in healthy adults by improving flow-mediated dilation; a measure of a blood vessel's ability to relax, due to enhanced carotenoid bioavailability with tomato processing and cooking in olive oil (Xaplanteri et al. 2012).

Phenolic compounds further play a major role in the sensory attributes of many food products, such as enhancing organoleptic properties in smoked food, cheese, and other dairy products (guaiacol, eugenol, syringol, cresol, and phenol); extending shelf-life of processed foods (catechins); controlling Maillard reaction (caffeic acid); and enhancing color development in wine and dairy products (anthocyanins). They can also cause food deterioration (e.g., beer haze [protein–phenolic compound interactions] and the development of off-flavor in fruit juices [conversion of ferulic acid to guaiacol]) (O'Connell and Fox 2001).

1.2.5 Fiber

Fiber, also known as "non-starch polysaccharide," is derived from the cell wall of plants such as cellulose, hemicellulose, and pectin. As with other carbohydrates, its primary function is to provide energy. It is a complex carbohydrate that is metabolized differently from other forms of carbohydrates, generally resistant to digestion and absorption. It is also classified as an unavailable carbohydrate with a different nutritional classification to available carbohydrates (starch and soluble sugars). Fiber is further classified as soluble (food gums/hydrocolloids and pectin) and insoluble (cellulose and hemicellulose). The soluble components of the dietary fiber is degraded by microflora, resulting in a substantial stimulation of microbial growth and an increased fecal bulk. Soluble fiber also affects glucose and lipid absorption into the bloodstream by lowering serum cholesterol. Insoluble fiber is resistant to breakdown by the microflora and has been identified for their role in laxation and bowel habit by retaining water within the cellular structure, intestinal transit time, production of short-chain FAs, and prebiotic effect (Havrlentová et al. 2011). Their indigestible and fermentable nature mainly defines their nutraceutical and functional properties. Apart from the physiological properties of this fibrous material, fibers are suitable fermentable substrates adjuncts for probiotics and encapsulation shells (Charalampopoulos et al. 2002; Havrlentová et al. 2011). Several studies have reported the hypocholesterolemic effect of fiber and its role in lowering blood pressure and inflammation, reducing the risk of CVD, and inhibiting and decreasing the growth of certain cancer cells and tumors (Havrlentová et al. 2011; Grooms et al. 2013). Table 1.16 provides the total dietary fiber content of some common cereal grains. Recently, a health claim for plums of "prune"

Table 1.16 Fiber content of cereal grains

Categories	Total dietary fiber (%, dwb)
Legumes	13.6–28.9
Rye	15.5
Corn	15
Triticale	14.5
Oat	14
Wheat	12
Sorghum	10.7
Barley	10
Rice	3.9

Source: Charalampopoulos et al. 2002. Reproduced with permission of Elsevier.

cultivars (*Prunus domestica* L.) in maintaining normal bowel function was approved by the EFSA. In order to obtain the claimed effect, about 100 g of dried plums (prunes) must be consumed daily (EFSA 2012). Prunes contain both dietary soluble and insoluble fiber. The cholesterol-lowering effect of fiber may be related to β-glucan mediation in increasing bile acid secretion. Beta-glucan is recognized as the main functional component of some cereal fibers. A recent study by Grooms et al. (2013) involving 23,168 subjects over the period 1999–2010 strengthened the association between low dietary fiber intake and reduction of cardio-metabolic risks such as metabolic syndrome, cardiovascular inflammation, and obesity. The limited digestion and low glycemic index of fiber helps in glycemic (blood sugar) control by slowing the release of energy, delaying gastric (stomach) emptying, retarding the entry of glucose into the bloodstream, and lessening the postprandial (post-meal) rise in blood sugar, thereby stabilizing blood sugar in diabetic individuals or those predisposed to diabetes (Childs 1999; Grooms et al. 2013). Keeping blood sugar low and stable may also impact the production of advanced glycation end products (AGEs), known for their role in vascular (atherosclerosis) and renal complications and for their association with diabetes and aging (Raj et al. 2000; Daroux et al. 2010; Yamagishi 2013). In weight control and obesity, fiber may help to control hunger, regulate food intake, increase satiety, reduce overconsumption, and displace other nutrients such as lipids. Despite this knowledge, several investigators have found that dietary fiber intake consistently falls below the recommended amount (Grooms et al. 2013). The Institute of Medicine recommends dietary fiber intake levels according to age and sex: 38 g per day for men aged 19–50 years, 30 g per day for men 50 and over, 25 g for women aged 19–50 years, and 21 g per day for women over 50 years. The data from the recent study reveals that the mean dietary fiber intake was only 16.2 g per day across all demographics during that study time period (Grooms et al. 2013). Health Canada suggests the consumption of 26–35 g of fiber daily by healthy adults and 25–50 g per day by people with diabetes. Present fiber intake in Canada only averages 4.5–11 g a day, according to Health Canada. Fiber is ubiquitous in grains, legumes, and vegetables. Wheat bran and whole grains, as well as the skins of many fruits and vegetables, and seeds, are rich sources of insoluble fiber. Soluble fiber is found in oats, legumes (peas, kidney beans, and lentils), some seeds, brown rice, barley, oats, fruits (e.g., apples), some green vegetables (e.g., broccoli), and potatoes.

FDA has permitted product labels to carry health claims stating that oatmeal and oat cereals may reduce the risk of heart disease – as part of a diet low in fat and cholesterol. Products made of whole-grain oats or oat fiber with a minimum of 0.75 g β-glucan per serving size qualify for the FDA health claim. Table 1.17 provides the β-glucan content of some common cereal-based foods. Oats and barley have the highest β-glucan content among cereals.

Table 1.17 Soluble β-glucans content in some cereal-based food

Cereal	Food	Soluble β-glucans (g/100 g dry weight)
Wheat	Whole meal	0.39
	Bran	1.38
	Groat	0.42
Barley	Whole meal	3.35–3.95
	Bran	4.14
	Groat	5.28
Oat	Whole meal	2.66–4.51
	Groat	3.5–5.0
	Bran concentrate	7.48–17.0
	Flakes	2.64–4.6

Source: Reproduced from Cereal β-glucans and their significance for the preparation of functional foods – a review by Havrlentová et al. with permission from Czech Journal of Food Sciences.

Health Canada suggests increasing the amount of fiber by eating more grains and unpeeled but well-washed fruit and vegetables. Fiber is utilized in the formulation of breakfast cereals and bread, and commercial preparations such as Agarol® (agar) and Metamucil® (psyllium). The strong gelling and water-absorbing capacities of psyllium may prevent the incorporation of the required amount in one serving of a food product to permit the use of the cholesterol-lowering claim on the food label (Childs 1999; Anderson et al. 2000).

1.2.6 Prebiotics and Probiotics

Prebiotics and probiotics have received tremendous interest and have found wide application in the food industry, as is evident in consumer acceptance and the volume of production worldwide. According to BCC reports, probiotics was a US$21.6 billion industry in 2011 (BCC Research 2011c), and it is expected to reach US$31.1 billion by 2015, with a CAGR of 7.6% for the next 5-year period. Unlike other nutraceuticals and functional products, probiotic foods account for 90.1% of this value, followed distantly by 6.4% for supplements. The FAO/WHO define *probiotics* as live microorganisms that when administered in adequate amounts confer a health benefit on the host (FAO 2001), while a *prebiotic* has been defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon and thus improves host health (Gibson 2004). The importance of probiotics and prebiotics as nutraceutical and functional foods stems from their ability to improve intestinal microflora, and to reduce and inhibit the growth of pathogenic strains such as *E. coli* and *Salmonella* (known for their roles in causing infectious diseases in the GI tract), allergic disorders, diarrhea, and inflammatory bowel disease (Gibson 2004; Champagne et al. 2005; Di Criscio et al. 2010). For dietary products, bacterial count of at least 106 CFU ml^{-1} is commonly accepted. The effect of probiotics is strain specific. Bacterial strains from the genera *Lactobacillus*, *Bifidobacterium*, and *Bacillus* are three known sources of probiotics. Three new bacterial strains with potential probiotic effects – *Lactobacillus paracasei* CNCM I-4034, *Bifidobacterium breve* CNCM I-4035, and *Lactobacillus rhamnosus* CNCM I-4036 – isolated from the feces of breastfed infants have recently been identified (Muñoz-Quezada et al. 2013). *Saccharomyces boulardii*; a yeast strain is commercially available as a probiotic for human use (Martins et al. 2005). Currently, several strains of *Saccharomyces cerevisiae* (905 and L11), *Kluyveromyces marxianus* L10, and *Kluyveromyces lactis* L13 from different environments (insect association, tropical fruit, and cheese) are being considered for use as potential probiotics (Martins et al. 2005; Binetti et al. 2013). Known examples of prebiotics include non-digestible oligosaccharides such as inulin (fruits and vegetables), fructo-oligosaccharides, galacto-oligosaccharides, lactulose, and resistant starch (Table 1.18) (Charalampopoulos et al. 2002; Śliżewska et al. 2012; Al-Sheraji et al. 2013). The limited digestibility in the small intestine and fermentation by intestinal bacteria of these non-digestible oligosaccharides in the colon may slow energy release in individuals predisposed to diabetes, increase satiety, and reduce hunger. They may also play an important role in colonic health and other GI illnesses by stimulating the beneficial activity and proliferation of specific members of the intestinal microflora, prevent colonization by potential pathogens, produce beneficial short-chain FAs (such as acetic acid, propionic acid, and butyric acid, which are used by the host organism as an energy source), and stimulate calcium absorption from food (Bosscher et al. 2006; Al-Sheraji et al. 2013). Prebiotics and probiotics in foods and beverages and as supplements may enhance health independently, or in combination (also referred to as *synbiotics*) (Champagne et al. 2005; Di Criscio et al. 2010).

1.3 Production of Nutraceuticals and Functional Foods

Nutraceuticals and functional foods may be produced with the same ingredients in various formats to meet specific physiological requirements and needs of target groups. As an example, functional foods may be formulated as liquid shakes for infants and senior citizens and as solids for adults. Liquid foods permit easy ingestion in infants and children, whereas the solid form may be more convenient for adults. Nutraceuticals and functional food ingredients

Table 1.18 Sources and production of prebiotics

Prebiotic	Source	Production
Inulin	Chicory, *Agave tequilana*	Extraction from raw material
Fructooligosaccharides	Asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato, and rye	• Transglycosylation of the polysaccharides • Transfructosylation of sucrose using β-fructofuranosidase • Hydrolysis of chicory inulin
Xylooligosaccharides	Bamboo shoots, fruits, vegetables, milk, honey, and wheat bran	• Enzyme hydrolysis of xylan-containing lignocellulosic material • Chemical fractionation of xylan from lignocellulosic material followed by enzymatic hydrolysis of xylan • Hydrolytic degradation of xylan by steam, water, or dilute solutions of mineral acids
Galactooligosaccharides	Human's milk and cow's milk	Enzymatic hydrolysis (transglycosylation) of lactose by β-galactosidase
Cyclodextrins	Water-soluble glucans	Transglycosylation and hydrolysis of starch using cyclodextrin glucosyltransferases
Raffinose oligosaccharides	Seeds of legumes, lentils, peas, beans, chickpeas, mallow composite, and mustard	Water or aqueous methanol or ethanol extraction
Soybean oligosaccharide	Soybean	Extraction from the by-product of soybean protein isolate/concentrate production (whey)
Lactulose	Lactose (milk)	Alkali isomerization of lactose glucose moiety to fructose residue
Lactosucrose	Lactose	Transglycosylation of lactose and sucrose
Isomaltulose (Palatinose)	Sucrose, honey, sugarcane juice	Enzymatic rearrangement of the glycosidic linkage in sucrose from α (1, 2)-fructoside to α (1, 6)-fructoside followed by crystallization

Table 1.18 (*continued*)

Prebiotic	Source	Production
Maltooligosaccharides	Starch	Debranching of starch by pullulanase and isoamylase, combined with hydrolysis by various α-amylases
Isomaltooligosaccharides	Starch	• Transglucosidation (transgalactosylation) of starch maltose using α-glucosidase • Enzymatic synthesis from sucrose
Arabinoxylooligosaccharides	Wheat bran	Enzymatic depolymerization of raw material (lignocellulosic materials)
Enzyme-resistant dextrin	Potato starch	Chemical modification of starch

Source: Śliżewska et al. 2012. Reproduced under a Creative Commons License; http://creativecommons.org/licenses/by/3.0/ and Al-Sheraji et al. 2013. Reproduced with permission of Elsevier.

and products may be formulated dry as wettable or dispersible powders, or wet as soluble, suspension, emulsifiable concentrate, dispersion, or encapsulated agents, using processes such as emulsification, agglomeration, retorting, spray-drying, extrusion, precipitation, fermentation, and ultrafiltration to preserve, modify, or deliberately incorporate the active ingredients. Detailed information of these methods have been reported in the literature (Ozdemir and Cevik 2007; Barbana and Boye 2010; Mochida et al. 2010; and references therein), and further relevant details are provided elsewhere in this book. Functional foods may be incorporated and consumed in a muffin, other bread products, baked goods, or breakfast products. Second-generation products (e.g., soy foods such as soymilk, miso, tofu, and tempeh) are also very popular.

Both polar and non-polar solvents such as ethanol, chloroform, petroleum ether, hexane, and acetonitrile are commonly used in different ratios with or without water to extract ingredients with functional and nutraceutical properties such as phenolics and anthocyanins (Dunford et al. 2010; Plaza et al. 2010). The pre-treated/non-treated raw material is exposed to the solvent, which preferentially extracts compounds of interest and also other agents such as flavors and colorings. The extracted sample may be separated by centrifugation or filtration to remove solid residue, and the solvent is evaporated and the extract recovered and concentrated. The main drawbacks to solvent extraction in some instances include the toxicity and danger of these solvents to humans and environment, and the labor-intensive and extensive downstream processes required to rid the product of the solvents used.

Acids, bases, and enzymes have been used to partially or extensively hydrolyze proteins to produce hydrolysates and release bioactive peptides (Clemente 2000; Humiski and Aluko 2007; Potier and Tomé 2008; Rui et al. 2012). Enzymes have received increased attention due to the mildness of their reactions and the production of fewer undesirable side reactions. GI enzymes, such as trypsin, pepsin, chymotrypsin, peptidases, and pancreatin, as well as commercial endo- and exopeptidases such as Alcalase™, Flavourzyme™, and Thermolysin™, have been used sequentially or in combination to hydrolyze the peptide bonds in proteins. Enzymatic hydrolysis is a useful tool in producing bioactive peptides with preferred characteristics such as molecular weight (MW), size, functional properties, and potential health benefits (Clemente 2000; Potier and Tomé 2008). Fermentation of milk with proteolytic starter cultures may also be used to generate bioactive peptides. Alpha and β-amylases, isoamylase, pullulanase, and amylopullulanase are some examples of enzymes used to hydrolyze starch and other polysaccharides (Tomasik and Horton 2012; Al-Sheraji et al. 2013; Śliżewska et al. 2012).

Soy concentrate (65–90% protein) is produced by water or alcohol extraction to remove soluble carbohydrates and also to improve functionality. This process may, however, denature some proteins and reduce the concentration of isoflavones (Table 1.9; Bhathena and Velasquez 2002). The use of techniques such as milling, air classification, salt extraction (micellization), isoelectric precipitation, chromatography (ion exchange), electrodialysis, ultrafiltration, and other membrane technologies have resulted in various purities (concentrate or isolate) and overall quality of the protein fraction (Boye et al. 2010; Barbana and Boye 2013).

Most nutraceuticals and functional ingredients found in fruits, vegetables, legumes, and animal-derived sources may have low bioavailabilities, partially as a result of their slow release from the food matrix (Kurzer and Xu 1997; Rao et al. 1998; Benakmoum et al. 2008; Xaplanteri et al. 2012). Processing technologies used in the development of nutraceutical and functional food ingredients often strive to improve product bioavailability or create novel foods enriched with isolated and/or concentrated fractions of the bioactive ingredients. As an example, enzymatic deglycosylation has been used to increase the bioavailability and antioxidant activity of flavonoids (Park et al. 2006; Christensen 2009). Additionally, it should be mentioned that observed *in vitro* effects may not be directly correlated with *in vivo* effects due to degradation, fragmentation, or modification in the GI tract or other parts of the body (Anguelova and Warthesen 2000; Reboul et al. 2005; Binetti et al. 2013). Probiotics must also survive in the GI environment and maintain at least one beneficial function (e.g., colonization resistance against pathogenic microorganisms, immunomodulation or nutritional contribution) (Martins et al. 2005) to be considered useful.

Various formulation options have been explored in the quest to produce simpler, safer, and more efficacious food products with uniform appearance and acceptable taste. The influence of particle size, pH, solubility, hydrophobicity/hydrophilicity, and stability of both the bioactive and excipient (inert) components on bioavailability is a critical factor to consider during formulation. Formulations are usually developed to be close to the preparation that will ultimately be on the market. In addition to a series of downstream processes, several tests may be carried out to determine the effects of temperature, humidity, oxidation, or photolysis (ultraviolet light or visible light) on the stability of a product, as well as the loading efficiency, dissolution, and release rates in encapsulated formulation (Brownlie 2007; Hébrard et al. 2010; Wichchukit et al. 2013). For nutraceuticals, dose and site-specific and release profiles are very crucial. Acid-labile ingredients must be protected from gastric pH, and thus digestive aids may be used in the formulations to preferentially help them dissolve faster and be rapidly released as small-sized nanocapsules with shorter gastric resident times, allowing them to traverse the stomach quickly to reach the site of interest (Hébrard et al. 2010). Alternatively, bioactive ingredients may be formulated for extended or delayed release (Brownlie 2007). Sufficient numbers of live probiotic microorganisms, as an example, may reach the large intestine (colon) when protected from gastric pH, bile, and other nutrients/compounds in the gut (Hébrard et al. 2010; Wichchukit et al. 2013).

Processing affects the quality and properties of bioactive ingredients to different extents with varying nutritional implications (Rao et al. 1998; Anguelova and Warthesen 2000; Bhathena and Velasquez 2002; Reboul et al. 2005). Fish oil refining processes such as neutralization, degumming, and winterization help to improve sensory attributes, such as off-flavors and off-taste, and/or safety, but they may also lead to chemical reactions such as autoxidation, hydrolysis, isomerization, polymerization, and pyrolysis, which can influence the quality of the oil. Fermented, sprouted, and germinated immature/green beans of the same legume can contain varied amounts of bioactive compounds, as shown in Table 1.19. Unprocessed soybeans contain 1.2–4.2 mg/100 g of isoflavones, whereas some high-protein soy products such as soy flour and textured soy protein contain 1.1–1.4 mg/g dry weight of isoflavones. Second-generation soy foods such as tofu, yogurt, and tempeh burger contain varied amounts of isoflavones, since most of the components of the matrices in these foods are non-soybean constituents. The presence (glucoside) or absence (aglycone) of the sugar moiety will further affect the absorption and bioavailability of many flavonoids in humans. Glucose-conjugated isoflavones are highly polar and water-soluble compounds that are hardly absorbed by the gut. This consequently reduces

Table 1.19 Isoflavone content of some selected foods (mg/100 g)

Food Product	Daidzein	Genistein	Glycitein	Total Isoflavones
Dairy				
Ensure plus, liquid nutrition	0.20	0.35	0.00	0.60
Ensure, liquid nutrition	1.40	2.58	0.28	4.33
Non-dairy creamer, with added soy flour or soy protein	0.06	0.14	–	0.21
Baby Food				
Infant formula, Abbott nutrition, SIMILAC, ISOMIL, with iron, powder, not reconstituted	6.03	12.23	2.73	25.82
Infant formula, Abbott nutrition, SIMILAC, ISOMIL, with iron, ready-to-feed	0.73	1.37	0.21	2.21
Infant formula, ENFAMIL Next Step, powder, soy formula, not reconstituted	7.23	14.75	3.00	25.00
Infant formula, PBM products, ULTRA bright beginnings, soy, liquid concentrate	0.98	2.69	0.35	3.81
Infant formula, PBM PRODUCTS, Ultra bright beginnings, soy, powder	5.70	13.55	2.05	28.01
Infant formula, PBM products, Ultra bright beginnings, soy, ready-to-feed	0.75	1.60	0.28	2.63
Fats and Oils				
Mayonnaise, made with tofu	5.50	11.30	–	16.80
Soups, Sauces, and Gravies				
Black bean, sauce	5.96	4.04	0.53	10.26
Miso soup	0.78	0.73	0.03	1.52
Miso soup mix, dry	29.84	40.0	–	69.84
Sausages and Luncheon Meats				
Frankfurter, beef	1.00	0.80	0.10	1.90
Frankfurter, beef, fat free	0.60	1.00	0.10	1.70
Breakfast Cereals				
Cereals ready-to-eat, Kashi Golean by Kellogg's	8.40	7.70	1.40	17.40
Cereals ready-to-eat, KELLOGG'S, Smart start soy protein	41.90	41.90	10.20	93.90
Cereals ready-to-eat, KELLOGG'S, KELLOGG'S start (purchased in the United Kingdom)	0.01	0.01	–	0.02
Cereals ready-to-eat, NESTLÉ'S Shreddies (purchased in the United Kingdom)	0.02	0.04	–	0.06
Vegetables and Vegetable Products				
Clover sprouts, raw	0.04	0.21	–	0.25
Clover, red	11.0	10.0	–	21.0
Soybeans, green, cooked, boiled, drained, without salt (includes edamame)	7.41	7.06	4.60	17.92
Soybeans, green, raw (includes edamame)	20.34	22.57	7.57	48.95
Soybeans, mature seeds, sprouted, cooked, steamed	5.0	6.7	0.8	12.50
Soybeans, mature seeds, sprouted, raw	12.86	18.77	2.88	34.39

Table 1.19 (*continued*)

Food Product	Daidzein	Genistein	Glycitein	Total Isoflavones
Nuts and Seeds				
Nuts, pistachio nuts, raw	1.88	1.75	0.00	3.63
Seeds, flaxseed	0.02	0.04	0.06	0.12
Legumes and Legume Products				
Bacon bits, meatless	64.37	45.77	8.33	118.50
Bacon, meatless	2.20	5.66	1.50	9.36
Beans, common, raw (*Phaseolus vulgaris*)	0.29	0.30	0.00	0.59
Beans, kidney, red, mature seeds, raw	0.01	0.01	–	0.02
Beans, navy, mature seeds, raw	0.01	0.20	–	0.21
Beans, scarlet runner, mature seeds, cooked	0.04	0.05	–	0.09
Beans, scarlet runner, mature seeds, raw	0.05	0.07	0.00	0.12
Broadbeans (fava beans), mature seeds, fried	0.00	1.29	–	1.29
Broadbeans (fava beans), mature seeds, raw	0.33	0.15	0.28	0.63
Chicken nuggets, meatless, canned, prepared (Worthington FriChik)	4.35	9.35	0.90	14.60
Chicken nuggets, meatless, canned, unprepared (Worthington FriChik)	3.45	7.90	0.85	12.20
Chicken patties, meatless (Morningstar farms	1.80	2.20	0.40	4.40
Chik Patties Original)				
Frankfurter, meatless (purchased in Germany)	5.78	6.43	0.06	12.27
Instant beverage, soy, powder, not reconstituted	40.07	62.18	10.90	109.51
Miso	16.43	23.24	3.00	41.45
Natto	33.22	37.66	10.55	82.29
Sausage patties, meatless (Morningstar Farms Veggie, sausages, patties)	2.00	2.30	0.30	4.60
Sausage, meatless	4.46	9.23	2.30	14.34
Soy cheese, American	5.75	8.70	3.50	17.95
Soy cheese, Monterey Jack, fat-free	7.80	8.80	2.10	18.70
Soy cheese, mozzarella	1.14	2.60	2.28	6.02
Soy drink	2.75	5.10	–	7.85
Soy fiber	18.80	21.66	7.90	44.43
Soy flour (textured)	67.69	89.42	20.02	172.55
Soy flour, defatted	64.55	87.31	15.08	150.94
Soy flour, full-fat, raw	72.92	98.77	16.12	178.10
Soy flour, full-fat, roasted	89.46	85.12	16.40	165.04
Soy meal, defatted, raw	80.77	114.71	16.12	209.58
Soy paste	19.71	17.79	6.05	38.24
Soy protein concentrate, aqueous washed	38.25	52.81	4.94	94.65
Soy protein concentrate, produced by alcohol extraction	5.78	5.26	1.57	11.49
Soy protein drink	27.98	42.91	10.76	81.65
Soy protein isolate	30.81	57.28	8.54	91.05
Soy sauce made from soy and wheat (shoyu)	0.78	0.39	0.14	1.18
Soy yogurt	13.77	16.59	2.80	33.17

(*continued overleaf*)

Table 1.19 (*continued*)

Food Product	Daidzein	Genistein	Glycitein	Total Isoflavones
Soy-based liquid formula for adults, Abbott nutrition, Enrich	0.14	0.40	–	0.54
Soybean butter, full fat, Worthington Foods, Inc.	0.22	0.30	0.05	0.57
Soybean chips	26.71	27.45	–	54.16
Soybean, curd, fermented	12.18	21.12	2.30	34.68
Soybeans, flakes, defatted	37.47	91.22	14.23	131.53
Soybeans, flakes, full-fat	21.75	39.57	1.12	62.31
Soybeans, green, mature seeds, raw	61.70	60.07	7.07	128.83
Soybeans, mature seeds, canned	26.15	25.15	6.10	52.82
Soybeans, mature seeds, dry roasted (includes soy nuts)	62.14	75.78	13.33	148.50
Soybeans, mature seeds, raw (Australia)	39.88	65.64	17.12	120.84
Soybeans, mature seeds, raw (Brazil)	29.09	67.57	13.10	99.82
Soybeans, mature seeds, raw (China)	53.38	57.98	11.71	118.28
Soybeans, mature seeds, raw (Japan)	45.95	74.33	9.01	130.65
Soybeans, mature seeds, raw (Taiwan)	27.77	45.88	13.24	85.68
Soybeans, mature seeds, raw (United States)	61.33	86.33	13.33	159.98
Soymilk (all flavors), low-fat, with added calcium, vitamins A and D	1.01	1.51	0.04	2.56
Soymilk (all flavors), non-fat, with added calcium, vitamins A and D	0.30	0.41	0.00	0.70
Soymilk skin or film (Foo jook or yuba), cooked	17.81	25.15	2.69	44.67
Soymilk skin or film (Foo jook or yuba), raw	80.03	101.40	15.43	196.05
Soymilk, made from soy isolate (purchased in Australia)	2.80	3.10	–	5.90
Soymilk, original and vanilla, fortified or unfortified	4.84	6.07	0.93	10.73
Sufu	7.50	5.46	0.78	13.75
Tempeh	22.66	36.15	3.82	60.61
Tempeh burger	6.40	19.60	3.00	29.00
Tempeh, cooked	13.12	21.14	1.39	35.64
Tempeh, fried	32.90	39.90	–	72.80
Tofu yogurt	5.70	9.40	1.20	16.30
Tofu, dried–frozen (koyadofu)	29.59	51.04	3.44	83.20
Tofu, firm, cooked	10.26	10.83	1.35	22.05
Tofu, fried	13.80	18.43	2.93	34.78
Tofu, okara	3.62	4.47	1.30	9.39
Tofu, salted and fermented (fuyu)	20.72	23.83	4.95	48.51
Tofu, silken	9.15	8.42	0.92	18.04

Table 1.19 (*continued*)

Food Product	Daidzein	Genistein	Glycitein	Total Isoflavones
Tofu, smoked	7.50	5.60	–	13.10
Tofu, soft, Vitasoy-silken	8.59	20.65	–	29.24
Veggie burgers or soy burgers, unprepared	2.36	5.01	0.55	6.39
Baked Products				
Bread, soy and linseed (purchased in Australia)	4.87	9.13	0.67	14.67
Bread, white, commercially prepared, with added soy flour or soy protein	0.74	0.68	0.10	1.48
Bread, whole grain, commercially prepared, with added soy flour or soy protein	0.29	0.28	0.00	0.57
Bread, whole meal, commercially prepared, with added soy flour or soy protein	0.16	0.14	–	0.30
Doughnuts, cake-type, plain (includes unsugared, old-fashioned)	2.58	2.44	0.29	5.31
Doughnuts, with added soy flour or soy protein	1.30	3.22	–	4.52
Sweets				
Desserts, frozen, Glace Soymilk	7.00	6.20	0.90	14.00
Desserts, frozen, Tofutti non-dairy original premium	1.10	1.70	0.10	2.90
Pudding, made with soymilk	3.50	5.63	0.00	9.13
Cereal Grains and Pasta				
Noodles, egg, dry, enriched	0.01	0.01	–	0.02
Rice, brown, long-grain, raw	0.03	0.04	–	0.07
Semolina, enriched	0.01	0.02	–	0.03
Fast Foods				
Jack in the Box, Beef monster taco	2.60	13.10	0.20	15.90
Pizza, with added soy flour or soy protein	0.23	0.24	0.47	3.00
Subway, meatball sandwich	3.00	2.70	0.30	6.00
Snacks				
Bar, Tiger's Milk protein rich	4.90	5.90	0.70	11.50
Formulated bar, Balance yogurt honey peanut flavor	11.80	13.60	1.20	26.60
Formulated bar, Cliff Luna nuts over chocolate flavor	8.10	8.40	1.20	17.70

Source: USDA (2008). Courtesy of USDA-Agricultural Research Service.

their biological activity compared to the unconjugated aglycone. In some instances, glycosylated isoflavones may be deconjugated to aglycones by colon microbial families. Non-fermented soy food (tofu) has high amounts of glucosides, while fermentation has been shown to increase the levels of the bioactive unconjugated isoflavone aglycones in soybean (tempeh). Processing of soybean can result in various amounts of isoflavone genistein and daidzein in the finished product (Table 1.18) (Bhathena and Velasquez 2002). Minimal processing results in soy flour with malonyl isoflavones isomers (6′′-O-malonyldaidzin and 6′′-O-malonylgenistin), whereas texturing by heat treatment during extrusion converts malonyl isoflavones to their acetyl forms (6′′-O-acetyldaidzin and 6′′-O-acetylgenistin). Textured soy protein (50–70% protein) may be used as a meat substitute in hotdogs, hamburgers, and sausages, and to fortify numerous products, as shown in Table 1.19. Soy protein isolate (≥90% protein) has been used to enrich energy bars, sports drinks, infant formula, cereals, granola bars, imitation dairy products, mayonnaise, ice cream, cheese, and even doughnuts.

Lycopene in tomatoes can be concentrated by processing into a juice, sauce or paste as ketchup, spaghetti, and pizza sauce. The content and bioavailability of lycopene is also markedly modified by processing and the chemical environment (Table 1.20; Rao et al. 1998; Anguelova and Warthesen 2000; Reboul et al. 2005; Xaplanteri et al. 2012). The presence of environmental factors such as air, light, and temperature may result in autoxidation and reversible isomerization of the predominant *trans* lycopene isomers in fresh tomatoes to the more oxidizable *cis* isomers, which may cause a decrease in total lycopene content and proportion of *trans* isomers, color loss, and development of grassy off-flavors (Anguelova and Warthesen 2000). The increase in bioavailability with processing may involve the breakdown of cell walls, which weakens the bonding forces between lycopene and tissue matrix, making lycopene more accessible. Dehydrated and powdered tomatoes have poor lycopene stability due to the conversion of the *trans* isomers to the more oxidizable bioavailable *cis* isomers (Rao et al. 1998). The enrichment of tomato paste with tomato peel increased the content of lycopene and β-carotene by >50% and ~100%, respectively, without any sensory changes (Reboul et al. 2005).

Cereal grains such as oats are milled into powder/flour or used for porridge (rolled oats: de-husked oat groats, steamed and rolled into flat flakes), muesli cereals (mixture of uncooked rolled oats, nuts, and fresh or dried fruits), baked goods (bread, muffin, and cakes), toppings (on pastries and desserts), pasta and noodles, soups, as meat extenders in meat products (sausage, burgers, nuggets, meatballs), as fat replacers (Nutrim®; concentrated β-glucan) in cheese, snack bars (bar-shaped pressed–baked muesli or granola), or incorporated into granola (mixture of uncooked rolled oats, nuts, and honey) for increased convenience. These processes may influence the quantity and bioavailability of the active compounds in cereals.

Table 1.20 Lycopene content of tomatoes and tomato products

Group	Product	Lycopene (ppm) mean ± SEM, n=5
Products for food preparation	Tomato paste	365.0 ± 3.6
	Tomato puree	365.0 ± 3.6
	Crushed tomatoes	223.8 ± 0.9
Sauces	Tomato sauce	130.6 ± 1.2
	Spaghetti sauce	191.2 ± 1.3
	Pizza sauce	121.7 ± 0.8
	Seafood sauce	185.6 ± 2.5
	Chilli sauce	168.3 ± 1.6
Condiments	Tomato ketchup	123.9 ± 2.1
	Light ketchup	141.1 ± 2.1
	Barbecue sauce	42.9 ± 0.6
Readily consumed	Tomato juice	101.6 ± 0.6
	Condensed soup	72.7 ± 0.2
	Ready to serve soup	44.1 ± 0.6
	Clam cocktail	43.3 ± 0.2
	Bloody Mary mix	42.3 ± 0.3

Source: Rao et al. 1998. Reproduced with permission of Elsevier.

There is growing interest to develop formulations that are protected and effectively delivered at specific targets and at controlled rates (Champagne and Fustier 2007; Ozdemir and Cevik 2007; Kuang et al. 2010; Jung et al. 2013). Several encapsulation procedures have been established, and predominant among them are emulsion, extrusion, and spray drying (Jantzen et al. 2013). Spray drying is routinely used to convert liquids into dry powders and for its dual functionality to both form a capsule and dry in a single step. Several shell materials such as polysaccharides, proteins, waxes, FAs, gums, and their derivatives that do not elicit immune responses and have low toxicities in humans have been approved for food use (Kuang et al. 2010; Nesterenko et al. 2013). The shell may have a hydrophobic cavity and hydrophilic exterior where lipid-based compounds interact with the interior of the shell to form an inclusion complex that dissociates on target. Encapsulation may improve the stability of the active ingredient under conditions encountered in food processing and storage conditions (temperature, oxygen, light), or in the GI tract (pH, enzymes, presence of other nutrients). Encapsulation has been used to maximize the proportion of the active ingredient that remains available following intake and improve gastric residence time, product permeability, solubilization at the site of action, bioavailability, and potential health benefits (Lee et al. 2007; Shen et al. 2010). Emulsions and self-microemulsifying formulations have been introduced using surfactants to increase bioavailability and absorption of poorly water-soluble ingredients such as isoflavones (Lee et al. 2007; Kuang et al. 2010; Shen et al. 2010).

Table 1.21 Beneficial effects of probiotic encapsulation

Benefits	Product
Facilitates the production of oxygen-sensitive cultures	Dried probiotic culture
Facilitates the recovery of centrifugation-sensitive cultures	
Facilitates the recovery of high EPS-producing cultures	
Less contamination problems	
Cultures can be air-dried	
Improved survival on exposure to gastric and bile solutions	Nutraceutical
Improved stability during storage in dried form	
Improved acidification rate	Dried sausages
Improved survival on heating	Biscuits, powder
Improved survival on freezing	Ice cream, milk-based medium, cranberry juice
Improved retention in the finished product	Cheese
Protection against bacteriophages and yeast contaminants	Fermented milks
Improved survival during storage	Yoghurt, mayonnaise, milk

Source: Champagne and Fustier 2007. Reproduced with permission of Elsevier.

The technique creates fine droplets of emulsion when reconstituted with water or with body fluids. Encapsulation can enhance the delivery of viable cells into dairy and bakery products during processing and storage, and equally improve their survival and facilitate controlled delivery in the GI tract following consumption (Champagne and Fustier 2007) as well as other benefits (Table 1.21).

Jung et al. (2013) detailed the preservation and increased bioactivity and bioavailability of microencapsulated green tea catechin. Green tea catechin reportedly has a protective effect on the cardiovascular system by mitigating the adverse features of myocardial fibrosis and high serum uric acid levels and increasing hepatic catalase activity. Carotenoids may also be encapsulated due to their highly unsaturated structure to improve thermal or oxidative stability during processing and storage, as well as to reduce the production of undesirable flavors and loss of potential health value (Champagne and Fustier 2007; Ozdemir and Cevik 2007). Some unprocessed functional foods may contain bitter endogenous compounds while processing, or deliberate addition may introduce bitterness-eliciting compounds (Table 1.22). Unlike pharmaceuticals drugs, the health claim appeal of functional foods and a positive perception of their benefits and sensory attributes are important to encourage their acceptance, purchase, and commercial success; various de-bitterness techniques and bitterness-modifying additives have therefore been used to enhance taste (Champagne and Fustier 2007; Gaudette and Pickering 2013;

Table 1.22 Some commercial functional food products containing endogenous or added bitter-eliciting functional ingredients

Product	Active functional ingredient	Company
Broccoli sprout juice	Glucosinolates (sulforaphane)	Garden Gate Farms, Ontario, Canada
Green tea energy drink Enviga™	Green tea phenolics (can include epigallocatechin gallate and (+)-catechin)	The Coca-Cola Company, Georgia, United States
Catechin-enriched green tea powder	Green tea phenolics	Shizuoka Tea, Japan
Matcha green tea yogurt	Green tea phenolics	Trader Joe's®, California, United States
Yogurt with green tea extract Silhouette 0+®	Green tea phenolics	Danone, Incorporated, Quebec, Canada
Chocolate bar CocoaVia®	Cocoa flavonoids (can include epicatechin and procyanidin B2)	Mars, Incorporated, Virginia, United States
Chocolate dietary supplement drink CirkuHealth™	Cocoa flavonoids (can include epicatechin and procyanidin B2)	Mars, Incorporated, Virginia, United States
Water Vitaminwater™-"sync" berry-cherry	Berry phenolics	Glaceau Vitaminwater®, New York, United States
Grape skin powder Cabernet Grape Powder – used to fortify breads	Grape phenolics (can include trans-resveratrol, quercetin, (+)-catechin, and (-)-epicatechin)	Vinifera For Life Canada™, Ontario, Canada
Sports drink Xilarate™ Sports Power Fluid	Grape-seed extract	Xilarate, Ontario, Canada
Polyphenolic-enhanced wine	Resveratrol	The Wine Doctor Resveratrol Enhanced Wines, NSW, Australia
Nutrition bar soy protein bar	Soy isoflavones	GeniSoy®, Oklahoma, United States

Source: Gaudette and Pickering 2013, Table 1. Reproduced with permission of Taylor & Francis Group; www.tandfonline.com

Nesterenko et al. 2013) and to remove molecules that cause off-flavors or undesirable tastes of certain functional ingredients.

Although it is reasonable and useful to consume more oil-rich fish and fish products, as another example, dietary preference and sensory attributes (smell and taste) may present a barrier to consumers' choices. Encapsulation has been used to circumvent these unacceptable effects (Champagne and

Fustier 2007), and encapsulated PUFAs have been used to enrich or fortify various food products. Fish oils and vegetarian algae-based PUFAs are incorporated into a wide range of products such as beverages and juices, baby food and pediatric juices, breads and bakery products, dairy products, processed meats, and cooking oils. Complexation with cyclodextrin provides an alternative method similar to encapsulation to improve the physicochemical characteristics of some bioactive substances in order to enhance their absorption and distribution to target tissues (dos Santos et al. 2011; Beig et al. 2013).

1.4 Current Formulation Trends and the Modern Marketplace

Robust science continues to demonstrate the beneficial health effects of bioactive proteins and peptides, PUFAs, fibers, phytochemicals, probiotics, and prebiotics in improving general health and reducing markers and risks of certain illnesses. Combined with a thriving and still largely untapped market for some of these nutraceuticals and functional foods, food manufacturers seeking higher profit margins from the sale of these food products are using various formulations and production technologies to expand growth opportunities. Important drivers and challenges include increasing production costs, sustainability concerns, growing health issues, and changes in consumer preferences including an increased desire for product differentiation and efficacy (Siró et al. 2008). Additionally, manufacturers are looking for alternative improved methods to the current labor-intensive and time-consuming purification procedures that must meet strict quality standards. Manufacturers are using superior handling techniques to improve stability during processing, and to enhance recovery of the final product. These approaches may prove to be cost-effective in the long term, while creating a competitive advantage in the marketplace to ensure sustained growth and consumer satisfaction. It is estimated that the total cost of developing and marketing functional foods may far exceed the US$1–2 million required to develop conventional new food products (Siró et al. 2008), owing to the more arduous requirements such as proof of product efficacy and hurdles to obtain health claim approvals for use on packaging in the marketing of the former.

In the future, new formulation and product development will likely focus on products for healthy aging, healthy breakfast and snacking, cognitive function, muscle building, and personalized nutrition, among others. Currently, examples of popular products developed to address market needs include fortified orange juice with calcium to serve lactose-intolerant consumers; soy-based products as sources of protein for vegetarians; ω-3-enriched eggs or pasta or preformed DHA products; and products fortified with EPA and/or DHA for those who do not like seafood, vegans, individuals who are allergic or cannot eat fish, those concerned about mercury levels in foods, and those who elect not to include fish in their diets.

Current formulation trends seek to offer products in which natural health choices and convenience have converged. Ready-to-drink (RTD) tea is one of the fastest-growing beverage categories globally, due to consumer preferences and convenience. Nestea® and its different varieties are produced by The Coca-Cola Company and Nestlé. These beverages are a combination of the strong antioxidant powers of tea and the benefits of drinking water. Several brands and forms of tea (e.g., black, green, herbal, fruit/spice-flavored and decaf, HONEST, and Celestial Seasonings' "Brew Over Ice") are available.

Healthy-ingredient snacks are shaping up as a new consumer trend and an emerging functional food market to contrast the well-known sweet and salty attributes of snacks. Functional foods as snacks and mini-meals including nuts and seeds have been formulated and packaged as bars, vegetable/fruit chips, dried fruit, and nut and seed blends to provide greater convenience. Probiotics are commonly consumed as part of fermented foods, such as kefir, kurut, and yogurt, or as dietary supplements. Other products such cheese, fermented milk, yog-ice cream, cheese-based dips, probiotic fermented lactic beverages, probiotic fiber-enriched fermented milks, starch-based dairy desserts, and non-fermented frozen vegetarian desserts are also commercially available (Di Criscio et al. 2010; Escobar et al. 2012; Minervini et al. 2012; Castro et al. 2013). Food manufacturers are finding new ways to incorporate alternative sources of functional ingredients into mainstream products, such as energy bars, breakfast cereals, meat alternatives, and beverages. Several probiotic foods and beverages have been formulated to contain other bioactive compounds such as fruits, vegetables, and herbs known for their minerals, vitamins, dietary fibers, and phytochemicals content with multi-functional health benefits provided in a single food (Table 1.23).

Foods such as yoghurt have low content of phenolics (Karaaslan et al. 2011). A recent study described the supplementation of yoghurt with grape extracts or grape callus cultures for their inherent phenolic compounds and potential free radical scavenging activity (Karaaslan et al. 2011; Chouchouli et al. 2013). Blending of milk and legume proteins into cheese may be used to reduce fat and cholesterol content without adversely affecting texture. Breakfast meals are being formulated to contain a combination of one or more of products such as whole grains, fiber, protein, omega-3 FAs, and antioxidants.

Table 1.24 provides a sample of products formulated with bioactive peptides as well as ingredients for various applications. Alternative protein ingredients are growing in importance, and they include plant protein sources such as grains and legumes, consumed as is and formulated into processed foods. Legume flours from pulses have been introduced into the food processing chain, where they contribute protein, starch, and fiber (Boye et al. 2010). Pulse proteins especially represent a small but growing segment of the overall protein market, a market dominated for many years by soybean and animal

Table 1.23 Examples of commercial probiotic products

Brand/trade name	Description	Producer
Actimel	Probiotic drinking yogurt with *Lactobacillus casei Imunitass®* cultures	Danone, France
Activia	Creamy yogurt containing *Bifidus ActiRegularis®*	Danone, France
Gefilus	A wide range of *Lactobacillus rhamnosus* GG (LGG) products	Valio, Finland
Hellus	Dairy products containing *Lactobacillus fermentum ME-3*	Tallinna Piimatööstuse AS, Estonia
Jovita Probiotisch	Blend of cereals, fruits, and probiotic yogurt	H&J Bruggen, Germany
Pohadka	Yogurt milk with probiotic cultures	Valašské Meziříčí Dairy, Czech Republic
ProViva	Refreshing natural fruit drink and yogurt in many different flavors, containing *Lactobacillus plantarum*	Skåne mejerier, Sweden
Rela	Yogurts, cultured milks, and juices with *Lactobacillus reuteri*	Ingman Foods, Finland
Revital Active	Yogurt and drink yogurt with probiotics	Olma, Czech Republic
Snack Fibra	Snacks and bars with natural fibers and extra minerals and vitamins	Celigüeta, Spain
SOYosa	Range of products based on soy and oats, including a refreshing drink and a probiotic yogurt-like soy–oats product	Bioferme, Finland
Soytreat	Kefir-type product with six probiotics	Lifeway, United States
Yakult	Milk drink containing *Lactobacillus casei* Shirota	Yakult, Japan
Yosa	Yogurt-like oats product flavored with natural fruits and berries containing probiotic bacteria (*Lactobacillus acidophilus*, *Bifidobacterium lactis*)	Bioferme, Finland
Vitality	Yogurt with pre- and probiotics and omega-3	Müller, Germany
Vifit	Yogurt drink with *Lactobacillus rhamnosus* GG (LGG), vitamins, and minerals	Campina, The Netherlands

Source: Siró et al. 2008. Reproduced with permission of Elsevier.

Table 1.24 Commercially available functional foods or ingredients containing food-derived bioactive peptides

Product name	Type of food	Health claim	Manufacturer
BioZate	β-LG hydrolysate (whey-derived peptides)	Hypotensive	Davisco, United States
Calpico (Europe) or Calpis AMEAL S (Japan)	Fermented milk (β- and $_{K}$-casein)	Hypotensive	Calpis Co., Japan
Casein DP Peptio Drink	Soft drink (casein-derived dodecapeptide)	Hypotensive	Kanebo, Japan
C12 Peptide	Ingredient (casein-derived dodecapeptide)	Hypotensive	DMV, Netherlands
Evolus	Fermented milk, calcium enriched (β- and $_{K}$-casein)	Hypotensive	Valio, Finland
Peptide Soup	Soup (Bonito-derived peptides	Hypotensive	NIPPON, Japan
BioPURE-GMP	Whey protein hydrolysate (glycomacropeptide)	Anticarcinogenic, antimicrobial, antithrombotic	Davisco, United States
Capolac	Ingredient (CPP)	Aids mineral absorption	Arla Foods, Denmark
CE90CPP	Ingredient (20% CPP)	Aids mineral absorption	DMV, Netherlands
Kotsu Kotsu calcium	Soft drink (CPP)	Aids mineral absorption	Asahi, Japan
Tekkotsu Inryou	Soft drink (CPP)	Aids mineral absorption	Suntory, Japan
PeptoPro	Ingredient (beverages, gels)	Improves athletic performance	DSM Food Specialists, Netherlands
ProDiet F200	Milk drink, confectionary (α_{S1}-casein (fragment 91–100))	Reduces stress	Ingredia, France
Glutamin peptide WGE80GPA WGE80GPN WGE80GPU	Dry milk protein hydrolysate (glutamine-rich peptides)	Immunomodulatory	DMV, Netherlands

Source: Hartmann and Meisel 2007. Reproduced with permission of Elsevier.

protein. It has become increasingly important as a replacement for soy in many foods over time due to the similarity in their properties and the allergenicity of soy and dairy protein (Table 1.19).

While milk and milk products continue to be the main vehicle for administering probiotics and prebiotics, non-dairy matrices such as fruits and vegetables, cereal grains, and meat have been considered for the delivery of probiotics (Siró et al. 2008; Rivera-Espinoza and Gallardo-Navarro et al. 2010). Innovations in the development of non-dairy-based probiotic foods are receiving attention due to dietary habits and preferences, and also because of lactose intolerance and allergenicity to dairy products that might prevent dairy usage by certain segments of the population (Vasudha and Mishra 2013). These matrices may be sources of other bioactive ingredients such as prebiotics, proteins, and fibers. Traditional fermented foods are a source of mixed cultures of yeast, fungi, and bacteria (lactic acid bacteria); some of them have been shown to exhibit probiotic characteristics (Table 1.25). Soybean, wheat, rye, millet, sorghum, and maize cereals have been used to replace dairy products to make beverages such as Boza, Bushera, Mahewu, Pozol, and Togwa (Vasudha and Mishra 2013). Several non-dairy, fruit- or vegetable-based beverages such as Hardaliye that contain probiotics are commercially available. Grainfields Wholegrain Liquid is a non-dairy beverage formulated with fermented grains, beans, and seeds of malted organic oats, maize, rice, alfalfa seeds, pearl barley, linseed, mung beans, rye grain, wheat, millet using lactobacillus (*Lb.*), and yeast cultures such as *Lb. acidophilus*, *Lb. delbreukii*, *Saccharomyces (Sc.) boulardii*, and *Sc. Cerevisiae*. Biola juice is a 95% fruit drink made with *Lb. rhamnosus* GG with no added sugar, by Tine BA, in Norway. It is available in orange–mango and apple–pear flavors. Rela fruit juice (*Lb. reuteri* MM53), BioGaia ProDentis lozenges (*Lb. reuteri* Protectis), BioGaia ProTectis straw (*Lb. reuteri* Protectis), and BioGaia ProTectis drops (*Lb. reuteri* DSM 17938 (*Lb. reuteri* Protectis)) are a few of the beverages and supplements from Biogaia, Sweden (Biogaia Global). Vita Biosa is a beverage made in Denmark from a mixture of fermented aromatic herbs and other plants, using a combination of lactic acid and yeast cultures.

"Ancient grains" such as kamut, teff, quinoa and amaranth, buckwheat, spelt, chia, and freekeh are called "super grains" due to their perceived health benefits. They are available as whole kernel, cut, flakes, crushed, and in flour forms, and have been employed in formulating various products such as soups, side dishes, hot cereals, ready-to-eat cereals or snack mixes, meat-free dishes (veggie-burgers, meatballs, or tacos), breads, and cookies.

Formulating synbiotic fermented milks, using strains of *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium sp*. as probiotics, and fructo-oligosaccharides, galacto-oligosacharides, lactulose, and inulin-derived products as prebiotics, is another trend (Champagne et al. 2005; Di Criscio et al. 2010). In addition to their individual advantages, the combination of probiotics and prebiotics in a synergetic mix may improve the survival of the

Table 1.25 Potential probiotic traditional fermented foods

Product	Probiotic microorganisms	Substrates
Adai	Lactic acid bacteria (LAB)	Cereal, legume
Agbelima	*Lactobacillus (Lb.) plantarum*, *Lb. brevis*, *Lb. fermentum*, *Leuc. mesenteroides*	Cassava
Atole	LAB	Maize
Ben-saalga	LAB	Pearl millet
Boza	*Lb. plantarum*, *Lb. brevis*, *Lb. rhamnosus*, *Lb. fermentum*, *Leuc. mesenteroides subsp. dextranium*	Cereals
Dosa	*Leuc. mesenteroides*, *Lb. fermentum*, *Sacch. cerevisiae*	Rice and Bengal gram
Idli	*Leuc. mesenteroides*, LAB, yeast	Cereal, legume
Ilambazi lokubilisa	LAB	Maize
Kecap	LAB	Wheat, soybean
Kenkey	*Lb. casei*, *Lb. lactis*, *Lb. plantarum*, *Lb. brevis*, *Lb. acidophilus*, *Lb. fermentum*, *Lb. casei*, yeast	Maize
Kimchi	*Lb. plantarum*, *Lb. curvatus*, *Lb. brevis*, *Lb. sake*, *Leuc. mesenteroides*	Vegetables
Kishk	LAB	Cereal and milk
Kisra	*Lactobacillus* sp., *Lb. brevis*	Sorghum
Koko	*Lb. fermentum*, *Lb. salivarius*	Millet
Mahewu	*Lb. bulgaricus*, *Lb. brevis*	Maize
Mawe	*Lb. fermentum*, *Lb. brevis*, *Lb. salivarius*, *Sacch. cerevisiae*	Maize
Ngari	*Lactococcus lactis* subsp. *cremoris*, *Lactococcus plantarum*, *Enterococcus faecium*, *Lb. fructosus*, *Lb. amylophilus*, *Lb. coryniformis* subsp. *torquens*, and *Lb. plantarum*	Fish
Ogi	*Lb. plantarum*, *Lb. fermentum*, *Leuc. mesenteroides*, and *Sacch. cerevisiae*	Maize
Sauerkraut	*Leuc. mesenteroides*, *Lactococcus lactis*, LAB	Cabbage
Som-fug	LAB	Fish
Tarhana	*Streptococcus thermophilus*, *Lb. bulgaricus*, *Lb. plantarum*	Parboiled wheat meal and yogurt
Tempeh	LAB, *Lb. plantarum*	Soybean
Uji	LAB	Maize, sorghum cassava, finger millet

Source: Rivera-Espinoza and Gallardo-Navarro 2010. Reproduced with permission of Elsevier.

probiotic with a readily available substrate for fermentation, resulting in a better microbial balance in the colon, which can provide protective effects against colonic carcinogenesis.

Several leading food companies such as Nestlé, DANONE Group, Kraft Foods, Unilever, PepsiCo, Coca-Cola, and Heinz are devoting part of their portfolios to the "Health and Wellness" market. While treating disease remains the primary pursuit of the pharmaceutical industry, some pharmaceutical companies such as Novartis Consumer Health have shown some interest in developing functional and nutraceutical foods. They are attracted to this portfolio due to the relatively shorter development times and lower product development costs compared to pharmaceutical products and their extensive experience in organizing clinical trials to back up health claims of a specific product (Siró et al. 2008). Some food industries such as DSM and ADM are also developing stronger links with pharmaceutical companies.

Becel pro-activ® is produced by Unilever as a functional variety of Becel® margarine with plant sterols. In light of this, Becel pro-activ® holds an EFSA-approved claim for cholesterol-lowering effects. Benecol® is a brand of products from the Finnish company Raisio Group, which also produces yogurts, spreads, yogurt drinks, cream cheese spreads, milk and soy drinks, bread, and oatmeal formulated with camelina oil as a source of PUFAs. Camelina oil contains ~57.4% of PUFAs with 38% as ALA (ω-3) and 17% LA (ω-6) (Health Canada 2010). Benecol®, Promise activ®, and HeartRight® contain 0.85, 1.0, and 1.7 g per tablespoon plant sterols, respectively. Blue Band Goede Start is another product from Unilever. It is a white bread fortified with fiber, prebiotic (inulin), vitamins (B1, B3, and B6), and minerals (iron and zinc).

Balade ™ light butter is a low-cholesterol butter (5 mg per teaspoon) made by removing about 90% of milk fat cholesterol by crystalline β-cyclodextrin (cyclic oligomers of glucose, sugar molecule bound in a ring). Beta-cyclodextrin has a hydrophobic core and a hydrophilic outside. Due to the bulkiness and hydrophobicity of cholesterol, it easily lodges inside the cyclodextrin ring and is then removed (Rodal et al. 1994; Alonso et al. 2009; dos Santos et al. 2011). Other spreads such as Olivio, Country Crock Omega Plus (or Plus Light), Promise, Land O'Lakes Margarine, Fleischmann's, I Can't Believe It's Not Butter! Mediterranean Light, and Earth Balance all claim to contain ω-3 (due to the presence of ALA from the vegetable oils used).

Largo® is a fortified juice produced in Estonia. It contains functional ingredients such as inulin, l-carnitine, vitamins, calcium, and magnesium. Other examples of functional drinks include cholesterol-lowering drinks (made with a combination of ω-3 and soybean), "eye health" drinks (made with lutein), or "bone health" drinks (made with calcium and inulin). Beta-glucans have been used to produce low-fat ice creams and yogurts. BioGaia® currently produces infant formula, colic drops, chewable tablets, lozenges, chewing gums, and drops with specially added active live cultures of *Lactobacillus reuteri Protectis*.

Escalating demand for conventional ingredients is putting pressure on prices, while demands for specific ingredients such as whole grains and gluten-free grains are also on the rise. Additionally, to help improve waste disposal in pursuance of corporate sustainability and growing regulatory pressure around waste disposal (Vriesmann et al. 2012), food manufactures such as Barry Callebaut have used valorization as a source of ingredients in food processing. Cocoa husks account for approximately 52–76% of the weight of the fruit (Donkoh et al. 1991). Barry Callebaut has filed several patents for various by-products that can be obtained from cocoa processing, such as fiber and antioxidants from cocoa shell/hull/husk, and to grind the shells into a powder for use as a cocoa replacer, fat bloom inhibitor, and ingredient in other foods. Kraft Foods filed a patent in 2005 for a method for extracting theobromine-enriched fractions and polyphenol-enriched fractions from cocoa shells. Tomato processing generates a large amount of waste made up of skins, seeds, and pulp (Kalogeropoulos et al. 2012). These three components account for about 10–30% of the total weight of tomato while the skins and seeds account for about 1–4% (Benakmoum et al. 2008). The by-products of tomato processing contain significantly lower amounts of lycopene, but increased amounts of tocopherol, polyphenol, β-carotene, terpenes, and sterols that seem to withstand industrial processing methods, and possess similar antioxidant activities to unprocessed tomatoes. Low-quality edible oil formulated with tomato skins enriched the oil with β-carotene and lycopene more than when tomato purée was used (Benakmoum et al. 2008).

While some information on these methods has been reported, detailed descriptions of several other emerging formulations is still at the developmental stage, with strong intellectual property positions. Some formulation and processing technologies' trends of interest include pressurized solvent extraction (PLE) and a variation of conventional solvent extraction using high temperatures (50–200°C) and pressures (1,450–2,175 psi); this maintains the solvent in the liquid state during the whole extraction procedure, and allows faster extraction by using lesser amounts of solvents, resulting in higher product yields. Increasing the temperature decreases the dielectric constant and polarity of a solvent; thus, by varying temperature, polar and non-polar compounds can be extracted. Due to the high temperatures employed, PLE is not suitable for thermolabile compounds. Water or other GRAS solvents, such as ethanol, can be used.

Gamma irradiation has been used to extend the shelf life of grape pomace and improve anthocyanin content (Ayed et al. 1999). Lower storage temperatures have been used to reduce isomerization and oxidation. Enzymatic liquefaction with pectinases and cellulases has been used to enhance the release of phenolics from apple pomace (Will et al. 2000), with improved juice yields for extended fields of application. In another interesting study, pulsed electric field (PEF) was used on tomato to induce a stress response that enhanced metabolic activity and accumulated secondary metabolites (Vallverdú-Queralta et al. 2013). The use of moderate-intensity pulsed electric field (MIPEF) made it possible to obtain tomato juices with high carotenoid content, while using high-intensity pulsed electric fields (HIPEF) helped to maintain higher contents of carotenoids (10–20%) during storage compared to thermally treated and untreated juices (Vallverdú-Queralta et al. 2013).

One of the critical parameters preventing the adoption of novel technologies and broad commercial implementation in specific applications has been technological/scale-up boundaries (Siró et al. 2008). Harmonizing regulatory processes across major international markets may also be beneficial to the success of functional foods and nutraceuticals at the global level.

1.5 Conclusion

Epidemiological and clinical studies across multiple geographical locations have generally shown neutral or beneficial effects of the consumption of certain types of foods on health and wellness and reduction of risk factors for certain diseases. Health-conscious consumers have developed an awareness and overall positive perception of functional foods and nutraceutical products derived from such food sources. Whereas much progress has been made in the last few decades in this market sector, there remains significant opportunity for further research and development. Substantiation of health claims, identification of new bioactives, robust processing technologies, and development of shelf-stable, tasty, and convenient products are examples of areas needing continued attention. Further innovation and introduction of products with well-substantiated health claims are therefore anticipated in the coming decades.

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2

Functional and Sustainable Food – Biophysical Implications of a "Healthy" Food System

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2.1 Introduction

Interest in healthy foods and the linkages between dietary preferences and human health have increased in recent years. Worldwide, an increasing number of studies are characterizing the functionality of specific food items and their potential contributions to human health. Pomegranates, pears, beets, carrots, and tomatoes are among the many foods that have been acknowledged for their health benefits.

Public and scientific interest in functional foods is expected to accelerate as the world population continues to grow, as life expectancy increases, and as greater numbers of people embrace Western standards of living. Identification and marketing of new "functional food" items to meet consumer demands is likely to be financially profitable. The number and variety of such products is rising.

Concurrent with the growing interest in functional foods, some academic, policy, and public discussions have focused on the environmental consequences of food production and consumption, on issues of food security, and on the extent to which the prevailing global food system is sustainable (Ericksen 2008; Godfray et al. 2011). These discussions have been prompted by a number of biophysical and economic signals that both impact and are

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impacted by global food systems. For example, renewable and non-renewable resource depletion, increasing energy prices, processes of environmental degradation, and climate change.

In response to concerns about the environmental and sustainability implications of food production, a growing number of food producers and processors are exploring dimensions of their sustainability, monitoring and reporting some of their ecological impacts, such as greenhouse gas (GHG) emissions, and pursuing the means to reduce those impacts. Nonetheless, as global demand for foods including "functional foods" continues to rise, the overall footprint of food continues to grow.

In the twenty-first century, understanding, analyzing, and building sustainable food systems is crucial. Assessing the natural capital required to supply the food demands of individuals and of societies can provide critical information and a base from which to explore ways of using the natural capital more sustainably. In this chapter, I argue for the need to consider both the individual immediate health benefits of various "functional foods" and the wider biophysical impacts of their production. If increased production of functional foods is promoted for health and economic benefits, it is important to investigate the consequences for ecological sustainability. After introducing some of the wider challenges of food system sustainability, the chapter will embrace a life cycle approach to explore some of the biophysical aspects of functional food production. That part of the chapter will focus on a single major agriculture item considered in the literature as "functional food" – tomatoes. It will analyze the carbon and ecological footprints of tomatoes. It will then highlight the complexity and challenges to characterizing this functional food as "sustainable." I will conclude the chapter by suggesting some potential directions for integrating both the concerns for human and environmental health.

2.2 Background

In recent decades, growth of the global human enterprise has been accelerating. The world's population has more than doubled in less than five decades to over 7 billion at the beginning of the twenty-first century. Supporting the demands of this growing population, some with increasing material standards of living, has resulted in increased global food production. During the last 50 years, food production has expanded two and a half fold, and water use has doubled (MEA 2005). While there is little question that such growth has contributed to human well-being and improved standards of living in much of the world, it has also dramatically increased the degradation of critical ecosystems and related life support functions (UNEP 2002). The Millennium Ecosystem Assessment (MEA) summarized its findings this way: "At the heart of this assessment is a stark warning. Human activity is putting such a strain on the natural functions of the Earth that the ability of the planet's ecosystems to sustain future generations can no longer be taken for granted" (MEA 2005).

Human societies have always depended on biophysical goods and services (e.g., clean air and water, food, and materials) produced by both local and global ecosystems. Over time, that dependence has increased and changed: first, we consume more than we did in the past, both per capita and in the aggregate (French 2000; Meadows et al. 2004; MEA 2005; Brown 2006; FAOSTAT 2013). This means that we extract ever-larger quantities of resources and place ever-greater demands on life-support services, trends that are now compromising the ability of ecosystems to support future demand. Second, recent decades have witnessed a significant increase in the spatial separation between human populations and the ecosystems that serve them. For most of human history, people supported themselves mainly on resources and assimilative capacities provided by local ecosystems. With increasing global economic integration, this dependence has been extended to sources and sinks in distant parts of the world (Princen 1999; French 2000; Rees 1994; 2004; Kissinger and Rees 2010).

Geographic and climatic circumstances combined with sociocultural and political arrangements have determined the diet composition and access to food of societies throughout human history. While trade always existed as a means of food supply, it was mostly supplementary to domestic sources. In recent decades, processes of globalization, combined with technological and shipping developments, have allowed international trade to become a central means of supplying the needs and wants of billions of consumers all over the world. At the beginning of the twenty-first century, the diet of people in all wealthy nations and, increasingly, in other parts of the world has very little to do with their geographic locations or climatic circumstances. Food commodities are being grown and shipped from one part of the world to markets in another. While the global food system has several merits for nations, businesses, and individual consumers, several constraints to this system should be considered. Evidence for peak oil (Campbell and Laherre 1998; Bardi 2009) suggests that we might be approaching the end of the cheap energy era. Consider also the rising energy prices as a result of political unrest in different parts of the world, increasing processes of climate change, and the degradation of agricultural/food supplying regions (MEA 2005). All are undermining the sustainability of the global supply system and raise some questions about food security (e.g., Ericksen 2008; Godfray et al. 2011; Huang et al. 2011). All suggest the need to examine ways to develop more efficient and sustainable food supply systems.

In response, some innovative national and international food policy ideas are emerging – for example, the adoption of food-related-carbon taxes, the implementation of carbon footprint standards, and commodity carbon labeling. All policies can alter the relative prices of commodities according to their carbon content. They can also influence consumer food choices by encouraging or fostering specific food preferences. However, to support the development of these and other policies to create more efficient and sustainable food supply systems, it is essential to increase our understanding of the current food supply system. In response to a range of environmental and sustainability challenges, researchers are investigating various dimensions of food–environment linkages.

2.2.1 Land, Water, and Energy – The Biophysical Footprint of "Functional Food"

Local and global food systems rely on natural capital (e.g., land, water, energy) to produce the foods demanded by individuals and entire societies. An assessment of existing natural capital stocks (quantity and quality) and of natural capital requirements for food production can provide individuals and policy-makers with a base of information from which to explore ways of using the natural capital more sustainably. The "footprint" family of tools can contribute to such an assessment. The carbon footprint measures energy use in production systems; the ecological footprint measures energy and land use; and water is accounted for in the water footprint.

The carbon footprint is the measurement used for calculating GHG emissions, throughout the life cycle (LCA) of food, from the field to the landfill (e.g., ISO 1997; Andersson et al. 1998; Roy et al. 2008). The carbon footprint formulates a total number comprised of the main GHGs, namely, carbon dioxide (CO_2), nitrous oxide, and methane. Each gas is converted into units of CO_2, and the total sum of the gases is presented in terms of CO_2 equivalent, also called *global-warming potential*. One component of the carbon footprint that has received some specific attention is the energy and GHG emissions related to food shipping – or what has come to be known as "Food Miles."

Ecological footprint analysis (EFA) can be used to quantify the biophysical "load" that any food production system (food production, processing, distribution, waste disposal) imposes on local and global ecosystems (Rees 2001). The method uses data on energy and material consumption, waste generation, and ecosystem productivity to estimate the total ecosystem area (hectares) required on a continuous basis to maintain that production system and to assimilate certain quantities of its wastes, wherever on earth that system is located (Rees 1992; Rees and Wackernagel 1994; Wackernagel and Rees 1996; WWF 2010). EFA can thus explicitly connect people's food consumption, product by product, to appropriated ecosystem areas, and reveal the gap (positive or negative) between local demand and global ecosystem area availability. EFA of production systems that serve a specific population (regional, national, etc.) can highlight that population's trade-based dependence on distant ecosystems.

When considering the identification and promotion of specific functional foods from an ecological perspective, it is appropriate to ask: do some functional foods have smaller footprints than others? Footprint tools can produce valuable information about the biophysical aspects of specific food commodities, but it is important to acknowledge that they do not cover all potential environmental implications of food production and consumption. Several other implications should be considered, such as soil degradation, habitat change, and various air pollutants.

2.3 Functional Food Footprint – The Case of Tomatoes

One of the most commonly consumed agricultural commodities, tomato, is considered a functional food item. Worldwide in 2011, approximately 160 million tonnes of tomatoes were grown (FAOSTAT 2013). Major producing regions include China and India (40%), the Middle East and North Africa (15%), Europe (12%), and North America (10%). Following the USDA data (2013), each American consumes on average 9 kg of fresh tomatoes plus 12 kg of processed tomatoes (e.g., tomato paste, ketchup, juice), equal altogether to approximately 40 kg of fresh tomatoes per capita per year.

Several studies have examined the health benefits of tomato consumption (e.g., Tonucci et al. 1995; Stewart et al. 2000; Etminan et al. 2004; Canene-Adams et al. 2005; USDA 2013). Tomatoes are major sources of potassium, folate, and the vitamins A, C, and E. Whereas tomatoes contain similar amounts of potassium and folate as other commonly consumed vegetables, they are superior sources of alpha-tocopherol and vitamin C. In addition to their micronutrient benefits, tomatoes contain valuable phytochemicals, including carotenoids and polyphenols. For instance, carotenoids (such as the red-pigmented lycopene), carotene (a pro-vitamin A compound), phytoene, and phytofluene are all found in abundance in raw tomatoes and tomato products. Of lycopene consumption, 85% comes from tomato sources. These tomato phytochemicals are thought to contribute to the reduced risk of human ailments such as cardiovascular disease (CVD) and prostate cancer.

As a significant part of the Western food basket, tomatoes have been the subject of several studies examining their life cycles and footprints. The following paragraphs summarize findings on the energy/GHG and land emissions associated with tomato production and consumption in different parts of the world.

2.3.1 The Carbon Footprint of Tomatoes

Interest in the emission of GHGs as a major driver of global climate change has led several researchers to study the GHGs emitted along the life cycle of tomatoes, from the growing phase to processing and shipping. The following table presents summary results on the energy/carbon footprint of tomatoes from various studies.

While we are probably far from understanding the carbon footprint of each production system, these studies illuminate the carbon footprint of some tomato production systems in various locations. Data presented in Table 2.1 indicate that there are some significant differences between the carbon footprints of tomatoes analyzed in different parts of the world and between different production systems (greenhouse, field, etc.). As consumers in various parts of the world enjoy tomatoes (and other commodities) from different other parts of the world, the true carbon footprint of the tomatoes depends on their type/breed, their production systems (greenhouse or field), and source of supply (Williams et al. 2006).

Table 2.1 Energy inputs (MJ) and the carbon footprint (CO_2e) of fresh tomatoes

Country	Energy inputs (MJ/kg)	Carbon footprint (CO_2e/kg)	Source
United States – Florida (field)	7.1 MJ	0.46 kg CO_2e	Reinhardt 2008
United States – California (field)	3.4 MJ	0.26 kg CO_2e	Reinhardt 2008
Canada – Ontario (greenhouse)	52.7 MJ	2.88 kg CO_2e	Hendricks 2012
Canada – British Columbia (greenhouse)	n/a	1.7 kg CO_2e	Wong and Hallsworth 2012
Mexico (field)	3.14 MJ	0.21 kg CO_2e	Reinhardt 2008
Jamaica	n/a	1.47 kg CO_2e	Woerishofer 2011
Spain (greenhouse)	7 MJ	1.2 kg CO_2e	Antón et al. 2005
Spain (greenhouse)	4.4 MJ	0.27 kg CO_2e	Williams et al. 2008
Netherlands (greenhouse)	31 MJ	2.0 kg CO_2e	Torrellas et al. 2012
Italy (greenhouse)	15.5 MJ	0.9 kg CO_2e	Cellura et al. 2012
France (greenhouse)	31.6 MJ	2.02 kg CO_2e	Boulard et al. 2011
United Kingdom	36.2 MJ	2.11 kg CO_2e	Williams et al. 2008
Sweden	49 MJ	2.69 kg CO_2e	Fuentes and Carlsson-Kanyama, 2006
Denmark	60 MJ	3.62 kg CO_2e	Fuentes and Carlsson-Kanyama, 2006
Turkey (field)	0.93 MJ	0.09 kg CO_2e	Karakaya and Ozilgen 2011
Turkey (greenhouse)	1.26 MJ	N/A	Ozkan et al. 2004

In the global food system, another component of the tomato footprint that needs to be considered is the distance the tomatoes have traveled from the field to our plates – food miles. Studies in recent years have calculated the food miles of various food commodities originating in different parts of the world. In a recent study, I followed Canada's import-related food miles over 1 year (Kissinger 2012). The study found that, on average, imported tomatoes to Canada generates over 400 grams of CO_2 per kilogram of tomatoes.

2.3.2 The Ecological Footprint of Tomatoes

The ecological footprint of tomatoes integrates the agricultural land required for growing the tomatoes and the forest land (i.e., energy land) required to sequester the CO_2 emitted along the life cycle of the tomatoes. Wada (1994) compared the ecological footprint of two types of tomato production systems in British Columbia. He found that, while greenhouse tomatoes are indeed more efficient in terms of the area of cropland they capture, their energy/carbon footprint is much larger per unit of production.

In a different study on the ecological footprint of Canadian food consumption, I calculated the ecological footprints of various agricultural and food products (Kissinger 2013). The study analyzed the crop and energy land required for different tomato commodities consumed in Canada. I found that the land footprint of 1 kg of fresh tomatoes is 1.24 global square meters (gm^2), processed tomatoes 2.1 gm^2/kg, and ketchup as high as 6.7 gm^2/kg.

Using data from the papers cited in Table 2.1, I used EFA to calculate the cropland, energy land, and ecological footprints of tomatoes per unit of production from some major producing regions (Table 2.2).

In the preceding paragraphs, I have described and presented the carbon and ecological footprints per unit of tomato produced in different parts of the world to illustrate some of the biophysical impacts related to tomato production. Next, I turn to an analysis of the footprints associated with a single country's annual consumption of tomatoes. The case of Canada's tomato consumption is also used to highlight some directions for minimizing carbon and ecological footprints of food production.

2.3.3 Toward a Sustainable System – The Case of Canada's Tomato Supply System

Canada is a major producer and exporter of a wide range of agricultural and food commodities including cereals, legumes, meat, and oils (FAOSTAT 2013). However, analyzing food consumption in Canada highlights that, while most food staples consumed in Canada are from domestic sources, a significant chunk of the nation's food supply comes from all over the world. On average, during the last decade, more than 80% of fruits and about 45%

Table 2.2 The ecological footprint of fresh tomatoes (global square meter per kg)

Country	Cropland (gm^2/kg)	Energy land (gm^2/kg)	Ecological footprint (gm^2/kg)
United States – Florida (field)	0.55	1.03	1.57
United States – California (field)	0.75	0.58	1.34
Canada – Ontario (greenhouse)	0.04	6.43	6.47
Canada – British Columbia (greenhouse)	0.05	3.80	3.85
Mexico (field)	0.30	0.47	0.77
Mexico (greenhouse)	0.14	0.58	0.72
Jamaica	1.29	3.28	4.57
Spain (greenhouse)	0.11	2.68	2.79
Spain (greenhouse)	0.03	0.60	0.63
Netherlands (greenhouse)	0.04	4.47	4.51
Italy (greenhouse)	0.23	1.65	1.88
United Kingdom (greenhouse)	0.05	4.71	4.76
Sweden (greenhouse)	0.04	6.01	6.05
Denmark (greenhouse)	0.04	8.09	8.13
Turkey	0.28	0.20	0.48

of vegetables consumed in Canada were imported from different parts of the world. Even for some agricultural and food commodities that are grown locally, some quantities are still imported. For example, 35% of oils, 17% of legumes, and 15% of meat consumed are imported (FAOSTAT 2013).

Several factors need to be considered in order to explain Canada's food supply in general and in the context of this chapter: (1) Canada's geographic location and climatic conditions limit its ability to grow many products; (2) the diverse ethnic make-up of the Canadian society influences and shapes food preferences; (3) a high standard of living, combined with the influence of strong commercial marketing campaigns, have generated a system that demands fresh food commodities year round with no connection to domestic growing seasons (e.g., tomatoes from Mexico, California, or even Israel); (4) the attempt to diversify commodities, increase consumer choice, and increase profit means that grocery shops will quite often sell a domestic commodity as well as competing import versions of the same commodity.

The case of Canadian tomatoes is particularly interesting because, despite unfavorable climatic conditions, the Canadian tomato production sector has used technological advances to become a significant producer of tomatoes consumed within Canada (Chart 2.1), in addition to being an exporter of fresh and processed tomatoes. On average, each Canadian consumes 7.4 kg of fresh tomatoes annually, plus an estimated 8 kg of processed tomatoes. Overall, Canadians annually consume over a million tonnes of tomatoes (Statistics Canada 2012). Chart 2.1 presents the different sources of supply. Following the sources of supply composition, Table 2.3 presents the carbon and ecological footprints of Canadian tomato consumption.

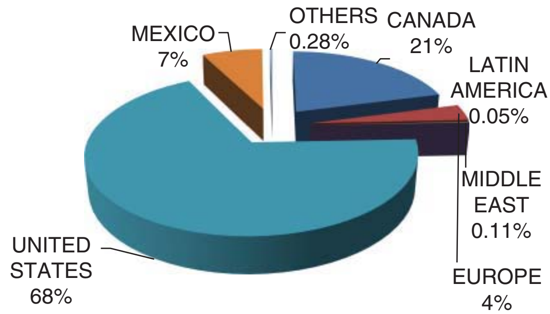

Chart 2.1 Canada's tomato supply by source.

Table 2.3 Canada's tomato footprint

	Carbon footprint (Tonnes of CO_2e)	**Ecological footprint (Global Hectares)**
Canada	759,800	1,801,800
United States	450,000	1,200,000
Mexico	60,000	79,500
Europe	53,600	120,000
Middle East	1,300	2,500
Latin America	900	1,950
Total	**1,325,600**	**3,205,750**

The preceding analysis provides us with a snapshot of Canada's annual tomato footprint in recent years. It integrates energy and CO_2 emissions along the commodity's life cycle (growing, processing, and shipping), as well as the area of crop land required to grow the commodity, and forest land to sequester the CO_2 emitted. It suggests that Canadian consumers' demand for tomatoes involves a significant demand from nature. However, the analysis can also signal directions to reduce that footprint, for example, by changing consumption habits, modes of production, or sources of supply.

The idea of getting back to local production as a means to reduce the footprint of consumption has been promoted in recent years. However, while there are several good reasons to increase or at least maintain local production, from a biophysical footprint approach, as suggested by the case analyzed in this chapter, that is not always the most efficient or sustainable approach. As shown in Table 2.1, the carbon footprint of 1 kg of Californian fresh tomatoes is 0.26 kg of CO_2, compared to 2.88 kg of CO_2 for Ontario's tomatoes. When integrating food miles, that is, shipping the tomatoes all the way from California to Ontario, an additional 0.76 kg of CO_2 should be added to the Californian tomato, totaling 1.02 kg of CO_2 per kg of tomatoes. It follows that, despite the distance and the significant contribution of shipping to the commodity's carbon footprint, increasing imports of tomatoes from California can reduce Canada's carbon footprint.

However, when considering also the agricultural land required in each producing region (California and Ontario), a different picture emerges, one that reflects the different production methods (field tomatoes in California vs. greenhouse tomatoes in Ontario). Ontario's greenhouse tomatoes yields are about 20 times higher than California's fields tomatoes. It follows that California has a cropland footprint of 0.75 gm^2 per kg vs. only 0.04 gm^2 per kg in Ontario. This suggests that, in order to minimize a commodity's footprint, several factors need to be considered, including energy and GHG emissions along the product life cycle, which includes shipping and the area of land required for different production systems. Both are influenced and shaped by the specific climatic conditions, available resources (e.g., land and water), rate of technology, and economic development in each producing region.

While indeed shipping distance influences the size of the carbon and ecological footprints, another factor that needs to be considered is the mode of transportation. Most of North American inland shipping of tomatoes (as with many other vegetables and fruits and their products) is by truck, which uses high rates of fuel per tonne-km; the use of rail can reduce the fuel-related part of the footprint significantly (Kissinger 2013). Perhaps the most efficient mode from a carbon footprint perspective is sea freight, which implies that importing tomatoes from Europe might generate lesser emissions than importing from Mexico. Consider that the carbon footprint of 1 kg of tomatoes from Mexico (travels to Ontario by truck) generates 0.75 kg of CO_2 along its life cycle (out of which 0.54 kg of CO_2 is from shipping). The same tomatoes from southern Spain, traveling by sea vessel, generate a carbon footprint of 0.40 kg of CO_2 per kg (out of which only 0.13 kg of CO_2 is from shipping). Thus, the same commodity chain using different modes of transportation will generate different outcomes. For example, air cargo from Spain to Ontario will have a carbon footprint of 5.24 kg of CO_2 per kg of tomatoes, while rail shipping from Mexico can reduce that footprint to 0.24 kg of CO_2.

As the preceding discussion reveals, the severity of ecological impacts associated with tomato production varies with factors such as production location, production systems, and distribution/shipping. These factors can be targeted to reduce the overall impacts of tomato and other functional food production.

2.4 Summary

In this chapter, I argue for the need to integrate the immediate human health benefits of various "functional foods" with the environmental and longer-term pressures that these food items generate. Such integration will allow us to identify those items that are not only functional but also sustainable. While indeed this is true for all types of foods, functional foods are a critical focus, because they have already received attention from governmental ministries, businesses enterprises, as well as individual consumers for their unique potential contribution for human health. Individuals who are considering the composition of their diets and are choosing various functional foods for their health benefits are already taking an active step that reflects their awareness. These individuals are a potential audience for also considering the environmental implications, as suggested in this chapter.

As suggested and illustrated in this chapter, once the environmental impacts of consuming functional foods have been measured, it is possible to consider the means to reduce the footprint of various functional food items along their life cycles. These means can be promoted by the various relevant stakeholders – government ministries and agencies, commercial enterprises, consumer organizations, and individual consumers. Means can target various ends: regulative, educational, and technical. Each approach on its own, and of course all three together, can contribute to promoting more efficient and sustainable food systems.

(a) Regulative – As widely acknowledged in the literature, present economic systems do not sufficiently reflect the external environmental costs of food consumption. Internalizing some of the environmental costs into product price (e.g., in the form of a carbon tax) can contribute to a more efficient supply system, one that at least partially has the potential to generate smaller footprints. (b) Educational – Consumers informed of food footprint analysis may voluntarily change their food purchasing habits. One educational measure is eco-labeling of commodities, so that consumers can make more sustainable purchasing decisions. To date, most such educational measures have been promoted by international and domestic NGO's. However, in some parts of the world (mostly in the European Union), we can see more active involvement of governmental policies to encourage voluntary (and slowly even mandatory) reporting on commodity footprints. (c) Technical – Both regulative and educational measures, if they influence consumers' choices and government/corporate policies and actions, have the potential to stimulate technological changes along a product's life cycle to minimize the product's footprint. Examples of technological changes include more efficient modes of production (using less energy, water, and land), and increasing energy efficiency in land and sea freight transportation.

The Canadian case used here illuminates the complexity of the food system and the need for further research and analysis of different commodity chains. As the world's population continues to grow, and as food consumption levels rise, the ability to account for the natural capital required to meet the demands of people all over the world and to manage that supply is essential. As interest in functional foods grows, pressure may mount to increase their production. Footprint analysis, as demonstrated here in the case of tomatoes, can measure biophysical impacts of functional foods, and can also highlight points in the life cycle at which production of those foods can be made more sustainable. Sustainably produced functional foods will meet the objectives of both individual human health and global ecological health.

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3
Key Considerations in the Selection of Ingredients and Processing Technologies for Functional Foods and Nutraceutical Products

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3.1 Introduction

There is an ancient Chinese saying, "medicine and food are isogenic." This philosophy has been in practice for centuries in Japan, China, India, and other eastern countries. Ancient sages in India preached the importance of diet in disease prevention and health promotion. However, as the centuries have passed, this philosophy of "food as medicine" has been diluted, while the food processing methods and traditional techniques that were used to improve the bioavailability of nutrients in food were modified with time and became more and more modernized. After the Industrial Revolution and economic growth in the early nineteenth century, the focus of food industries was to supply nutritionally and sensorily desirable products to please the growing population. People all over the world enjoyed the rich dietary life for decades until the twentieth century, when incidences of so-called lifestyle-related diseases became familiar and a public concern. It was then

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that the importance of diet in disease prevention and health promotion came back to the limelight once again.

For several years, the focus of the scientific community was on the identification of essential elements in food, such as vitamins, to prevent various dietary deficiency diseases (undernutrition). One of the best examples is the discovery of oryzanin (Vitamin B_1), which was isolated by Professor Umetaro Suzuki from rice bran at the University of Tokyo (Hardy, 2000). However, as diseases such as diabetes, arteriosclerosis, osteoporosis, cancer, and food allergies surfaced and were related to dietary practices in our daily life, the emphasis on nutrient deficiency dramatically shifted toward excessive nutrition or "overnutrition." As scientists have started to identify physiologically active components in foods that could potentially reduce or eradicate the risk of chronic diseases and with a growing health conscious population, technological advancement and introduction of new food regulations have started a trend of food products now known as *functional foods* and *nutraceuticals*.

The term *nutraceutical* is a contraction of the words "nutrition" and "pharmaceutical," and was coined by DeFelice and the Foundation of Innovation in Medicine in 1989. The term *functional foods* was first used in Japan in the 1980s for foods fortified with special constituents possessing beneficial physiological effects (Childs, 1997; Siró et al., 2008). While there is no universally acceptable definition for *nutraceuticals* and *functional foods*, Health Canada defines *nutraceutical* as "a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical is demonstrated to have physiological benefit or provide protection against chronic disease," while a functional food is seen as "a food similar in appearance to, or may be, a conventional food, which is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic diseases beyond basic nutritional functions." It is noteworthy that all foods are functional to some extent and have primary, secondary, and tertiary functions identified as nutritive, organoleptic, and preventive, respectively (Table 3.1).

Most of the nutraceuticals identified in the last few decades can be classified on the basis of their origin. Table 3.2 presents a few of the isolated nutraceuticals from plants, animals, and microbial sources. Several conventional and novel extraction techniques have been investigated to obtain these valuable bioactive compounds to be used in pharmaceutical and food industries. Many of those bioactive compounds are extracted using decades-old solid–solvent extraction technique, which requires removal of natural products from a source using organic solvents including ethanol, methanol, water, or hexane, coupled with heating and constant agitation (Cheng et al., 2007; Singh et al., 2011). This traditional technique is both time consuming and requires large quantities of solvent, and also has lower yield, making it uneconomical with the added purification steps needed to remove the organic solvents prior to application in food products. Increased interest in nutraceutical science in

Table 3.1 Classification of functional foods

Type of functional food	Example
Fortified products	Fruit juices fortified with vitamin C
Enriched products	Margarine with plant sterol ester, probiotics, prebiotics
Altered products	Fibers as fat releasers* in meat or ice cream products
Enhanced commodities	Eggs with increased omega-3 content achieved by modifying feed

*Intake of soluble fibers reduces hunger and increases satiety. Animal and human studies have suggested that they also reduce plasma cholesterol by their ability to bind bile acids in the gastrointestinal tract and reduce their reabsorption. To maintain the required bile acid level in the body, the bile excreted by binding with fiber is replaced by utilization of hepatic cholesterol for bile synthesis. This in turn results in the reduction of low-density lipoprotein (LDL) and total cholesterol levels (Horton et al., 1994; Marlett et al., 1994).
Source: Adapted from Siró et al. 2008. Reproduced with permission of Elsevier.

the late 1990s steered development of novel extraction techniques, including microwave-assisted extraction (MAE) (Ballard et al., 2010; Barriada-Pereira et al., 2003; Nemes and Orsat, 2011; Singh et al., 2011), supercritical fluid (András et al., 2005; Carvalho Jr et al., 2005; Chan and Ismail, 2009; Cherchi et al., 2001; Dean and Liu, 2000), pulsed electric field (da Cruz et al., 2010), ultrasound (Dey and Rathod, 2013), and accelerated solvent extraction (Brachet et al., 2001). Introduction of these techniques shortened extraction time, increased yield, and reduced organic solvent consumption and contamination.

This chapter considers the theoretical and technical advancements of some of these processing techniques and also provides a comprehensive analysis of operational and regulatory challenges faced in their application in food and pharmaceutical industries.

3.2 Processing Technologies for Functional Food Bioactive Components and Nutraceutical Products

The processing of nutraceuticals can be divided into four steps, namely: pretreatment, extraction, isolation/purification, and encapsulation (Routray and Orsat, 2012). Pretreatment includes homogenization, grinding, milling, maceration, and drying. All the pretreatment steps, except drying, increase the contact surface area between the extracting solvent and sample containing the desired compounds. Drying is an important unit operation that helps to increase the shelf life of the raw material. Freeze-drying is often employed to prevent any reduction in quantity and quality of heat-sensitive nutraceutical compounds such as phenolics, flavonoids, bioactive proteins, etc. (Routray and Orsat, 2012; Stalikas, 2007).

Table 3.2 Examples of nutraceutical substances classified on the basis of origin

Plants	Animal	Microbial
Allicin	Vitamin B_{12}	*Bifidobacterium bifidum*
Ascorbic acid	Eicosapentaenoic acid (EPA)	*Streptococcus salvarius* (subs. Thermophilus)
Capsaicin	α-Lipoic acid	*Lactobacillus acidophilus* LA-5
Cellulose	Lecithin	*Bifidobacterium infantis*
Gallic acid	Zinc	*Saccharomyces boulardii* (yeast)
Soy isoflavones	Coenzyme Q10	*Bifidobacterium longum*
Glutathione	Melatonin	*Lactobacillus plantarum*
Hemicellulose	Creatine	*Lactobacillus lactis*
Flax-lignans	Taurine	*Lactobacillus casei*
Lutein	Carnitine	*Lactobacillus rhamnosus*
Luteolin	Calcium	*Bacillus subtilis*
Lycopene	Choline	*Bifidobacterium Rosell-175*
Pectin	Glucosamine	*Lactobacillus Rosell-52*
Potassium	Selenium	*Bifidobacterium animalis DN 117 -001*
Quercetin	Minerals	
Selenium	Spingolipids	
Zeaxanthin	Chondroitin	
α-Tocopherol	Methylsulfonylmethane	
β-Carotene	S-Adenosyl methionine	
β-Glucan	Conjugated Linoleic Acid (CLA)	
γ-Tocotrienol	Docosahexenoic acid (DHA)	
δ-Limonene		
Resveratrol		
Flavonols		
Curcumin		
Piperine		
Vincristine		
Octacosanol		
Dehydroepiandrosterone		

Source: Robert, 2000; Mestre Prates and Mateus, 2002; Anekella and Orsat, 2013, 2014; Denery et al., 2004; Motta et al., 2012; Rapport and Lockwood, 2000; Rohdewald, 2002; Sanders et al., 1999; Soleas et al., 1997; Stramentinoli, 2001; Wadsworth and Lockwood, 2003.

Selection of an appropriate extraction method depends on several factors, such as the source and type of bioactive compound being extracted, desired recovery, and operational requirements. Numerous reviews and research studies have reported advancements on the use of different extraction techniques (Camel, 2001; Dai et al., 2010; Joana Gil-Chávez et al., 2013; Raynie, 2006; Singh et al., 2011).

3.2.1 Soxhlet Extraction

Solvent extraction is the most common method of extraction used for bioactive compounds including phenolics, flavonoids, and edible oils. Different types of solvent extraction methods are used, of which the Soxhlet method is the most common and is used as a standard technique for comparison with advanced novel extraction techniques (Routray and Orsat, 2012). The conventional Soxhlet system requires the solid sample to be placed in a thimble-holder, which is filled with condensed solvent coming from a distillation flask. When the solvent is boiled, the vapors pass through the condenser and fall by gravity on the sample. When the boiling height of the solvent reaches the overflow level, the solution that is passing through the thimble-holder is aspirated into the distillation flask. The solute and solvent are separated by distillation. The solute of interest, being far less volatile than the solvent, is left behind, and only the fresh solvent is passed back into the sample solid bed (Figure 3.1). This whole operation is repeated several times until complete extraction is achieved (Wang and Weller, 2006).

Various extraction parameters influence extraction yield during the Soxhlet process, including matrix characteristics, solvent composition, and operational parameters such as temperature. The particle size of the solid sample influences the diffusion of bioactive compounds from the matrix into the extraction solvent. The choice of solvent depends on its flammability, boiling point, toxicity, and its ability to solubilize the targeted nutraceutical compounds. Different solvents extract different compounds preferentially, and the extracted compounds must be easily recovered while any environmentally damaging impacts must be as minimal as possible. Hexane is frequently used for extraction of edible oil from plant and microbial sources (Shen et al., 2009; Zarnowski and Suzuki, 2004). However, it is listed as one of the 189 hazardous air pollutants by the US Environmental Protection Agency (Mamidipally and Liu, 2004). Alternative solvents such as chloroform and methanol have been used to extract phenolic compounds such as chicoric acid from *Echinacea purpurea*, and cynarin from *Echinacea angustifolia* roots (Perry et al., 2001). Hanmoungjai et al. (2000) used water to extract rice barn oil, and they reported that the oil extracted using water had a lower content of free fatty acids and color-imparting components as compared to oil extracted using hexane (Hanmoungjai et al., 2000).

The overall recovery of solute extracted using the Soxhlet process is often low and of reduced quality because most the nutraceutical compounds are heat sensitive and the high temperatures used during extraction decrease the overall yield and quality, while the toxicity of some of the extracting solvents also poses a health and environmental hazard. Hence, the major drawbacks of

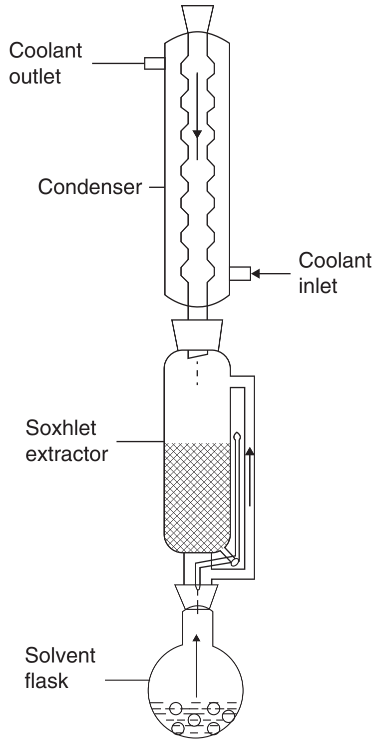

Figure 3.1 Conventional Soxhlet extraction apparatus.

the Soxhlet extraction process can be summarized as: (i) long processing times; (ii) large solvent volume requirements; (iii) high temperature with low yields of thermolabile bioactive components; and (iv) high processing costs. These drawbacks could be mediated by using novel extraction techniques such as MAE, supercritical and subcritical fluid extraction, and combined extraction techniques.

3.2.2 Microwave-Assisted Extraction (MAE)

Microwave energy has been extensively used for extraction of nutraceuticals from plant matrices (Ballard et al., 2010; Routray and Orsat, 2012; Singh et al., 2011). Application of microwave energy has been shown to increase the extraction yield compared to conventional methods (Barriada-Pereira et al., 2003; Dai et al., 2001; Kaufmann and Christen, 2002). Microwaves are electromagnetic waves within the frequency band of 300 MHz–300 GHz. When a microwave passes through a medium, its energy is absorbed and converted into thermal energy (Zhang et al., 2011). The ability of a medium to absorb and convert microwave energy into heat is governed by its dielectric properties. The dielectric property of a medium is described by the complex relative permittivity ε^* (relative to that of free space) in the relationship $\varepsilon^* = \varepsilon' - j\varepsilon''$, where $j = \sqrt{(-1)}$, ε', is the dielectric constant that describes the ability of the material to absorb microwave energy, and ε'' is the dielectric loss factor that is concerned with the conversion of absorbed microwave energy to heat. The relation between dielectric constant and loss factor is defined by the tangent of loss angle ($\tan\delta = \varepsilon'/\varepsilon''$), which, along with dielectric constant, defines the attenuation of microwave power within a biological matrix (Singh et al., 2011; Vadivambal and Jayas, 2008). At the industrial scale, the microwave frequency of 915 MHz is applied due to its high penetration depth, while 2,450 MHz is used in domestic microwave ovens and for analytical extraction purposes. The microwave ovens used for extraction of nutraceutical compounds can be of monomode or multimode cavities. Monomode cavities generate a frequency that can excite only one mode of resonance, and is used in focused microwave extraction systems. In a multimode system, the incident microwave can affect several modes of resonance, and this superimposition of modes allows homogenization of the electric and magnetic fields. The very first analytical application of microwave extraction was conducted by Abu-Samra et al. in 1975 for meat analysis (Abu-Samra et al., 1975). Commercially, two types of microwave extraction units are available: the closed-vessel system, commonly known as MAE, and the open-vessel system, known as *focused-microwave-assisted solvent extraction system*.

The conversion of microwave energy into thermal energy occurs via two mechanisms: dipole rotation and ionic conduction. When the radiation frequency corresponds to the rotational motions of the molecule, energy transfer takes place, resulting in a homogeneous heating of the solvent (Kubrakova and Toropchenova, 2008). Ionic conduction and increased temperature caused by microwave energy increase the penetration of the solvent into the matrix and facilitate a synergistic combination of heat and mass transfer gradients working in the same direction. This synergy results in a volumetric dissipation of heat (Nemes and Orsat, 2011). During MAE, considerable pressure builds up inside the biomatrix, which modifies the cell structure, allowing better penetration of solvent into the matrix, thus enhancing the extraction yield (Kratchanova et al., 2004). Extensive reviews providing details on the principle and application of MAE can be found in the literature (Al-Harahsheh and Kingman, 2004; Mandal et al., 2007).

Factors Affecting Microwave Extraction The performance of an MAE process depends on a number of factors, including solvent choice, microwave power output, extraction temperature, extraction time, and sample characteristics. These operational parameters, dictating the efficiency of the extraction using MAE, are very similar to conventional Soxhlet extraction. The most important factor that affects MAE process is the choice of solvent. A proper solvent choice provides higher extraction efficiency, which depends on its interaction with the plant matrix and its dielectric properties. Dielectric properties of the solvent play an important role in defining its ability to extract selected nutraceuticals from plant matrices. The higher the dielectric constant and dielectric loss factor of the solvent, the higher is the capacity of the solvent to absorb microwave energy and convert it into heat. This property of the solvent can be modified by combining it with other solvents, which leads to varying selectivity of the solvent for different nutraceutical compounds (Routray and Orsat, 2012). Alfaro et al. (2003) investigated the influence of the dielectric properties of both solvent and plant matrix on the extraction of essential oil from ginger (*Zingiber officinale*). They modified the dielectric properties of the matrix using ethanol or water to enhance the extraction efficiency. They concluded that enhancement of the matrix with ethanol or water and the dielectric properties of the extraction solvent played a decisive role in improving the extraction efficiency when compared to conventional Soxhlet extraction. Csiktusnadi Kiss et al. (2000) investigated the efficiency and selectivity of 30 different extracting solvents for the MAE extraction of color pigments from paprika powder. Their results revealed that MAE extraction efficiency depends significantly on the dielectric constant of the solvent mixture. In more recent studies, Nemes et al. (Nemes and Orsat, 2011) evaluated the applicability of MAE extraction of secoisolariciresinol diglucoside (SDG). Their results showed that MAE of SDG achieved a 6% increase in the extraction yield (21.45 mg SDG per gram defatted flaxseed meal [DFM]) using 0.5 Molar sodium hydroxide (NaOH) as the extraction solvent. They reported that MAE of SDG was governed by the interaction between microwave energy and NaOH concentration (molarity); Figure 3.2 presents the effect of operating factors including microwave power, extraction time, power mode, and NaOH molarity on the extraction yield of SDG (Nemes and Orsat, 2011).

In another study, Zhou and Liu (2006) evaluated the effect of various solvent mixtures of ethanol and hexane in the extraction of solanesol from tobacco leaves. They reported that use of hexane and ethanol in the ratio of 1:3 gave the best extraction efficiency. When only ethanol was used as the extraction solvent, the yield decreased because of lower solubility of solanesol in ethanol. They reported that the addition of 0.05 Molar NaOH further improved the extraction efficiency to 0.91% (weight solanesol/weight tobacco), as compared to the 0.75% obtained during MAE process using hexane and ethanol as the extraction solvents. The addition of salts to the extraction solvent mixture increases the heating rate during the MAE process, because, in addition to the dipole rotation, ionic conductivity also leads to heat generation in dielectric heating. Another factor that influences the extraction efficiency of the MAE process is the solvent-to-sample ratio. In conventional extraction processes, large volumes of solvents are used to increase the extraction recovery, but

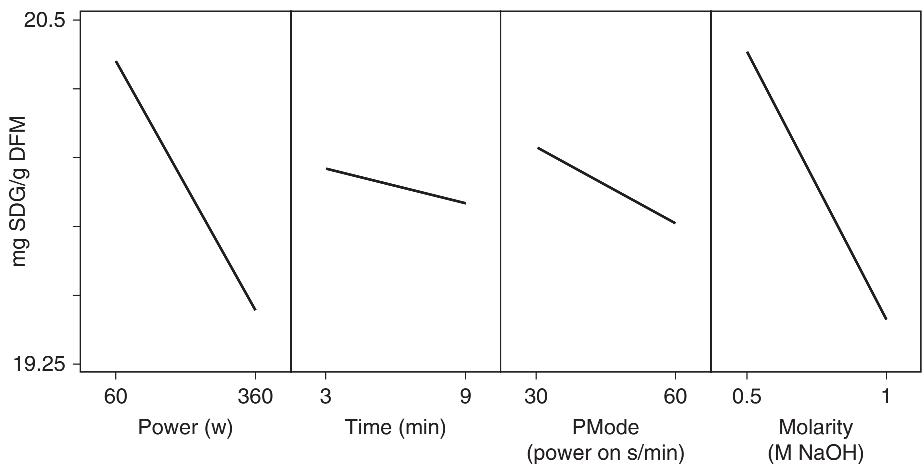

Figure 3.2 Effect of the operating factors Power, Time, Power Mode, and Molarity on the extraction yield of secoisolariciresinol diglucoside (SDG). *Source*: Nemes and Orsat 2011.

in the case of MAE, the solvent volume is an important factor, and several studies have reported that a solvent-to-sample ratio of 10:1 (ml/mg) to 20:1 (ml/mg) is optimal (Nemes and Orsat, 2010; Talebi et al., 2004). Use of higher volume of solvents will also increase the energy and time required to recover the nutraceuticals from the solvent during purification steps.

Extraction temperature is another important factor that influences the extraction yield of the MAE process. The dielectric properties of the solvent used for the extraction will govern the extraction temperature. For the extraction of thermolabile bioactive compounds, the choice of solvent becomes very crucial, and the use of a combination of different solvents to lower the dielectric properties ensures that the solvent temperature remains lower, to keep solutes cooler once they are extracted into the solvent. In this scenario, the microwave energy interacts more with the plant matrix, leading to an enhanced release of bioactive components into a relatively cooler solvent (Singh et al., 2011). The solvent choice also affects the solubility and selectivity of the analytes into the extraction solvent. Both polar and non-polar solvents can be used in microwave extraction. Bioactive compounds such as phenolics and flavonoids vary a lot in their polarity. Extraction of flavonoid aglycones such as isoflavones, flavanones, and flavones (Routray and Orsat, 2012) requires less polar solvents, while more polar solvents are required for flavonoid glycosides (Kothari and Seshadri, 2010). Microwave power and temperature are interrelated. It controls the partition of analytes between the sample and solute, and also affects the extraction temperature (Ma et al., 2009). In general, it can be conceived that increasing the incident microwave power will increase the rate of extraction and enhance the extraction yield

(Hu et al., 2008). However, an increase in the incident microwave power can decrease the extraction yield (Ma et al., 2009). Studying the effect of incident microwave power on MAE yield, Singh et al. (2011) optimized the MAE of phenolic compounds from potato peels. Their results showed that an increase in microwave power reduced the extraction yield of ascorbic acid due to the damaging high temperature achieved during the extraction process (Figure 3.3). They also stated that solvent concentration (methanol and water mixture) influenced the extraction yield, as the microwave-energy-absorbing ability of the solvent mixture varied with the proportion of water added to methanol. Table 3.3 presents the optimal conditions and predicted extraction yields of various phenolic compounds, including ascorbic, chlorogenic, caffeic, and ferulic acid from potato peel waste (Singh et al., 2011). Increasing the microwave incident power improves the extraction yield and results in shorter extraction time, but efficiency only increases till an optimal temperature is reached, and then it starts decreasing because the selection of ideal microwave power and temperature depends on the stability and selectivity of the target nutraceutical compound (Routray and Orsat, 2012).

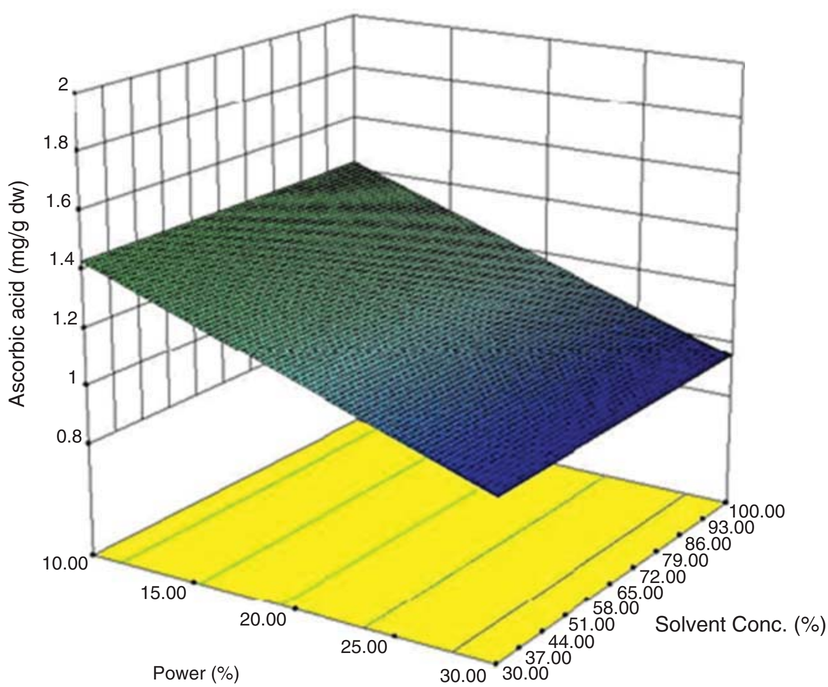

Figure 3.3 Effect of solvent (methanol) concentration and microwave power on ascorbic acid content of potato peel extracts. *Source*: From Singh, A. et al. (2011).

Table 3.3 Optimized MAE conditions and predicted extraction yield of ascorbic, chlorogenic, caffeic, and ferulic acid from potato peel waste

Phenolic compounds	Solvent concentration (%v/v)	Extraction time (min)	Power level (W)	Predicted extraction yield (mg/g dry weight)
Ascorbic acid	100	15	10	1.44 ± 0.5
Chlorogenic acid	100	5	10	1.35 ± 0.18
Caffeic acid	100	15	10	1.33 ± 0.06
Ferulic acid	100	15	10	0.5 ± 0.02

Source: Singh et al. 2011.

Overexposure to microwave radiation has been found to decrease the extraction yield because of the loss of chemical structure of the bioactive compound (Kaufmann and Christen, 2002). In 2011, Song et al. studied the effect of different microwave power levels on the extraction of total phenolics from *Ipomoea batatas* leaves. They reported that when other extraction parameters were set as: extraction time 90 seconds, ethanol proportion 70% (v/v), and solid-to-sample ratio of 30 ml/g, the total phenolic recovery increased with increase in microwave power till it reached 350 W, and later on decreased with further increase in microwave power levels (Song et al., 2011).

As with the Soxhlet method, the characteristics of the matrix, such as size, have a significant influence on the efficiency of the MAE process. Fresh plant material is not suitable for extraction using the MAE process, and the extraction yield of fresh plant is lower compared to the dry plant matrix. The particle size of the dry plant matrix also influences the MAE process, and smaller-sized particles increase the contact area between the plant matrix and the extraction solvent, which enhances the extraction yield (Brachet et al., 2002; Sparr Eskilsson and Björklund, 2000).

Due to its advantages such as shorter extraction time, reduced solvent usage, enhanced extraction yield, and selective extractability over traditional solid–solvent extraction systems, MAE can be considered as a potential alternative for extraction of nutraceuticals (Table 3.4). However, the utilization of organic solvents requires additional purification steps, whereas MAE using non-polar solvents for extraction has poor efficiency (Nemes and Orsat, 2012; Singh et al., 2011; Wang and Weller, 2006). Organic solvents, such as methanol, ethanol, ethyl acetate, hexane, acetone, and so forth, have been widely used for the MAE process, but their application in the food industry is limited due to stringent regulations to ensure food quality and safety. For example, some chemicals used during the extraction process may be toxic in nature, and if the extracts are added to food without proper purifications they may lead to various diseases and ailments of public concern.

Table 3.4 Recent studies on the use of MAE for extracting nutraceuticals from different natural matrices

Processing conditions	Materials	Bioactive compounds	Reference
Ethanol, 60 °C, 4.6 min at 150 W	Grape seeds	Polyphenols	Li et al., 2011
Ethanol, 30-s cycle at 855 W	Peanut skin	Polyphenols	Ballard et al., 2010
Water, 2 h, 40 W	*E. senticosus*	Essential oil	Bajpai et al., 2013
Water, 20 min, 200 W	*Uncaria sinensis*	Catechin, caffeic acid	Tan et al., 2011
Ethanol, water, 15 min, 1,300 W	*Cryptotaenia japonica Hassk*	Flavonoids	Lu et al., 2013
Ethanol (70%), 15 min, 850 W	*Radix puerariae*	Polyphenols	Guo et al., 2001
Water, 12 min, 200 W	Orange	Essential oil	Farhat et al., 2011
Dichloromethane, water, 5 min, 130–390 W	White cabbage	Sulforaphane	Tanongkankit et al., 2013
Ethanol, 30 min, 70 °C, 500 W	*Ligustrum lucidium* Ait	Oleanolic and ursolic acid	Xia et al., 2011b
Ethanol, 10 min, 50 °C, 500 W	*Sophora flavescens*	Oxymatrine	Xia et al., 2011a
Methanol/water, 16.34 min, 71.11 °C, 167.03 W	Broccoli	Phenolic compounds	Jokić et al., 2012
Ethanol (32.6%), 6 min, 121 W	Grape seeds	Phenolic compounds	Krishnaswamy et al., 2013

3.2.3 Supercritical Fluid Extraction

Nutraceuticals extracted from natural sources are key elements of functional foods. Several fortified and enriched functional foods contain bioactive compounds extracted from plants and animals. Keeping health and environmental concerns in mind, there is a worldwide pressure on food industries to adopt new sustainable technologies (Aleksovski and Sovová, 2007). The supercritical fluid extraction technology provides an excellent alternative to conventional solvent extraction methods. Since the late 1970s, supercritical fluids have been used to extract natural products, but their application was limited to only a few products (Ab Rahman et al., 2012). Supercritical fluid extraction (SFE) technique utilizes the thermodynamic properties of the solvents near their supercritical point. This state is achieved when the solvent's temperature and pressure are raised over its critical value. Several supercritical fluids including carbon dioxide (CO_2), ethane, butane, pentane, water, etc., are used in SFE processes (Herrero et al., 2010).

The supercritical state of the fluid has several advantages: (a) the solvating power of the supercritical fluid is higher and can be easily controlled by varying the operating conditions of pressure and temperature; (b) the diffusivity of supercritical fluid is better compared to organic solvents due to lower surface tension and viscosity; (c) application of supercritical fluids leaves minimal to no residue in the final product, and is non-toxic and environmentally friendly; (d) use of CO_2 with appropriate co-solvent such as water, ethanol, and methanol enhances the selectivity of the extraction process (Giannuzzo et al., 2003).

The SFE system works on the principle of distribution of the analyte between two phases, the separation and stationary phases (Bernardo-Gil et al., 2011). During phase equilibrium between liquid and gas, the partition of the liquid phase increases with an increase in pressure and decreases with an increase in temperature, and if the temperature and pressure are increased simultaneously the transport properties of both liquid and gas increases, resulting in convergence (Herrero et al., 2006; Herrero et al., 2010). Several comprehensive reviews are available on SFE, describing their operational principle and applications (Eggers, 1996; Herrero et al., 2006; Herrero et al., 2010).

Even though SFE can be considered as a significant alternative to MAE and solid–liquid extraction processes, it comes with its own challenges. Several factors have to be considered to develop a successful SFE process, including the choice of the supercritical fluid, matrix characteristics and preparation, and the type of co-solvents used to enhance extraction yield. Similar to the MAE process, the choice of solvent, that is, supercritical fluid, plays an important role in determining extraction efficiency and selectivity. In recent years, CO_2 has been used as a major solvent for SFE processes. The thermodynamic and chemical properties of CO_2 place its critical state at a temperature of 340 K and pressure of 7.3 MPa; its non-flammable and non-toxic nature make it an appropriate solvent for extraction of nutraceuticals. Supercritical CO_2 has been widely used in the extraction of hydrocarbons (Vilegas et al., 1997). Its applicability for nutraceuticals such as phenolics and alkaloids is limited due to its non-polar nature. To extract polar compounds, polar supercritical fluids including Freon-22 and nitrous oxide have been considered, but due to the high toxicity and environmental concerns, their application is very limited (Hamburger et al., 2004; Lang and Wai, 2001). Few researchers have used supercritical water as an alternative for the extraction of polar compounds, because superheated water (water under pressure, above 100 °C, and below critical temperature of 374 °C) has higher extractability for polar compounds; however, at this high temperature, thermolabile compounds degrade and their extraction yield decreases, and also water at this high temperature is corrosive in nature and might cause damage to the extraction vessel, making the extraction process uneconomical with respect to processing costs (Lang and Wai, 2001). To overcome this obstacle, researchers have added modifiers such as methanol, ethanol, acetone, water, etc., to CO_2. Addition of modifiers improves the extraction efficiency of the SFE process, but it also requires changes in the operating conditions such as temperature and pressure, which may result in denaturation of heat-sensitive nutraceuticals (Hamburger et al., 2004).

In contrast to MAE, fresh plant materials are commonly used in the SFE process, but their high moisture content may cause mechanical difficulties and lower extraction efficiency, because water-soluble solutes tend to partition into the aqueous phase. To overcome this issue, silica gel is added to the plant material to retain moisture during the SFE process (Lang and Wai, 2001). Another important characteristic of the plant matrix is the particle size – as in the case of MAE, smaller particle size enhances the extraction yield by virtue of the increase in exposed surface area, significantly reducing the extraction time. In 2008, a study conducted by Nagy et al. revealed that particle size influenced the SFE of volatile oil from *Capsicum annuum* L., and that the smaller particle size gave a higher extraction yield (Nagy and Simándi, 2008). Their result was in accordance with Del Valle et al. (2003a), who assessed and modeled the SFE kinetics of pre-pelletized Jalapeno peppers. They studied the effect of particle size and superficial solvent velocity at 40 °C and 120 or 320 bar, and reported that the rate of extraction increased with a decrease in particle size or an increase in superficial velocity at 120 bar. At 320 bar, the effect of superficial velocity was negligible. Several studies have, however, reported that particle size had no significant effect on extraction yield. Coelho et al. (2003) studied the effect of particle size on extraction yield of volatile oil from *Foeniculum vulgare*, and they observed that particle size of the fruit had no significant effect on the extraction yield. Similar results were obtained by Uquiche et al. (2004) in their study on extraction kinetics of red pepper oleoresin with supercritical carbon dioxide.

Other significant factors that affect the efficiency of SFE include the temperature and pressure required to generate the supercritical fluid. Any change in operating conditions may lead to a decrease in overall efficiency of the SFE process, since the solvating power, diffusivity, and selectivity of the supercritical fluid are governed by changes in temperature and pressure. For selective extraction using SFE, it is desirable to extract the compound right above the critical point where the targeted compound becomes soluble in the fluid (Del Valle et al., 2008; Del Valle et al., 2003b). The selectivity of the extraction fluid during the extraction of bioactive compounds is a function of the extraction pressure and deviation from ideal pressure, which if not properly monitored could lead to an increase in the extraction of other compounds. An increase in temperature decreases the density of supercritical carbon dioxide (at a given pressure), thus reducing its solvating power. Since the temperature of the SFE process is set to be in the vicinity of the critical point, and as low as possible, the only factor that can be used to fine-tune the selectivity of the supercritical fluids is pressure. As a general rule, the higher the pressure, the larger the solvating power of the fluid and the lower the selectivity (Reverchon and De Marco, 2006). In 2003, Del Valle et al. observed that, during the SFE extraction of α-acids from hops (*Humulus lupulus* L), an increase in pressure over 20 MPa at 313 K increased the extraction of undesirable compounds (Del Valle et al., 2003b). Figure 3.4 presents a typical extraction curve, which shows that changes in pressure significantly affect extraction yield. Del Valle et al. also evaluated the effect of process temperature and time on the yield of oleoresin at 120 and 200 bar pressure (Figure 3.5), and reported that, as the temperature increased beyond 40 °C, the solubility of the extract decreased at higher pressure (Figure 3.5). It can be concluded from their study that the effect of temperature does not follow a specific trend, as in the case of pressure, but it is rather the combined effect of pressure and temperature that affects the overall yield.

SFE has been widely used to extract lipids (Arnáiz et al., 2011; Couto et al., 2009) and essential oils (Berna et al., 2000; Ivanović et al., 2010) from plant sources, and SFE-CO_2 can be a viable alternative to conventional and MAE processes for the extraction of nutraceuticals from agricultural products (Table 3.5). It is important to note that the amount of nutraceuticals extracted from the raw materials using any extraction method is only a small fraction of its total weight; hence, the economic feasibility of the selected extraction method will depend on the overall yield, operation and maintenance cost, and the market demand of the extracted product. Nutraceutical products have a huge global market (i.e., worth billions of dollars), with sustained market demands expected for years to come. SFE is an environmentally cleaner technology that provides higher yields compared to MAE and other conventional methods, but the complexity of the design and high operation and maintenance costs have limited its application to highly marketable products such as decaffeinated coffee, tea, and hops (Castro-Vargas et al., 2010; Herrero et al., 2006).

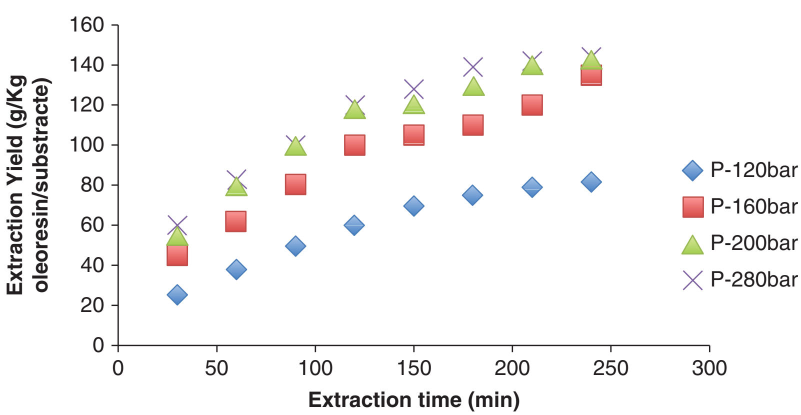

Figure 3.4 The effect of pressure and time on cumulative yield of hop oleoresin from cone pellets using supercritical CO_2 at 40 °C. *Source*: Data from Del Valle et al., 2003b. (For a colour version, see Plate 1).

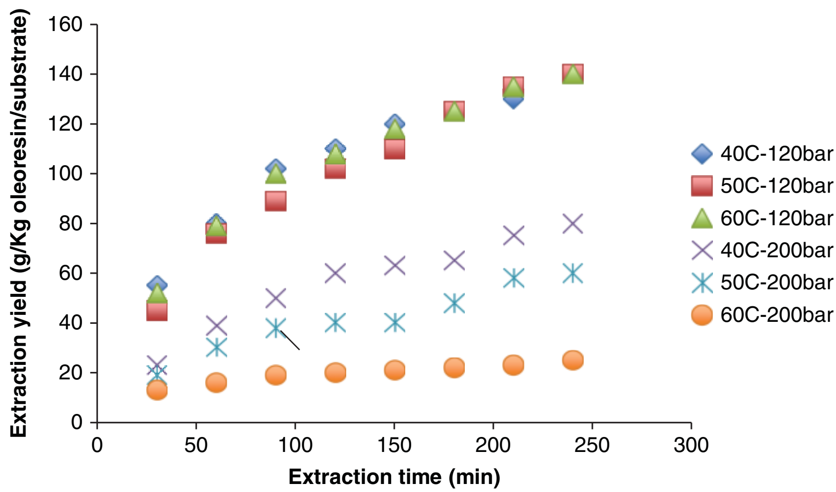

Figure 3.5 The effect of temperature (40, 50, and 60 °C), pressure (120, 200 bar), and time (30–240 min) on cumulative yield of hop oleoresin from cone pellets using supercritical CO_2. Data from Del Valle et al., 2003b. (For a colour version, see Plate 2).

Table 3.5 Recent studies on the use of SFE for extracting nutraceuticals from different natural matrices

Processing conditions	Materials	Bioactive compounds	Reference
SFE-CO_2, 40–80 °C, 200–350 bar	Tomato juice	Lycopene	Egydio et al., 2010
SFE-CO_2, 40–60 °C, 150–360 bar	Oregano leaves	Flavonoids	Cavero et al., 2006
SFE-CO_2, 40–60 °C, 148–602 bar	Roasted wheatgerm	Phenolic compounds	Gelmez et al., 2009
SFE-CO_2, 50 °C, 100–300 bar	Cretan barberry herb	Phenolic compounds	Kukula-Koch et al., 2013
SFE-CO_2, 30–130 °C, 20–30 MPa	*Amaranthus* seeds	Squalene and essential oil	Wejnerowska et al., 2013
SFE-CO_2, 40–60 °C, 100–500 bar	*Synecoccus sp.* (microalgae)	Carotenoids	Macías-Sánchez et al., 2007
SFE-CO_2/ethanol (10%), 313 K, 20–35 MPa	Grape bagasse	Polyphenols	Farías-Campomanes et al., 2013
SFE-CO_2, 50–60 °C, 150–300 bar	Grape (*Vitis vinifera*)	Antimicrobial extract	Oliveira et al., 2013

3.2.4 Combined Extraction Process

All extraction technologies, whether novel or conventional, have advantages and disadvantages. Furthermore, no single technique can be used universally for extraction. Therefore, alternative ways to improve the overall efficiency of an extraction process are regularly sought after. One such approach is a combined extraction process, which utilizes the full potential of conventional and novel extraction technologies by employing their core principle advantages to combat each other's disadvantages. For example, Soxhlet extraction process is time consuming, requires high volumes of organic solvents, and has lower yield compared to MAE process, but combining these two processes allows engineers to design a continuous process that is easy to scale up or down. Soxhlet process is widely applied for the extraction of fats and oils from plant material using n-hexane as the extraction solvent, but the non-polar nature of n-hexane makes it inappropriate for MAE process, requiring it to be mixed with a polar solvent such as water, ethanol, methanol, etc., which results in a biphasic extraction medium that ensures removal of both polar and non-polar compounds. The only limitation that this mixing of solvents causes is the addition of the separation and purification steps (Cravotto et al., 2008; Luque de Castro and García-Ayuso, 1998; Sporring et al., 2005).

In 1998, Luque et al. developed a focused microwave-assisted Soxhlet extraction (FMASE) process. In this innovative design, microwave energy was applied to the extraction chamber, and electric heating was applied to the distillation flask. The combination of these two energy sources accelerated the extraction process, and has since been used for the determination of oil content and fatty acid composition (García-Ayuso et al., 2000; Luque de Castro and García-Ayuso, 1998; Luque de Castro and Priego-Capote, 2010; Luque-García and Luque De Castro, 2004). In another study, Virot et al. (2007) developed a microwave-integrated Soxhlet (MIS) method for the extraction of fats and oil from plant and animal sources (Virot et al., 2007). Their design involved the use of polytetrafluoroethylene/graphite compounds, which help to diffuse heat generated by the microwave to the surroundings and the extraction solvent. Power consumption of the MIS method was 0.5 kWh, in contrast to 8 kWh for a conventional Soxhlet process. The combination of Soxhlet and microwave energy not only improved extraction efficiency but also reduced the amount of solvent required during the extraction process, with a solvent recovery potential of 90% (Virot et al., 2007).

Ultrasonication is another technology that can be used alone or in combination with other novel processes for the extraction of nutraceutical compounds from plant sources. It involves the application of sound waves of frequencies higher than 20 kHz, which creates mechanical vibrations in solids, liquids, and gases (Dey and Rathod, 2013). Sound waves generate expansion and compression cycles while traversing through the medium that create cavitation bubbles which implode, resulting in the generation of high shear forces. This high force disrupts the cell wall, allowing the extraction solvent to penetrate into the plant material (Luque-García and Luque De Castro, 2003). In comparison to conventional methods, ultrasound-assisted extraction (UAE) is fast and can generate higher extraction yield (Toma et al., 2001). However, operational parameters such as equipment design and extraction temperature limit the use of UAE commercially. In UAE, the generated sound wave is not uniformly distributed across the sample, and maximum ultrasound power is observed in the vicinity of the radiating surface. Additional agitation is required to disrupt the plant matrix and avoid generation of standing waves and solid free regions. Application of ultrasound generates heat and may denature thermolabile bioactive compounds (Romdhane and Gourdon, 2002;, Sališová et al., 1997). To combat these disadvantages, UAE processes could be combined with another novel technique – enzyme-assisted extraction (EAE). EAE is used as an alternative to conventional techniques and is based on the inherent ability of enzymes to function under mild processing conditions and catalyze reactions, as well as their specificity and regioselectivity (Puri et al., 2012). EAE has been applied for the extraction of bioactive compounds – such as carotenoids from marigold flower (Barzana et al., 2002) and tomato skin (Dehghan-Shoar et al., 2011); polysaccharides from sterculia (Wu et al., 2007); and polyphenols (Yang et al., 2010) and antioxidant pectic-polysaccharides from mangosteen (*Garcinia mangostana*) rind (Gan and Latiff, 2011). In the food processing industry, enzymes are widely used for the extraction of pectin (Ptichkina et al., 2008), oils (Mishra et al., 2005), flavors, and pigments from plant matrices (Passos et al., 2009; Sowbhagya and Chitra, 2010). Application of enzymes for the extraction process provides specificity, and can be used under a wide range of operational conditions, but the extraction parameters have to be optimized for each specific application. These parameters include temperature, pH, time, and enzyme concentration. For combined ultrasonic–enzyme-assisted extraction, enzymes such as cellulases, pectinases, and hemicellulases may be used to disrupt the structural integrity of the plant cell wall prior to UAE. Application of these enzymes reduces the UAE extraction time and also improves the extraction yield. UAE may also be used prior to enzymatic extraction as reported by Sharma and Gupta (2004), who demonstrated that ultrasonic treatment of almonds and apricot seeds prior to EAE reduced the extraction time from 18 to 6 hours (Sharma and Gupta, 2004).

EAE may also be combined with the MAE process. Ookushi et al. (2008) achieved higher extraction of β-glucan from *Hericium erinaceum* by application of proteolytic and chitin-degrading enzymes prior to the MAE process (Ookushi et al., 2008). In another study, Yang et al. (2010) optimized the microwave-assisted enzymatic extraction (MAEE) of antioxidants corilagin and geraniin from *Geranium sibiricum* Linne. They reported that the combination of cellulose enzyme to release the polyphenolic compounds bound to cell wall structures with MAE resulted in an increase of 64.01% and 72.95%, respectively, for the two antioxidants. They concluded that MAEE not only improved the extraction efficiency compared to control experiments, but also prevented chemical decomposition of the bioactive compounds (Yang et al., 2010).

There are several other methods, including pulsed electric field (PEF), that can be combined together or used as pretreatment prior to conventional or novel extraction techniques (Bazhal et al., 2003). PEF's non-thermal applications in inducing increased cell permeability make them an ideal pretreatment method to enhance mass transfer in food products. PEF works through electro-permeabilization (loss of permeability) of the cell membrane when an external electric field is applied. The intensity of the applied electric field pulses, their duration, and shape determine the permanence and size of the pore formed, which can eventually lead to cell lysis (Angersbach et al., 2000).

PEF has been widely used for food dewatering enhancement in drying processes (Arevalo et al., 2004; Lebovka et al., 2007). For extraction of nutraceuticals, the technique has been used to extract anthocyanin from grapes and red cabbage (Corrales et al., 2008; Gachovska et al., 2010). Anthocyanins are used as natural dyes and have potential health benefits as dietary antioxidants (Suda et al., 2003). Puértolas et al. (2013) studied the application of PEF in the extraction of anthocyanins from purple-fleshed potato. They investigated the effect of PEF on the solvent extraction yield of anthocyanins at different temperatures, using water and ethanol as solvents, by subjecting potato slices to a PEF treatment of 5–35 pulses of 3 microseconds duration for 24–105 microseconds at an electric field intensity of 5 kV/cm. The study found that the use of ethanol as an extraction solvent was more effective for non-treated samples than PEF-treated samples. This was attributed to the ability of PEF to permeabilize cells, and hence ethanolic denaturation of the cell membranes was less significant for the cells previously permeabilized by PEF. PEF pretreatment also increased anthocyanin extraction yield at lower temperature (60 mg/100 g [fresh weight] of anthocyanin at 25 °C), compared to non-treated samples. This observation is important for the extraction of thermolabile bioactive components because, in conventional solvent extraction systems, a higher temperature is required to induce cell permeabilization or lysis. The extraction yield of anthocyanin using ethanol and water as extraction solvents was similar, which led to the conclusion that PEF pretreatment allows a reduction in the use of organic solvents for extraction. In another study, Guderjan et al. (2007) investigated PEF as a pretreatment for the extraction of rapeseed oil by pressing and solvent extraction. Rapeseeds were subjected to PEF treatments of 50 pulses at 5 kV/cm and 60 pulses at 7 kV/cm, with a duration of 30 microseconds. Application of PEF induced irreversible permeabilization of cell membrane and enhanced the extraction yield of rapeseed oil. El-Belghiti et al. (2008) reported an improvement of the aqueous extraction of solute from fennel

(*Foeniculum vulgare*) by the application of a PEF of 40 kV/cm and 20–90 pulses at 20 °C (El-Belghiti et al., 2008).

PEF has been widely used for the enhancement of juice extraction from fruits such as apple, carrot, and beet (Bazhal and Vorobiev, 2000; Rastogi et al., 1999). Gachovska et al. (2006) studied the effect of PEF on extraction of juice from alfalfa. They subjected mashed alfalfa in a treatment chamber that was used both for high-electric-field treatment and juice extraction. Mechanical pressing and PEF treatment were carried out alternatively, leading to an enhancement of approximately 38% volume in the extraction of alfalfa juice, and achieving relatively higher quality as compared to non-treated alfalfa (Gachovska et al., 2006).

PEF could be a good alternative to conventional pretreatment methods of homogenization, grinding, milling, maceration, and drying, because it offers higher yield and quality, but practical implementation on an industrial scale is very limited, because of the complexity in the design of the treatment chambers that are used to process high volumes of raw materials. The application of high electric field, ranging from 5 kV to 30 kV, could also be a safety concern for workers (Donsì et al., 2010).

3.3 Delivery of Nutraceuticals in Food and Its Limitations

Over the years, the list of nutraceutical compounds identified and analyzed for potential health benefits has been growing steadily. Scientific evidences have been provided to support the concept of "food as medicine." Even though the very nature of action of these nutraceutical compounds is not yet fully understood, scientists and food engineers have recognized that their addition to food matrices creates novel functional foods and products that can produce physiological benefits, and which may reduce the risk of developing life-style-related diseases. The measure of the effectiveness of nutraceuticals added in food is related to their bioavailability and bioactivity. Nutraceuticals are stable when processed under certain conditions (e.g., controlled temperature, pressure, oxidation, light) employed during the processing of food; however, the environmental conditions (pH, presence of enzymes, other nutrients) within the gut can significantly affect their bioavailability (Chen et al., 2006; McClements et al., 2009). Therefore, it may be necessary for food engineers and scientists to devise mechanisms or delivery systems that will maintain their bioavailability and efficacy during processing until they are delivered to the physiological target within humans (McClements et al., 2009). Nutraceuticals and functional food components come in different forms and are unique in their molecular structure, polarity, and molecular weight. These differences confer varied physiochemical properties including solubility, partitioning, and chemical and optical stability

(McClements et al., 2009). Several polymer-based delivery systems are used in the biomedical and pharmaceutical sectors, but their applicability in food is difficult because they have to be compatible with the food system they are added to, and must also be safe for consumption (Chen et al., 2006). The design of the delivery systems depends on the physicochemical properties of the nutraceuticals and functional food components. Bioactive lipids, including carotenoids, conjugated linoleic acid (CLA), omega-3 fatty acids, and phytosterols, are difficult to deliver because of the following challenges: (a) bioactive lipids have low water solubility, hence they have to be encapsulated within delivery systems such as emulsions or microemulsions to make them dispersible in aqueous food products; (b) carotenoids exist in a crystalline form at room temperature, and hence their delivery requires elevated temperature, or they may influence the organoleptic quality and shelf-life stability of the food products; (c) the majority of bioactive lipids, such as omega-3 fatty acids, carotene, and lycopene, are thermolabile and photosensitive, and hence they have to be kept within a protective delivery system that can prevent their oxidation and maintain their thermal stability (McClements et al., 2009; Ubbink, 2002). Bioactive peptides, proteins, and amino acids are another group of nutraceuticals and functional components that require complex delivery systems (Tripathi and Misra, 2005). Since proteins and amino acids are associated with unique functional properties, it is necessary that their delivery systems make them compatible with the food matrix while keeping them non-reactive and preventing them from affecting the textural and physicochemical properties of the food. Thermal sensitivity of proteins makes them susceptible to food processing conditions; they can be easily degraded at elevated temperatures, which can affect their bioavailability and functionality (Chatterton et al., 2006). Peptides are susceptible to the enzymes present in the food and gut, while they may get hydrolyzed and lose their specific functionality under high acidic conditions; hence, their delivery vehicle has to maintain their stability within the stomach and release them only at the targeted site, where they are expected to be absorbed and successfully reach their end target. Bioactive carbohydrates or “dietary fibers” possess another major challenge for food developers, as their presence in food can adversely affect texture and sensory qualities. Unlike other nutrients, dietary fibers are not a single substance, but a term used for a group of plant polysaccharides that are not easily hydrolyzed by the endogenous enzymes secreted in the human digestive system. Structural polysaccharides, such as cellulose and hemicellulose (insoluble in water), and non-structural polysaccharides, such as pectin, β-glucans, and gums (soluble in water), are few of the dietary fibers used in the food industry. In recent years, scientists have proven an association of high dietary fiber intake with lower risk of several chronic diseases, and this has led to recommendations of increased dietary fiber intake in the regular diet. At present, several dietary fiber supplements such as psyllium (trademark “Metamucil or Fiberall) and methylcellulose (trademark Citrucel) are being marketed to increase the consumption of dietary fibers (Licari, 2006). However, they have received limited consumer acceptance, because of their ability to increase the viscosity of the aqueous food product. Exposure to water makes them swell prior to or during the digestion process, generating a gummy, chewy product, which has undesirable mouth feel and can even obstruct bowel movements, which adds to increased safety risk (Licari, 2006). Hence, they require a delivery system with the unique ability to encapsulate large quantities of dietary fibers, which will not affect the organoleptic and physico-chemical properties of the food products to which they are added and would be easily acceptable by consumers (Redgwell and Fischer, 2005).

Some of the delivery systems that are currently used or could be used to deliver bioactive compounds by the food industry are the following.

(1) Lipid-based nanostructured delivery system, commonly termed as "liposomes," which consist of one or two lipid bilayers fabricated from food-grade phospholipids derived from sources such as soy, dairy, or egg; they are suitable for the delivery of hydrophobic nutraceutical compounds (McClements, 2005).
(2) Emulsions (conventional emulsions, multiple emulsions, multilayered emulsions, solid–lipid particle emulsions), which are surfactant-coated lipid droplets dispersed in an aqueous medium. They are thermodynamically less stable than microemulsions, but have smaller particle size (<100 nm), giving them higher optical clarity and making them suitable for translucent food products such as juices. Multiple emulsions provide the ability to deliver two or more nutraceutical products that can adversely interact with each other (Benichou et al., 2004; Guzey and McClements, 2007; McClements, 2012, 2005).
(3) Micelles and microemulsions (McClements et al., 2009), which are oil-in-water emulsions with a particle size of < 50 nm, are thermodynamically stable and have high optical clarity. The only disadvantage they have is that the surfactants used to make them are usually not food-grade and can be injurious to health (McClements, 2012).
(4) Biopolymer-based delivery systems (proteins and polysaccharides) (Rosenberg and Young, 1993) are fabricated using various methods such as extrusion, coacervation, and emulsion templating. Some of the applications and advantages of the aforementioned delivery systems are as follows: (i) simple oil–water emulsion is widely used to encapsulate bioactive lipids and flavoring compounds, and (ii) biopolymer- and surfactant-based delivery systems are used for antioxidants, proteins, and dietary fiber delivery (McClements et al., 2009; Rosenberg and Young, 1993). The main advantage of these systems is that they provide the required thermodynamic stability, but have low loading capacity, and often affect the organoleptic and physiochemical properties of the food.

The formulation of successful delivery systems for nutraceutical and functional food components faces numerous technical, legal, and commercial challenges. Several challenges are due to the adverse alteration to the organoleptic quality of the food products to which they are added, while the most important of all the challenges is the efficacy of the individual nutraceutical components (McClements, 2012). As in the case of drug delivery, known amounts of drug doses are administered, which provides the base for scientists to estimate their efficacy against specific diseases. Since there is no calculated dose or time period defined for nutraceutical products, it is difficult for manufacturers to design delivery systems that can sustain prolonged storage, since nutraceuticals are incorporated in foods that are consumed in different amounts by different individuals at different times along with different processing methods (McClements, 2012).

Therefore, there is a need for extensive research in the field of designing appropriate delivery systems, which can maintain the quality of the supplied nutraceutical throughout the processing, storage, and consumption stages.

3.4 Conclusion

This chapter focused on various technologies that have been used to isolate bioactive compounds from natural sources. Several applications of these technologies have been described. However, some challenges related to these technologies must be overcome before they can be used reliably. Issues pertaining to production cost, scalability, product quality, and safety have to be addressed. No single technology is foolproof, and more research is required to improve the effectiveness of current technologies. Moreover, novel ideas such as combination extraction systems need to be looked at in more detail in order to address technological challenges.

Functional foods and nutraceuticals are a growing market. The design of suitable processing and delivery systems for nutraceuticals and functional food components of interest is still a growing field; extensive research will continue to be required to develop effective delivery systems and new functional foods and nutraceutical products. Additionally, regulatory reforms may be required to allow manufacturers to produce, introduce, and market novel functional food and nutraceutical products.

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4

Quality Evaluation and Safety of Commercially Available Nutraceutical and Formulated Products

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4.1 Introduction

The range of dietary supplements available to the consumer has been increasing, and nutraceuticals are an expanding sector of this market. They are being studied not only for use in diet supplementation for prophylaxis against a number of diseases, but also for development as possible medicines for the treatment of a huge range of major diseases, including cancer and Alzheimer's disease. Pharmaceutical companies and consumer food companies are increasingly moving into this area, either by expansion or by acquisition of nutraceutical suppliers.

Stephen De Felice, of the Foundation for Innovation in Medicine, was the first to use the term "nutraceutical" in 1989, defining it as "food, or parts of a food, that provide medical or health benefits, including the prevention and treatment of disease" (Rapport and Lockwood 2002). This definition is still generally adhered to today; however, certain examples are not currently dietary components. These nutraceuticals are generally sourced from plants and plant products – for example, soy and its isoflavones – but some are

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Table 4.1 The biological origins of the major nutraceuticals

Human metabolites	Plant constituents	Animal constituents
Branched-chain amino acids (BCAAs)	Bilberry anthocyanins	Conjugated linoleic acids (CLA)[1]
Carnitine	Carotenoid derivative	n-3-Polyunsaturated fatty acids (PUFAs)
Coenzyme Q10	Astaxanthin	Chondroitin
Creatine	β-Carotene	Glucosamine
Dehydroepiandrosterone (DHEA)	Lutein	
γ -Linolenic acid	Lycopene	
Lipoic acid	α-Tocopherol	
Melatonin	Zeaxanthin	
S-adenosylmethionine (SAMe)	Flax lignans	
	Grapeseed proanthocyanidins	
	Policosanol*	
	Similar homologous alkanols are widely available in waxy surfaces of many plant foods	
	Pycnogenol*	
	Composed of proanthocyanidins	
	Resveratrol	
	Soy isoflavones	
	Sterols/stanols – e.g., Sitosterol, sitostanol	
	Tea polyphenols – e.g., catechins	

*denotes non-food source.
[1]A range of semisynthetic derivatives are also available.

derivatives of naturally occurring substances in the human body and often found in foods, while others are of animal origin. Commercial sources of these two groups may be derived either from natural sources, or be semi-synthesised or manufactured in a biotechnological process. Table 4.1 outlines the origins of the major nutraceuticals. Table 4.2 lists representative chemical structures and dosage levels for major nutraceuticals. Dosage range is the currently advised level based on clinical evidence, where available.

Nutraceuticals are commercially available in a staggering range of products in myriads of formulations and strengths, list widely variable claims on their labels, and are available at widely differing prices. Their presentation is usually of the same appearance as for pharmaceuticals; however, they are generally not regulated as to their quality, label claims, and availability. Unlike the situation when medicating with pharmaceuticals, there is no evidence that a consumer will attain the same level of benefits from commercially available nutraceuticals as those available from any in a range of generic pharmaceutical products, essentially due to lack of government regulation.

Undoubtedly, there is some good-quality epidemiological and clinical evidence available for certain specified (proprietary) nutraceuticals produced by some manufacturers, and these are often the same products used for clinical trials, but the vast majority of commercial products are not the ones that resulted in the high-quality scientific data. Clinical trials are usually conducted on single compounds from a particular nutrient source (soy and teas are obvious exceptions), but commercially available products are usually multi-component mixtures of often unknown constitution.

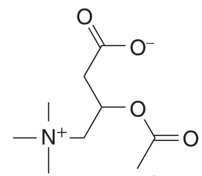
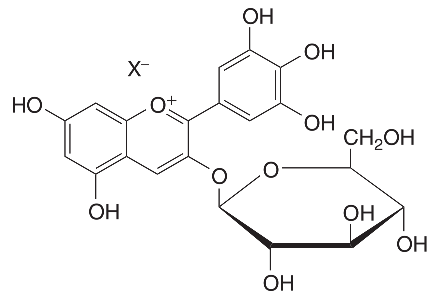
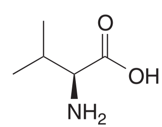
4.2 Contents of Single Components in Formulated Products

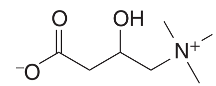
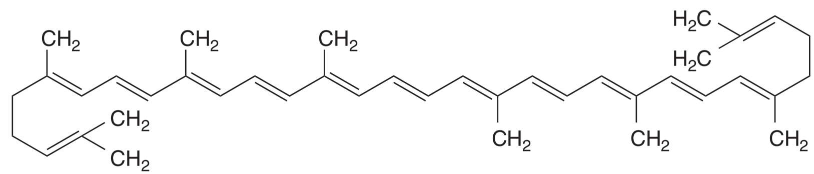
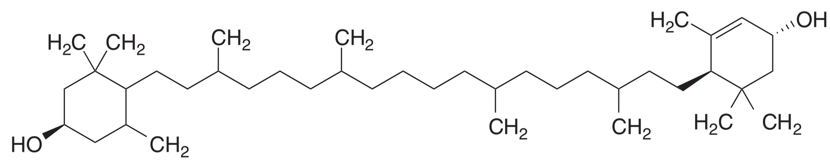
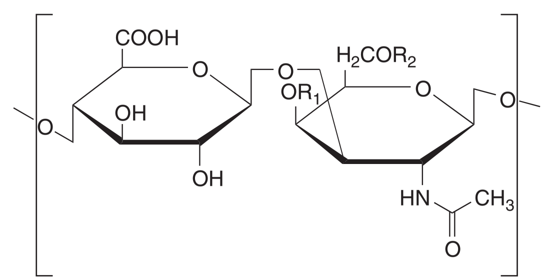
Many formulated nutraceutical supplements can be found in widely varying labelled ranges of contents – for example, the resveratrol content may be claimed to be present from 10 mg to 500 mg in different products. A similar broad range can be found in most commercially available nutraceutical product ranges. In addition to specified constituents, many single-constituent nutraceuticals (labelled as such) have actually been shown to be simple, non-fractionated extracts containing a complex constituent profile, and may contain up to 70% non-specified constituents, probably not fully elucidated by the manufacturer, nor evaluated for biological activity (Espin et al. 2007).

Standard good manufacturing practice (GMP) of pharmaceuticals requires that levels of 95–105% of claimed constituent levels be present, and this parameter has been calculated from published data on nutraceutical quality, and incorporated into Table 4.3. Methodological standards (assays) are increasingly becoming available (Anon. 2007b), and the two major reference texts are: (1) *The Handbook of Analytical Methods for Dietary Supplements* (Jaksch et al. 2005), which lists monographs on five nutraceuticals (chondroitin, coenzyme Q10, glucosamine, melatonin, and soy), and (2) the *United States Pharmacopeia/National Formulary* (Anon. 2007a), which has monographs on seven nutraceuticals (chondroitin, coenzyme Q10, glucosamine, lipoic acid, lutein, lycopene, and maritime pine extract [Pycnogenol]). These standard methods have been used for some of the analytical data laid out in Table 4.3, but other recently developed methods have also been employed to obtain data.

Two of the most popular products, and also the subjects of a number of investigations, are chondroitin and glucosamine, and these have been shown to have variable compliance with labelled contents (Table 4.3), probably caused by the use of a range of sources for starting materials and the chemical forms used. Analysis of chondroitin demonstrates a range of problems not seen in the majority of nutraceuticals; the molecular mass of the molecule is variable, dependent on biological origin, varying from 14 KDa to70 KDa (bovine, porcine, chicken, shark, or skate cartilage). Within the chondroitin samples, the specific ratio of different disaccharides also varies, and is further

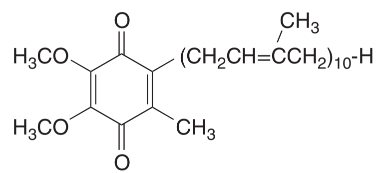
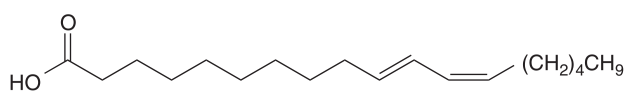
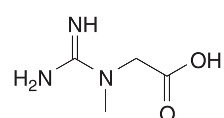
Table 4.2 Summary of therapeutic applications, representative chemical structures, and dosage levels for the major nutraceuticals

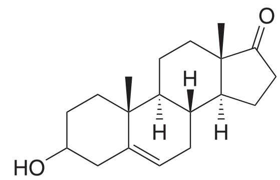
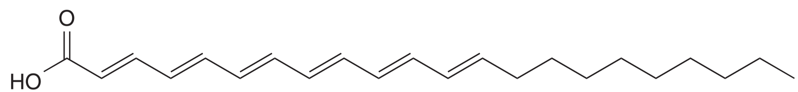
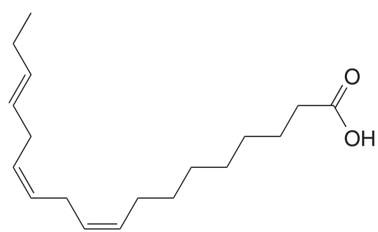
Nutraceutical	Structure	Source	Dosage Range (Lockwood 2007)
Acetyl carnitine	 acetyl carnitine	Brain, liver, kidney	1.5 – 3 g/day
Bilberry anthocyanins – e.g., Delphinidin-3-*O* – glucoside	 delphinidin-3-*O*-glucoside	*Vaccinium myrtillus*	120 mg/day
Branched-chain amino acid (BCAA) – e.g., valine	 valine	Dietary amino acids	7 – 20 g/dose

Carnitine	 carnitine	Heart, skeletal muscle	2–4 g/day
Carotenoids – e.g., lycopene	 lycopene	Wide range of foods	10–40 mg/day
Carotenoids – e.g., lutein	 lutein	Wide range of foods	10–40 mg/day
Chondroitin	Consists of 15–150 monomer units 	Bovine trachea, cartilage	1,200–1,500 mg/day

(continued overleaf)

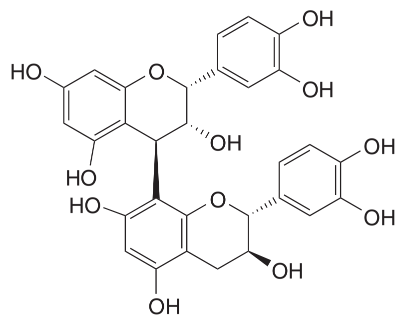
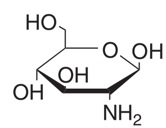
Table 4.2 (*continued*)

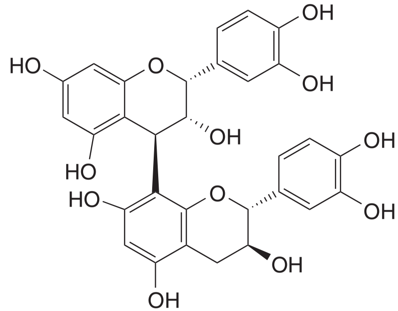
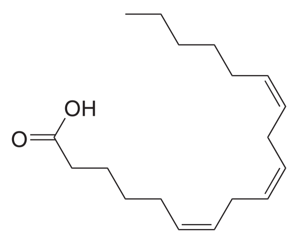
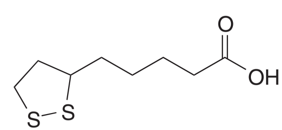
Nutraceutical	Structure	Source	Dosage Range (Lockwood 2007)
Coenzyme Q10	 coenzyme Q10	Wide range of foods	100–360 mg/day
Conjugated linoleic acid – e.g., trans, 9-cis, 11-octadecadienoic acid	 trans, 10-cis, 12-octadecadienoic acid	Beef, dairy products	1–2 g/day
Creatine	 creatine	Skeletal muscle	5–25 g/day

DHEA 3-hydroxyandros-5-en-17-one	 3-hydroxyandrost-5-en-17-one	Wild yams	5–25 mg/day
n-3-Fatty Acid – e.g., DHA	 docosahexaenoic acid	Fish, algae, plankton	2.5 g/day
Flax Alpha linoleic acid	 alpha linoleic acid	*Linum usitatissimum*	50–100 mg/day

(*continued overleaf*)

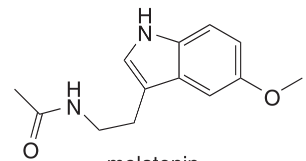
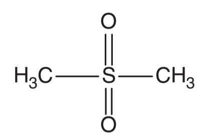
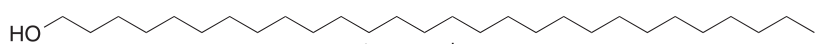
Table 4.2 (*continued*)

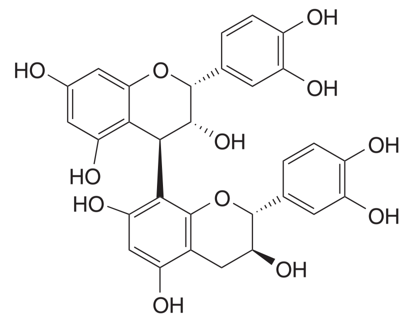
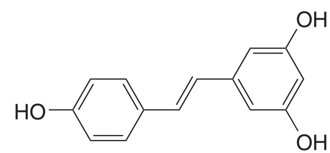
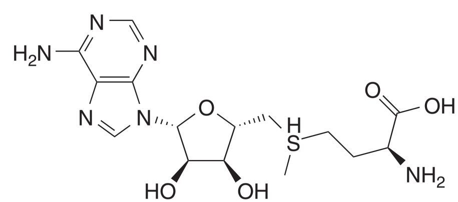
Nutraceutical	Structure	Source	Dosage Range (Lockwood 2007)
Flax Lignans	 secoisolariciresinol	*Linum usitatissimum*	Present in flaxseed oil at about 1%
Glucosamine	 glucosamine	Bovine trachea, shellfish	1,500 mg/day

Grapeseed proanthocyanidin extract	 proanthocyanidin B2	*Vitis vinifera*	50–100 mg/day
Gamma Linolenic acid	 gamma linolenic acid	*Oenothera biennis*, *Borago officinalis*	360–2,800 mg/day
Lipoic acid	 lipoic acid	Meat, liver	50–100 mg/day

(*continued overleaf*)

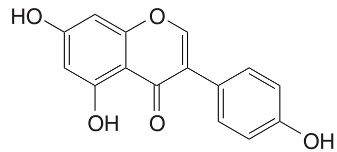
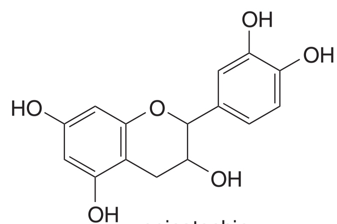
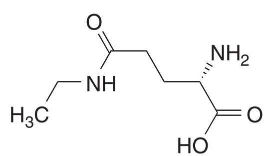
Table 4.2 (*continued*)

Nutraceutical	Structure	Source	Dosage Range (Lockwood 2007)
Melatonin	 melatonin	Bovine pineal glands	0.3 – 25 mg
MSM* Methyl sulphonyl methane	 methyl sulphonyl methane	Meat, milk, capers	Up to 2 g/day
Policosanol – e.g., octacosanol	 octacosanol	Sugarcane waste	100 mg/day

Pycnogenol	 proanthocyanidin B3	*Pinus pinaster*	25–200 mg/day
Resveratrol 3,4′,5-trihydroxy-stilbene	 3,4',5-trihydroxystilbene	Red wine, Polygonum capsidatum, etc.	15–200 mg/day
SAMe S-adenosyl methionine	 S-adenosyl methionine	Meat, yeast, vegetables	200–1,200 mg/day for osteoarthritis, 400–1,200 mg/day for depression

(*continued overleaf*)

Table 4.2 (*continued*)

Nutraceutical	Structure	Source	Dosage Range (Lockwood 2007)
Soy isoflavones – e.g., genistein	 genistein	Soy, fermented soy	30–50 mg/day
Tea extracts Catechin derivatives – e.g., epicatechin and theanine	 epicatechin  theanine	*Camelia sinensis*, green and black	50–100 mg/day catechin derivatives 50–200 mg/ week of theanine

*Methylsulphonylmethane (MSM) is present in minute amounts in milk and meat (animals) and capers (plant), but commercial material is manufactured from dimethylsulphoxide.

Table 4.3 Proportion of products shown to comply with accepted quality standards from a range of nutraceutical supplements

Nutraceutical	Origin	Numbers passed/ failed	Range of contents	References
Astaxanthin	United States	0/4	11–12%	Armstrong 2008
Branched-chain amino acids	Italy	3/0	97–104%	Cavazza et al. 2000
Carnitine	Czech	1/2	50–104%	Prokoratova et al. 2005
Carnitine	United States	0/1	35%	Prokoratova et al. 2005
Carnitine	Greece	2/0 capsules	98% each	Kakou et al. 2005
Carnitine	Greece	5/0 oral solutions	96–100%	Kakou et al. 2005
Carnitine	Spain	1/3 tablets 0/4 capsules	1–355% 159–488%	Sanchez-Hernandez et al. 2010a
Carnitine	Spain	4/0 tablets/ampoules 3/4 sachets/ oral solutions	100–102% 77–99%	Sanchez-Hernandez et al. 2010b
β-Carotene	Germany	1/10	61–137%	Aman et al. 2004
β-Carotene	Canada	2/4	86–111	Humayoun and Bryan 2008
Chondroitin	United States	1/2	101–103%	Adebowale et al. 1999
Chondroitin#	United States	2/2	Two, 32–36%	Adebowale et al. 1999
Chondroitin	Canada	2/5	33–109%	Russell et al. 2002
Chondroitin	United States	0/3	80–90%	Anon 2002
Chondroitin#	United States	3/9	80–120%	Anon 2002
Chondroitin	United States	4/7	9–112%	Eddington 2005
Chondroitin	South Korea	6/6	40–106%	Sim et al. 2005
Chondroitin	South Korea	0/7	1–21%	Sim et al. 2007
Coenzyme Q10	Japan	10/0	96–106%	Nishii et al. 1983
Coenzyme Q10	New Zealand	1/6	100–130%	Molyneux et al. 2004

(*continued overleaf*)

Table 4.3 (*continued*)

Nutraceutical	Origin	Numbers passed/ failed	Range of contents	References
Coenzyme Q10	United States	100% pass	95–105%	Johnson et al. 2005
Coenzyme Q10	United States	4/0	98–103%	Tang 2006
Coenzyme Q10	Japan	36/25	5–123%	Kettawan et al. 2007
Creatine	United States	1/7	83–106%	Wagner et al. 2001
Creatine	United States	1/2	Effervescent powders 99–100% "Serum" formulation, 1.7% of claimed level	Dash and Sawhney 2002
Creatine[1]	United States	4/2	94–126%, 100% in four	Persky et al. 2003a
Creatine	United States	0 pass	"Serum" formulation, 1.7% of claimed level	Dash and Sawhney 2002
Dehydroepiandrosterone DHEA	United States	6/10	Three contained 0%, and one 150%	Parasrampuria et al. 1998
DHEA	United States	14/30	One contained 0%	Thompson and Carlson 2000
Docosahexaenoic acid	United States	1/7	57–115%	Chee et al. 1990
Eicosapentanoic acid	United States	0/8	75–94%	Chee et al. 1990
Glucosamine	United States	2/4	77–117%	Adebowale et al. 1999
Glucosamine[#]	United States	2/2	90–108%	Adebowale et al. 1999
Glucosamine	United States	1 pass	101%	Anon. 2002
Glucosamine	United States	3/1	95–135%	Anon. 2002
Glucosamine	Canada	0/15	89–117%	Russell et al. 2002

Glucosamine[#]	United States	9/3	75–120%	Anon. 2002
Glucosamine	United States	6/0	99–103%	Shao et al. 2004
Glucosamine	United States Tablet	0/6	55–87%	Sullivan and Sherma 2005
Glucosamine	United States Capsule	0/3	41–87%	Eddington 2005
Glucosamine	United States	4/10	35–117%	Eddington 2005
Glucosamine	Iran	2/8	13–139%	Nemati et al. 2007
Glucosamine	United States	6/0	97–105%	Volpi 2009a
γ - Linolenic acid	Germany	5/14	73–107%	Ihrig and Blume 1994
γ - Linolenic acid	Australia	12/4	36–109%	Gibson et al. 1992
Lipoic acid	Austria	3/2	87–110%	Sitton et al. 2004
Lipoic acid	Austria	1/5	40–97%	Durrani et al. 2007
Lutein	United States	0/3	134–194%	Sechrist et al. 2002
Lutein	Canada	0/2	109–125%	Humayoun and Bryan 2008
Lutein/zeaxanthin	Germany	0/7	16–136%	Breithaupt and Schlatterer 2005
Lutein/zeaxanthin	United States	2/5	11–22%	Breithaupt and Schlatterer 2005
Lycopene	Canada	1/5	6–143%	Feifer et al. 2002
Melatonin	Italy	5/1	93–102%	Costantini and Paoli 1998
Policosanol	United States	Tab-53% pass Cap-54% pass Cap-40% pass	Octacosanol 58% 16% 24%	Irmak et al. 2006
Proanthocyanidins	Japan Grapeseed oil	0/2	ND	Nakamura et al. 2003
Oxyresveratrol	United States	-	Three of four contained 0%	Bertram et al. 2010
Resveratrol	United States	Wine/juice/capsule	96–102%	Zhu et al. 2000
Resveratrol	Italy	5/9	40–110% Two only traces	Rossi et al. 2012

(*continued overleaf*)

Table 4.3 (*continued*)

Nutraceutical	Origin	Numbers passed/ failed	Range of contents	References
Resveratrol	United States	2/2	94–97%	Babu et al. 2005
SAMe	United States	4/8	Levels from 40–120%	Anon. 2000
Soy isoflavones	31 US/1 UK	4/28^{2}	10%– 383%	Setchell et al. 2001
Soy isoflavones	Australia	2/8	< 1–100%	Howes and Howes 2002
Soy isoflavones	Finland	1/6	37–99%	Nurmi et al. 2002
Soy isoflavones	Various	2/12*	30–99%	Penalvo et al. 2004
Soy isoflavones	United States	2/11*	47–99%	Chua et al. 2004
Soy isoflavones	Austria	NS	91–109%	Kren and Poetsch 2006
Soy isoflavones	Germany	2/9	51–139%	Sturtz et al. 2008
Soy isoflavones	United Kingdom	2/17	40–130%	Clarke et al. 2008
Soy isoflavones	United Kingdom	1/16	5–133%	Clarke et al. 2008
Sterol/stanols	South Africa	0/5	73–106%	Nair et al. 2006
Green tea extracts	United States	0/4	9–48% catechin content of that stated on labels	Manning and Roberts 2003
Green tea extracts	United States	0/3	92–141%	Weiss et al. 2006
α-Tocopherol	Canada	0/7	59–149%	Feifer et al. 2002
α-Tocopherol	United States	4/10	0–119%	Remsberg et al. 2010

* – products specifying particular levels of isoflavonoids did not meet claimed levels.
# – combination product, chondroitin +glucosamine.

complicated by admixtures and other sources, such as avian material (Volpi 2007). Disaccharide compositional analysis of 12 Japanese chondroitin supplements showed two products falsely labelled, as being from shark as opposed to their actual bovine origin (Sakai et al. 2007). Another study revealed that commercially available chondroitin may actually consist of hyaluronic acid (Saad et al. 2005). Overall, published studies have shown that the quality of chondroitin sulphate in commercially available products is often poor. Pharmaceutical-grade chondroitin should be used for the manufacture of formulated products in order to guarantee standardised molecular structure, which will affect both pharmacokinetics and the overall activity of the product (Volpi 2009b).

Commercial carnitine has also been the subject of a number of investigations. Carnitine samples showed good compliance in two surveys, but not in two further reports. More recently, two Spanish reports have shown varying quality (Sanchez-Hernandez et al. 2010a and 2010b). Creatine products conformed poorly, as did DHEA, n-3 PUFAs, γ-linolenic acid, lutein and zeaxanthin, lycopene, β-carotene, and α-tocopherol. Complex materials such as soy isoflavones and tea extracts were also found to have poor compliance.

Detailed variation of product conformity is being published as shown in Table 4.4. An illustrative example of the level of detail is the data on formulated tablets of green tea extracts. Out of 19 green tea extracts formulated as tablets, 11 made label claims for (-) epigallocatechin gallate (EGCG) content (Seeram et al. 2006).

Table 4.4 shows that the levels of stated content achieved by these products were wildly variable; there have clearly been faults in the QC/QA departments. These products were purchased from a wide range of sources,

Table 4.4 EGCG content of label claim of green teas (% tablet weight)

Label claim (%)	Level determined (%)	Level determined/ label claim (%)
2.9	14.8	513
22.2	6.8	32.6
1.7	1.6	94
50	7.9	15
22.2	2.7	12
1.7	2.4	141
1.9	2.7	142
8.9	7.7	86
55	27.1	49
29.2	25.7	88
20.5	21.3	103

including stores and the Internet. Manufacturers who had supplied the products included some of the major brand names!

This investigation also found a clear correlation between biological activity in green tea supplements, specifically antioxidant activity, and the levels of biological markers, namely the total polyphenol content (Seeram et al. 2006).

A few individual nutraceuticals may be classed as medicines in certain countries, and as such are subject to medicine regulations. Evaluation of 10 Japanese pharmaceutical-grade coenzyme Q10 products showed complete compliance with standards (Nishii et al. 1983), and also in one survey of US products (Johnson et al. 2005), but not in three other surveys. Also, variability has been reported for other examples of products often classed as medicines, such as dehydroepiandrosterone (DHEA).

Examples of most formulated nutraceutical products have been tested over the last 15 years, although no examples of acetylcarnitine or MSM have been reported. The latter product is a branded formulation made by a single manufacturer, consequently subject to internal standards. Relatively high quality was found for melatonin products, but poor quality was found with soy isoflavones, proanthocyanidins, and α-tocopherol. Other reports on the quality of nutraceuticals recorded data on lutein, SAMe (Anon. 2000), and DHEA products (Parasrampuria et al. 1998; Thompson and Carlson 2000), but they show a similar picture of low-quality products available. Lack of label compliance for single-entity products is evident, but is even more pronounced for complex materials such as soy.

Commercial evaluation of a number of nutraceuticals has been published on the Internet over the last few years (ConsumerLab.com). Data has been produced for MSM, coenzyme Q10, SAMe, DHA/EPA, DHEA, lutein/zeaxanthin, creatine, and sterols, among other nutraceuticals (Lockwood 2007). GMP and European Pharmacopoeia standard designation of good quality (95–105%) have not been used, and variable parameters for the different entities were employed. Although products have been named, varying levels of constituents are no doubt confusing to subscribers.

From the consumer's viewpoint, looking at a range of commercial nutraceuticals shows that there is a marked difference in prices. The costs of a number of ranges have shown wide variations, as seen in Table 4.5. Chua et al. found that most soy products surveyed contained sub-standard levels of isoflavones,

Table 4.5 The price range of four nutraceuticals

Nutraceutical	Minimum cost	Maximum cost	Reference
Glucosamine	US$0.30	US$0.70	Anon. 2002
Glucosamine	£0.11	£0.61	Anon. 2003
Chondroitin	US$0.85	US$1.35	Anon. 2002
Soy isoflavones	US$0.10	US$2.19	Chua et al. 2004

and package costs varied by a factor of two times. More importantly, unit costs of 50 mg isoflavonoids varied by a factor of 22, from US$0.1 to US$2.19 (Chua et al. 2004)!

4.3 Contents of Active Constituents of Ranges of Nutraceuticals of Complex Composition

Commercially available, multi-component products derived from a single natural source are being advertised on the basis that individual components or the complete material may have health benefits. Data has been published for a number of examples, including bilberry, branched-chain amino acids (BCAAs), conjugated linoleic acid (CLA), flaxseed, grapeseed proanthocyanidin extracts, green teas, soy isoflavones, Pycnogenol, and resveratrol preparations. Published data on constituent levels has been driven both by the desire of biologists and clinicians to ascertain precise data concerning the biological/therapeutic activity of nutraceutical supplementation, and also by analysts with an interest in developing new analytical methods to investigate the ability and applications of developing technology.

4.3.1 Bilberry

Cassinese et al. (2007) assayed the anthocyanins (glycosides) of bilberry extracts, and at the same time determined the level of anthocyanidins (aglycones) that are markers of poor-quality products, the latter class of compounds being present due to hydrolysis caused either during storage, extraction, or manufacture. They identified and quantified 15 anthocyanins and five anthocyanidins and revealed that only 15% of the products tested had the same level of anthocyanins as test materials shown to be effective in clinical trials. Two products were found to be composed of constituents from berries other than bilberry. There were significant inter-batch variations, and one product had no anthocyanins. Labelling errors included botanical species, plant part, and, in some instances, there was no presence of purported active constituent indicated (Cassinese et al. 2007).

Workers using the United States Pharmacopeia/National Formulary methods for the quantification of anthocyanin contents of 10 commercially available products noted extreme variations in levels; anthocyanin values ranged from 0 to 44%, and anthocyanidins from 0 to 6.5% (Artaria et al. 2007).

4.3.2 Branched-Chain Amino Acids (BCAAs)

More favourable results were found from the analysis of BCAA products of Italian origin, where there were high levels of conformity with label claims (Cavazza et al. 2000).

4.3.3 Conjugated Linoleic Acid (CLA)

Individual and total CLA isomer levels in four US formulated supplements have been found to vary widely in both physical characteristics (colour and solubility) and total fixed oil and levels of individual CLA isomers. This is most likely due to the variability of fixed oil source, and the conditions of isomerisation (Yu et al. 2003).

4.3.4 Flaxseed

Over recent years, the therapeutic properties of α-linoleic acid and the lignans have been widely studied. Variability in the composition of three flaxseed cultivars have been shown to have different levels of activity in a number of biomarkers for atherosclerosis and mental stress, demonstrating the importance of phytochemical composition on biological activity (Spence et al. 2003). Surveys of labelled composition of proprietary breads and cereals containing flax have revealed extremely wide ranges of constituents. In one survey of breads fortified with flaxseed, 13 were labelled to contain 0–10.1% of flaxseed, and analysis of lignan aglycones resulting from fermentation revealed a wide range of levels of the metabolites, secoisolariciresinol, enterolactone (EL), and enterodiol. Total levels ranged from 1 to 32 μmol/100 g in bread, with the metabolite EL appearing as the major constituent in all samples. Similar investigation of six breakfast cereals revealed a metabolite level ranging from 2 to 48 μmol/100 g, with EL usually present as the predominant metabolite (Nesbitt and Thompson 1997). These results clearly demonstrate no clear link between labelled claims and actual levels of flax constituents. Nesbitt and Thompson also found significant differences in levels of individual lignans and in the proportions of individual lignans, and this was clearly influenced by the variety of flaxseed used.

4.3.5 Grapeseed

A total of 16 Spanish grapeseed products have been studied and shown to have widely differing antioxidant activity, and levels of active constituents, gallic acid, and cyanidins, with a factor of 12X different levels (Monagas et al. 2005). A further 10 products derived from grape skins were evaluated, and detailed quantitative data was shown for levels of 13 cyanidins (Monagas et al. 2006). Grape pomace, and its residues from wine making and juice pressing, were also found to contain the cyanidins, and it is likely that these fractions are used in commercial manufacturing of nutraceuticals.

4.3.6 Green Tea

Both green and, to a lesser extent, black teas are increasingly popular. Levels of claimed active compounds depend upon the method of preparation of the infusion, as well as the choice of tea type. Surveys of teas, and their preparations, have been reported to exhibit wide differences in the levels of catechin derivatives and also theanine (Manning and Roberts 2003). The catechins have been shown to be surprisingly stable during normal tea-making procedures, but long-term storage in aqueous media at extreme pH may cause greater degradation, as in previously prepared commercial products (Chen et al 2001). Catechins were shown to be stable in water at room temperature – brewing Longjing tea at 98 °C for 7 h caused 20% loss of catechins. Autoclaving at 120 °C for 20 min was found to cause epimerisation of EGCG to (-) gallocatechin gallate (GCG), which was found in a number of commercial tea drinks, probably caused by high temperature treatment. The stability of GCG itself is liable to further degradation, depending on the medium in which it is present, irrespective of a low pH. Levels of theanine, caffeine, and catechins have been determined in a range of six teas from Taiwan. Although caffeine levels were similar, wide variations were reported for theanine (20–92 mg/g) and individual catechins (Chen et al. 2003).

A total of 191 specific types of green teas have also been investigated, and Longjing teas showed higher levels of theanine, gallic acid, and certain catechin derivatives (Le Gall et al. 2004). Comparison of a range of tea bags containing green tea also revealed a wide variation in both individual and total catechin contents, the latter ranging from 6.9 to 48.3 mg/g (Manning and Roberts 2003). Catechin variation between 11 different teas and 14 commercial tea drinks has been reported, with catechin levels ranging from 2.4 to 144.4 mg/g and 2.6 to 341.7 mg/g, respectively (Chen et al. 2001). Similar wide variations were reported in a survey of 12 named black teas, with total catechin contents varying from 5.6 to 47.5 mg/g (Khokhar and Magnusdothir 2002), and further evidence of high variability of commercial products found total catechins in canned green tea drinks to range from 7.5 to 346.1 μg/ml, with extreme differences in the levels of individual catechins, (-) epigallocatechin (EGC), EGCG, (-) epicatechin gallate (ECG), and (-) epicatechin (EC) (Bonoli et al. 2003). Further variability has been reported in 18 teas and one green tea supplement, with levels varying from 21.2 to 103.2 mg/g for regular teas, and 4.6 to 39.0 for decaffeinated teas. This publication showed further evidence for the importance of the catechin levels, by finding a positive correlation between levels and antioxidant activity (Henning et al. 2003).

Aucamp and coworkers compared levels of both polyphenols and caffeine in fresh tea leaves, black tea, and bottled black tea. Caffeine levels were higher in teas than the commercial bottled product, which contained miniscule levels, but even lower levels of polyphenols. Generally, fresh leaves contained higher levels of polyphenols than commercial black tea (Aucamp et al. 2000). Similar discrepancies between caffeine and polyphenol levels were found when comparing commercially canned tea drinks with green teas (Horrie et al. 1997). Bonoli et al. (2003) also found much lower levels of polyphenols in canned green tea drinks than in freshly made infusions.

4.3.7 Pycnogenol

Although Pycnogenol is a proprietary product manufactured by one individual manufacturer, variations in constituent levels and proportions of components have been found to exist in 12 commercially available products, but no assessment of the proportion of claimed levels was stated. In addition, one formulated product was found to contain extra ingredients of Citrus origin (Chen et al. 2009).

4.3.8 Resveratrol

The content of resveratrol in grape products has been studied and showed a wide variation. White grape juice is known to contain lower levels than that from red grapes, and amounts differ widely with different varieties of vines (Nikfardjam et al. 2000). The resveratrol levels of grapes were shown to depend on the variety of the plant, and this survey revealed wide-ranging levels, ranging from 98 to 1,803 μg/100 ml (Burns et al. 2002). Rossi et al. found a positive relationship between resveratrol levels and both antioxidant and anti-proliferative activity (Rossi et al. 2012).

In addition to agricultural factors, the resveratrol content of grapes is determined by the infection of the grapes by the fungus *Botrys cinerea* prior to harvesting. This results in higher resveratrol content, but this procedure cannot be standardised. As a result, manufacturers enrich the content in formulated products by the addition of resveratrol obtained from the Japanese knotweed, *Polygonum cuspidatum* (Espin et al. 2007).

4.3.9 Soy Isoflavones

Soy flour, which is the origin of a number of commercial products, has been evaluated (Romani et al. 2010). Five samples were analysed for isoflavone levels, and were found to range from 94 to 120% of the labelled claims, revealing no extreme divergence from expected levels.

Wide variations in the levels of soy isoflavones in commercial food sources have been revealed in products. Levels of individual isoflavonoid glycosides and aglycones have been reported for soymilks, tofu, and fermented soy foods, including soy sauce, miso and tempeh, and meat analogue/hamburger products – and wide variations were evident. A range of commercial examples of 12 soy foods have been investigated and found to contain widely differing levels of the major isoflavonoids – daidzein, glycetein, and genistein (Preinerstorfer and Sontag 2004).

Another detailed analysis of 12 products surveyed the levels of the three most prevalent glycosides and their aglycones, and as expected revealed extreme variations, notably from 0.4 to 57 mg/g. Where actual levels of isoflavones were claimed, the levels were found to be low (Prabhakaran et al. 2006). Detailed analysis of the individual isoflavone contents of 13 soy supplements from Southeast Asia have been reported. In addition to major differences in individual levels of isoflavones, some products had lower total levels than claimed on labels, and there was a fivefold difference in the range of this parameter over the 13 products (Prabhakaran et al. 2006).

Variability in levels in soy-based infant formulas available in the United States has been reported with contents of isoflavones ranging from 214 to 285 µg/g in dry products, and 25 to 30 µg/ml in reconstituted products, probably caused by the use of different amounts of soy isolate in product formulation. Individual isoflavones were estimated, and genistin levels ranged from 75 to 134 µg/g, and diadzin levels from 31 to 76 µg/g (Murphy et al. 1997). Further work has also shown wide variation in total isoflavone content in commercial products, with up to 200% variation between products and up to 150% variation in values in one specific brand. Large variations were evident in both isoflavone content and individual isoflavones in near-identical food sources, using similar processing conditions as was found from the analysis of 85 soymilks (Setchell and Cole 2003). Contaminants in a range of soy isoflavone formulations have been shown to contain up to 40% of levels of non-soy components, some unidentified and others present as breakdown products (Chua et al. 2004).

In addition to the data on these nine complex products, there is virtually no other comparable published data on other food sources such as pomegranate, cranberry, and cocoa.

Another interesting finding reported in one analysis of formulated soy products was that, in addition to the variability of isoflavone constituents in the 14 formulations, there was also present up to six lignans, including secoisolariciresinol, albeit at much lower levels (Penalvo et al. 2004).

4.4 Bioavailability

Available published compositional data has been collated, and shows wide variability in the quality of products available to consumers. Studies on uniformity of products between batches are now appearing, but few bioavailability studies have been published to date.

Bioavailability is dependent on a number of factors, including solubility of the raw material. A wide range of pharmaceutically styled formulations of nutraceuticals are commercially available. Tablets and capsules are widespread, but there is increasing use of more novel dosage forms, including soft gels, various controlled release preparations, chewable tablets, liquids, chewing gums, patches, dissolving strips, oral sprays, and fizzing tablets. (Lockwood 2007). The bioavailability of nutraceuticals differs widely. Parameters affecting bioavailability include the chemical form of the active (e.g., glycoside or aglycone, salt form) type of formulation, and specific manufacturing details. Data for a great number of polyphenols present in plant extracts, as opposed to pharmaceutical formulations, has been published. The bioavailability of resveratrol is extremely low, and that for many compounds such as the isoflavonoids is dependant on whether present as the glycoside or aglycone. Pomegranate is recognised as one of the most powerful sources of antioxidants in plants when tested *in vitro*, but, in reality, the extremely low bioavailability *in vivo* means that the antioxidants are not absorbed (Espin et al. 2007).

A number of studies have been carried out on the bioavailability of several types of formulated nutraceuticals, showing wide variations, some with very low levels even when present in solutions as opposed to more sophisticated formulations. In addition to modifying the stability of the active component, particularly with liquid formulations, there is some probability that the bioavailability will be affected. This has been demonstrated for a number of nutraceuticals, including coenzyme Q10 (Miles et al. 2002) and creatine (Persky et al. 2003b). The bioequivalence of four coenzyme Q10 formulated products has been studied using 180-mg doses of four products. The absolute bioavailability of coenzyme Q10 is unknown as it is strongly lipophilic, and practically insoluble in aqueous solution, and consequently has poor bioavailability. A range of products formulated with emulsifying agents and oil-based vehicles, as well as fully solubilised formulations, were studied in an attempt to improve bioavailability. Improved bioavailability was recorded for the oil suspension in soft gelatine capsule, when compared to a standard dry formulation (Weis et al. 1994). Research into the variability of 10 coenzyme Q10 products available in New Zealand showed that there was at least a fourfold variation in the increase in plasma coenzyme Q10 levels achieved by the different products, and patients showed no increase in levels with the least effective products (Molyneux et al. 2004).

The pharmacokinetic data of 17 formulated creatine products, taken from six published studies, showed a wide range in levels of quoted pharmacokinetic parameters, maximum concentration (blood concentration–time profile) (C_{max}), area under the curve (AUC), half-life ($t_{1/2}$), clearance, and the volume of distribution. Overall, these levels showed variations of the order of 100%, and even comparable data from a single study using different volunteer groups (young or elderly) exhibited variations of up to 50% (Persky et al. 2003b).

4.5 Other Indicators of Quality

Manufacturers often supply formulations in dosages not usually employed in clinical trials, and often lower, and, therefore, unless consumers are aware of the levels used for the reported effects, they may be taking dosages below the required amount.

Standard assay procedures for pharmaceuticals and nutraceuticals include not only the levels of active ingredients, but also a range of further tests to give an indication of overall quality. Examples of these include disintegration, dissolution, weight variation, friability, and hardness, for tablet formulations. Data in this area has been collated, revealing wide differences (Lockwood 2007).

4.6 Possible Contaminants in Nutraceuticals

A range of breakdown products present in nutraceutical formulations have been reviewed (Lockwood 2007). These have been found to be either synthetic intermediates, or co-occurring related constituents in nutraceuticals. A number of evaluations of levels of some of these possible contaminants have been published (Lockwood 2007).

Overall, levels of contaminants reported in isolated formulations are low. The possible presence of dioxins and polychlorinated biphenyls (PCBs) in fish oil supplements (containing n-3-fatty acids) available in the United Kingdom was investigated by the FSA, and two out of 33 branded liquid and capsule products were withdrawn from sale due to levels that could lead to ingestion of twice the tolerable daily intake (FSA 2013).

Impure creatine supplements have been shown to contain creatinine, a metabolite of creatine, present in higher concentrations than the precursor, creatine. This may have occurred as a result of improper manufacturing or breakdown of the creatine, as could another manufacturing by-product, dicyandiamide. Creatine sold in liquid forms is believed to be more liable to breakdown into creatinine within the formulation. This is an issue because creatine dosages are relatively large – often as much as 20 grams/day (ConsumerLab.Com, 2004).

Two ranges of commercial carnitine supplements from Spain were analysed, and, in addition to the problem of variable levels of L-carnitine, all products were found to be contaminated with D-carnitine, sometimes present in higher concentrations than the L-isomer (Monagas et al. 2010a, Monagas et al. 2010b).

Three commercial melatonin preparations have been analysed, and a number of contaminants found in levels up to 0.5%. Six of these contaminants were related to impurities that had been previously discovered in a sample of L-tryptophan, associated with an eosinophilia–myalgia syndrome (Philen et al. 1993). Others have been found to contain lead (at 0.5 μg/daily dose) (ConsumerLab 2004).

One survey of nutraceutical supplements used by athletes found a risk of 25% contamination with prohibited substances, including contamination of carnitine, by the Cologne doping lab (Maughan et al. 2004).

Terrorism now involves wide-ranging illegal activities, and perceived risks exist in medicines, foods, and nutraceuticals, not only in the areas of quality control and assurance, but also for covert introduction of chemical and microbiological toxins as agents of terrorism or counterfeiting (Lachance 2004). Traceability is now standard in medicine supply, using a combination of global positioning, bar/chip coding, and hazard analysis critical control point management, coupled to nanotechnology marker assays. This development will certainly follow through into foods and nutraceuticals

4.7 Safety

Selected LD50 values of a wide range of nutraceuticals and doses found to be safe in animal studies have been reviewed (Davies et al. 2005). Even allowing for species differences, these selected nutraceuticals appear very safe at therapeutic levels.

4.8 Adverse Effects

Nutraceuticals are frequently involved in basic metabolic pathways in the body, and as such are closely involved in the metabolism of other nutrients. Availability of one nutrient may impair or enhance the action of another in the immune system, as reported for nutrients such as dietary fatty acids and vitamin A (Dillard and German 2000). Decreased absorption of any prescription drug may occur with concomitant dosing with certain nutraceuticals, such as that observed with flaxseed products (Ly et al. 2002).

Table 4.6 lists the incidence of a range of adverse effects of a number of nutraceuticals from data collected by the American Association of Poison Control Centers .

The most commonly cited adverse effects were drowsiness, lethargy, and headaches. Symptoms from moderate to severe were seen in a number of entities, and the authors warned that it was difficult to identify the cause in multi-component formulations, or if the product was incompletely labelled. Table 4.6 outlines the potentially serious adverse effects of nutraceuticals

Table 4.6 Incidence of adverse effects of a number of nutraceuticals

Symptom	Nutraceutical	Incidence Cases (Single Ingredients)
Drowsiness/lethargy	Melatonin	26 (22)
Headache	Creatine	35 (16)
Peripheral numbness or weakness, possible neuropathy or ischaemia	Glucosamine/ Chondroitin	1
Coma	Melatonin	1 (1)
Ataxia	Melatonin	1 (1)
Tachycardia	Melatonin	2 (2)
Hypertension	Melatonin	1 (1)
Conduction disturbances and dysrhythmias	DHEA	1
Anaemia	Glucosamine/ chondroitin	1
Anaemia with bleeding	Melatonin	1
Anaemia with hepatotoxicity	Melatonin	1
Electrolyte abnormalities	Creatine	1
Dyspnoea/shortness of breath	Melatonin	1 (1)
Urinary retention	Creatine	1 (1)

Source: Data from Palmer et al. 2003. Reproduced with permission of Elsevier.

found in the survey (Palmer et al. 2003). Melatonin accounted for 4% of the total adverse effects reported, which is surprising, considering that herbal remedies were also included in the survey.

Patients taking S-adenosyl methionine also reported mild-to-moderate gastrointestinal complaints, which have been claimed to of as high as 20% incidence, but in addition there is a possible risk of more significant psychiatric and cardiovascular adverse effects (Fetrow and Avila 2001). Similar gastrointestinal effects are reported to occur with L-carnitine, and patients with severe renal impairment should not be given high oral doses for long periods, because of the accumulation of the metabolites trimethylamine and trimethylamine-*N*-oxide, leading to a "fishy" odour. β-carotene supplementation may cause the skin to assume a slightly yellow discoloration. Bruising, dizziness, and arthralgia have been reported, though rarely.

α-Lipoic acid has been found to cause allergic skin reactions and possible hypoglycaemia in diabetic patients as a consequence of improved glucose utilisation associated with high doses (Packer et al. 1995). Insulin auto-immune syndrome has been associated with the use of lipoic acid as a dietary supplement.

There is evidence from both mice and human studies that a particular isomer of CLA (trans-10, cis-12 conjugated linoleic acid) may induce liver hypertrophy and insulin resistance (Larsen et al. 2003). It has been reported that hepatitis can be induced by use of shark cartilage (a source of chondroitin) over a 3-week period, after one patient experienced nausea, vomiting, diarrhoea, and anorexia (Ashar and Vargo 1996). Levels of endogenous metabolites may be adversely affected by the administration of certain nutraceuticals; glucose levels may be depressed by coenzyme Q10, and levels of thyroid hormone depressed by carnitine or soy products (Harkness and Bratman 2003).

The most commonly reported adverse effects with melatonin are headache, nasopharyngitis, back pain, and arthralgia. Melatonin should not be used in individuals with auto-immune disease or hereditary galactose intolerance disorders, LAPP lactase deficiency, or glucose–galactose malabsorption (Sweetman 2011). A complex relationship between melatonin and other antioxidants *in vitro* has been reported (Medina-Navarro et al. 1999), in which melatonin was shown to exhibit pro-oxidant qualities, possibly due to the formation of secondary oxidation products, such as endoperoxides.

One placebo-controlled clinical trial of isoflavones found that they caused endometrial hyperplasia, after 5 years, in six out of 154 women. This finding, coupled with the lack of clinical consensus concerning the health benefits of the isoflavones, has resulted in the American Heart Association not recommending the use of formulated products (Espin et al. 2007).

However, to put these findings into context, carnitine, soy isoflavones, proanthocyanidins, and ß-carotene are safely ingested on a daily basis by most people.

4.9 Drug Interactions

Interactions between nutraceuticals and prescribed medicines or even other nutraceuticals are being reported. Table 4.7 shows some of these interactions that occur with medicines.

Table 4.7 shows wide-ranging interactions between nutraceuticals and medicines, and it is therefore important that physicians are aware of the nutraceutical supplementation being taken by patients. Table 4.8 lists examples of reported interactions of nutraceuticals with either other nutraceuticals or foods.

Table 4.8 shows perhaps unexpected interactions between a range of nutraceuticals and other food constituents. This situation will also require more detailed investigation in the future, as consumption of nutraceuticals increases.

Melatonin should not be used in patients with hepatic impairment, because of reports of decreased clearance of prescription medicines in such patients. Cytochrome P450 isoenzymes CYP1A1 and CYP1A2 are involved in the metabolism of melatonin, and, consequently, other drugs that inhibit or induce these isoenzymes may affect melatonin levels (Sweetman 2011).

Table 4.7 Examples of interaction of medicines with a range of nutraceuticals

Medicine	Nutraceutical	Reference
Sodium valproate	Induction of carnitine deficiency	De Vivo et al. 1998
Pivampicillin	Reduction of carnitine levels	De Vivo et al. 1998
Isotretinoin	Reduction of certain effects of carnitine deficiency	De Vivo et al. 1998
Fluvoxamine	Increased levels of serum melatonin	Hartter et al. 2000
Clomipramine	Enhanced effects of SAMe	Iruela et al. 1993
Indometacin	Reduction in prostaglandin excretion when in conjunction with n-3-fatty acids or linolenic acid	Codde et al. 1995
Warfarin	Reduced response to treatment with coenzyme Q10	Landbo and Almdal 1998
Warfarin and aspirin	Reduced anticoagulant effect with policosanol	Harkness et al. 2003
Pentylenetetrazol	Possible enhancement of effects with L-carnitine	Kelly 1998
Cimetidine	Enhanced anti-ulcer effect with policosanol	Kelly 1998; Valdes et al. 2000
Nicoumalone	Enhanced anticoagulant effect with L-carnitine	Martinez et al. 1993
Phenothiazines	Suggested interaction with GLA-induced inhibition of CYP enzymes	Williamson 2005
Phenothiazines	Seizures reported in combination with GLA	Ulbricht and Basch 2005
Nifedipine	Increased heart rate and blood pressure in combination with melatonin	Lusardi et al. 2000
Morphine	Possible potentiation of analgesia with β-carotene	Penn 1995

Table 4.8 Interactions of nutraceuticals with either other nutraceuticals or foods

Nutraceutical	Nutraceutical or Food	Reference
Lutein	Reduction of β-carotene absorption	van den Berg 1998
β-Carotene	Possible interaction with lutein/lycopene	van den Berg 1999
β-Carotene	Levels of α-tocopherol affected	Simone et al. 2002
SAMe	Reaction with foods high in tyramine	Codde et al. 1985
CLA	Effects on gene expression in combination with n-3-fatty acids	Ide 2005

Data has also been published on the effects of certain medicines on nutraceuticals, and there are instances where medicines depress levels of nutraceuticals. Levels of coenzyme Q10 have been depressed by administration of a number of medicines including acetohexamide, statins, propranolol, phenothiazine, and tricyclic antidepressants, and both carnitine and acetyl carnitine levels are reportedly depressed by valproic acid (Harkness and Bratman 2003).

4.10 Conclusions

Nutraceuticals have applications in a number of therapeutic areas, and some of them show comparable efficacy to conventionally prescribed pharmaceuticals. The quality issue includes complexity and purification of raw materials, standardisation of complex products, the presence of contaminants, and varying effects of different formulations, and requires careful consideration. The number of formulated products exhibiting extreme deviation from label claims is particularly worrying. The majority of nutraceuticals are derived from natural materials, but this does not mean that they are devoid of adverse effects, and, as outlined, a number of these have been reported. Compared to both conventional pharmaceuticals and complementary medicines, nutraceuticals show lower incidences of adverse effects and drug interactions, but this is rising with increasing use. Clear government regulation is required in order to eradicate quality problems and limit adverse effects and drug interactions.

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5

Novel Health Ingredients and Their Applications in Salad Dressings and Other Food Emulsions

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5.1 Current Developments and Emerging Trends in Food Emulsion Products

Dressings and sauces generally include mayonnaise, spoonable and pourable salad dressing, and condiment sauces (e.g., ketchup, barbecue sauce, spaghetti sauce). Food dressings vary widely in their composition, texture, and flavor. Salad dressings and mayonnaise are defined by their ingredients and oil content. Specifically, salad dressings are generally considered to be emulsified foods prepared from a vegetable oil, an acidifying ingredient of vinegar or diluted vinegar optionally mixed with citrus juice from lemon or lime, a starchy paste, and an egg yolk component containing ingredients such as liquid egg yolks, frozen egg yolk, dried egg yolk, liquid whole egg, frozen whole egg, dried whole egg, or any of these ingredients with liquid egg white or frozen egg white (Burkes et al. 1993). Salad dressings can be typically divided into three categories: semisolid, pourable, and reduced-fat dressings, with the pourable dressings consisting of two classes: "creamy dressing" (e.g., blue cheese, Ranch, and Thousand Island) and "oily dressings" (e.g.,

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Russian, Italian, and French dressings) (Ford et al. 1997). Mayonnaises, on the other hand, are generally spoonable, less flavored when compared with salad dressings, and have relatively higher oil content with or without starch (Ma and Boye 2013).

There is increasing awareness among consumers of the impact of diet on health and about the critical role that foods play in preventing certain chronic diseases and a concomitant growth in demand for healthier food products. The development of food products with reduced contents of certain food constitutes such as fat, cholesterol, salt, and sugar has been a trend in food product innovation. A new market for no and low-fat emulsion and salad dressing products presents challenges to the food industry, as substitutes for fats need to be identified. Ingredients providing calorie reduction, improved texture or flavor, or functional characteristics are potential targets for such reduced-fat food emulsion products. The use of natural ingredients that are free from priority allergens is another trend driving current market innovation. Furthermore, there continues to be considerable demand for health-enhancing, physiologically active supplemented and fortified food products with proven benefits. For example, current market trends in the Western world include fortifying or supplementing foods with healthy ingredients and nutraceuticals (such as plant fibers, β-carotene, omega-3 [n-3 or ω-3], polyunsaturated fatty acids, and polyphenols) to enhance health benefits (Jackson and Paliyath 2011).

The growing market for salad dressing is in good agreement with consumers' desire for eating "healthy food" such as fresh vegetable – that is, salads. The US regulations for foods are published each year in the Code of Food Regulations (21 CFR Part 101). The three claims frequently encountered regarding food emulsion products are "reduced," "low," and "free" as applied to calories, sodium, fat, saturated fat, and cholesterol. The Nutrition Labeling and Educational Act of 1990 standardized serving sizes for different food-emulsion-type products (Table 5.1). Due to the changes in dietary consciousness of consumers, new products are being introduced

Table 5.1 Nutrition Labeling and Educational Act claims

Claim	Definition
Reduced	A nutritionally altered product contains at least 25% less of a nutrient or 25% fewer calories than a reference food.
Low	A reference amount (and 50 g of food if reference amount is small) contains ≤40 cal, ≤140 mg of sodium, ≤3 g of fat, ≤1 g of saturated fat, and ≤15% of calories from saturated fat, or ≤20 mg of cholesterol.
Free	A serving and the reference amount contains no or physiologically inconsequential amount: <5cal, 5 mg of sodium, <0.5 g of fat, <0.5 g of saturated fat, and <0.5 g trans fatty acids, <2 mg of cholesterol, or <0.5 g of sugars.

Source: Adapted from Ford et al., 1997. Reproduced with permission of Taylor & Francis Group.

in both low-calorie and regular versions, which are available at various supermarkets and in restaurants. Instead of being labeled as weight loss products, some of these products are being advertised and promoted as nutritious, lower-calorie, and good-tasting alternatives.

5.2 Emerging and Novel Ingredients in Food Emulsion Products

5.2.1 Fat Replacers and Mimetics

The reduction in fat content in food emulsion products may lead to the loss of important quality attributes, including texture, mouthfeel, and flavor. A variety of novel fat replacers and mimetics have been developed to achieve the desired texture and mouthfeel of fat without increasing caloric content.

Fat replacers and mimetics play a crucial role in ensuring acceptance of reduced-fat food emulsion products. The most important first step in identifying and using fat replacers and fat mimetics is to understand the role and contribution of fat in the food emulsion system. In salad dressings, fats provide body (viscosity and cling), creaminess (smoothness), lubricity (slipperiness), sheen (appearance), and flavor (intensity and duration) (Stauffer 1999). In most instances, the first impression a consumer has of a product is based on the product's appearance. Consumers would assume, for example, that reduced-fat products are less rich and creamy if they lack the gloss, sheen, or opacity that are common attributes of full-fat products.

Fat replacers, or fat substitutes, are defined as substances that substitute triglycerides in reduced-fat food systems. Huyghebaert et al. (1996) summarized the basic properties of fat replacers as follows: (1) lipid structure with a modified ester bond; (2) hydrophobic character; (3) appropriate composition of short- and long-chain fatty acids on the glycerol backbone in appropriate ratios to one another; (4) reduced calorie content due to enzyme resistance; and (5) similar functionality to that of natural fat. Typical examples of fat replacers include Olestra, Salatrim, and Caprenin. Table 5.2 presents the chemical components and characteristics of a variety of fat replacers developed for use in food emulsions. These fat replacers are either indigestible to humans (e.g., Olestra) or contribute lower calorie on a per-gram basis (e.g., Saltrim and Caprenin) (Kitty 1995). However, their chemical structure and chemical properties in terms of density, refractive index, surface tension, and polarity are often not too dissimilar to the original fat. Thus, food emulsions formulated with fat replacers generally exhibit similar properties and behavior to that made with the original fat. The majority of fat replacers consist of lipid-based components (esters or polyesters) that provide no or few calories. Preparation of these fat replacers generally involves esterification

Table 5.2 The chemical components and characteristics of some fat replacers and fat mimetics developed in food emulsions

Fat replacers/ fat mimetics	Category	Chemical components and characteristics
Olestra	Fat replacers	Manufactured by chemical reaction between sugars and fatty acids, specifically, it is a compound synthesized from sucrose, which can bond with six to eight fatty acids (normal fats usually have only three fatty acids), and has similar taste and mouthfeel to fat, however, without contributing to calorie or nutritional values.
Salatrim	Fat replacers	Modification of triglycerides.
Caprenin	Fat replacers	Caprenin is a randomized triglyceride primarily comprised of caprylic (C_8:0), capric (C_{10}:0), and behenic (C_{22}: 0) acids, which has reduced energy value compared with regular fat due to the incomplete absorption of its long-chain saturated fatty acids ($C_{20}-C_{24}$).
Simplesse	Fat mimetics	Protein-based material (usually manufactured from milk protein or egg whites), it can form uniform colloid systems due to their small size and round shape.
Modified starch	Fat mimetics	Manufactured by hydrolysis or substitution, and can provide mouthfeel similar to that of fat.
Maltodextrins	Fat mimetics	Nutritive polysaccharides derived from the acid or enzymatic hydrolysis of starch (made from wheat, corn, potato, oat, or tapioca starch). It can be used to replace fat in dry form or gel form by binding and controlling water, providing humectancy, and contributing a mouthfeel similar to that of fat.
Dietary fiber	Fat mimetics	Plant polysaccharides and lignin, which are resistant to hydrolysis by digestive enzymes. The use of dietary fiber can be attributed to the enhancement of consistency and texture of the water phase in the food system. It is an inexpensive, non-caloric bulking agent for partial replacement of fat in food emulsions and salad dressings.
Microcrystalline cellulose	Fat mimetics	Can provide the creamy mouthfeel of fat in oil-in-water emulsions; provides emulsion stabilization, control of water mobility, and viscosity increase, and supports suspension of solids.
Sucrose fatty acid esters (SFE)	Fat replacers	Have a high hydrophile–lipophile balance and act as emulsifiers, texturizers, and components that provide protective coating. Manufactured by transesterification or interesterification, or by enzymatic synthesis (with the use of lipases).
Medium-chain triacylglycerols (MCTs)	Fat replacers	MCTs are triacylglycerols that have fatty acid chains with carbon lengths less than 12.

of a carbohydrate or alkyl glycosides or polyol with the fatty acids found in conventional fats (Sandrou and Arvanitoyannis 2013). For example, Olestra is a sucrose polyester comprising a mixture of hexa-, hepta-, and octa-esters synthesized by esterifying sucrose with long-chain fatty acids in the presence of suitable catalysts (Table 5.2) (Huyghebaert et al. 1996).

Fat mimetics are ingredients that have similar organoleptic and textural properties as fats but are not fats. Some typical examples of fat mimetics as well as their characteristics are presented in Table 5.2. Most fat mimetics are either protein-based or carbohydrate-based, and they are unable to fully replace the functionality of fats in foods. Protein-based fat mimetics have limited applications as fat replacers due to their flavor-binding ability. Specific examples of protein-based fat mimetics are presented in Table 5.3. Simplesse is a microparticulated protein derived from egg whites, skim milk, and whey protein, whose round shape and uniform particle size imparts unique properties such as the smoothness and richness associated with fat. Simplesse has been widely applied in a variety of food products including cakes, soups, yogurt, and mayonnaise. Carbohydrate-based fat replacers, on the other hand, include modified starch ingredients produced by hydrolyzation or substitution. Gums, dietary fiber, and microcrystalline cellulose are also sometimes used as fat mimetics (Table 5.3). Due to the large varieties of carbohydrate-containing plants on earth and the huge numbers of methods that can be applied for starch modification (such as hydrolysis, crosslinking, and microparticulation), the functional properties and the performance of these carbohydrate-based fat mimetics vary to different extents. Nevertheless, they have similar mechanisms in their role as fat mimetics, that is, stabilizing substantial quantities of water in a gel-like matrix, and increasing viscosity and body, by contributing to lubricant and flow properties and by providing a creamy and slippery mouthfeel similar to fat.

In addition to the substitution of original fat with fat replacers and mimetics, the preparation of multiple emulsions provides another option of reducing fat content in oil-in-water emulsions (McClements and Demetriades 1998). Fat content can be reduced by replacing some of the fat inside of oil droplets with small water droplets (with the same outer droplet concentration and size), which is called water-in-oil-in-water multiple emulsion (Figure 5.1). These multiple emulsions generally have similar physicochemical and sensory properties as compared to the original oil-in-water emulsion. However, a major weakness is the short-term stability of these multiple emulsions, and, as a result, the approach is not widely used in the development of reduced-fat food products.

No single fat replacer or mimetic can contribute all the desirable sensory and functional qualities of fats in reduced-fat emulsion products. The degree of cohesiveness, viscosity, dryness, juiciness, and other physical properties of low-fat emulsion are determined by a variety of factors. The judicious combination of fat replacers or mimetics coupled with the selection of

Table 5.3 Examples of fat replacers and fat mimetics in different food applications

Class of fat replacers and fat mimetics	Trade name	Functional properties	Applications
Fat mimetics			
Carbohydrate-based polydextrose	Litesse, Sta-Lite	Viscosity enhancer, thickening agent	Dairy products, sauces, salad dressings, pudding
Modified starch	Amalean I and II, N-Lite, Instant-Stellar	Gelling, thickening, stabilizing, texturizer	Dairy products, frozen desserts
Maltodextrins	CrystaLean, Maltrin, Rice-Trim, Oatrim	Gelling, thickening, stabilizing, texturizer	Dairy products, salad dressings, sauces
Dextrins	N-Oil, Stadex	Gelling, thickening, stabilizing, texturizer	Salad dressings, puddings, dairy products, soups
Gums (xanthan, guar, locus bean, carrageenan, alginates)	Kelgogel, Keltrol, Viscarin, Novagel, Jaguar, Fibrex	Water retention, texturizer, thickener, mouthfeel, stabilizer	Salad dressings, formulated foods
Pectin	Grindsted, Slendid, Splendid	Gelling, thickening, mouthfeel	Soups, sauces, dressings
Cellulose (CMC, microcrystalline cellulose)	Avicel, Cellulose gel, Methocel, Just Fiber	Water retention, texturizer, stabilizer, mouthfeel, clouding agents	Dairy products, sauces, frozen desserts, salad dressings
Protein-based fat mimetics	Simplesse, K-Blazer, Dairy-lo, Veri-lo, Power-pro, Versapro	Mouthfeel, creaminess, viscosity	Mayonnaise, salad dressings, dairy products
Others	Titanium Dioxide	Appearance	Dairy products
Fat replacers/fat substitutes			
Fat-based fat replacers	Caprenin, Olean, Benefat, Dur-Em, Dur-Lo	Mouthfeel	Confections
Combinations	Prolestra, Nutrifat, Finesse	Mouthfeel	Ice cream, salad oils, sauces, mayonnaise

Source: Adapted from American Dietetic Association Reports (1998).

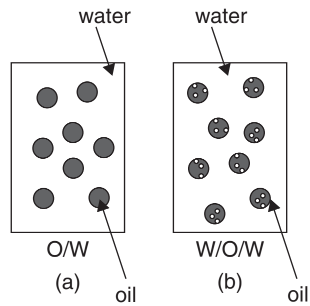


Figure 5.1 Schematic diagram of (a) oil-in-water (o/w) and (b) multiple water-in-oil-in-water (w/o/w) emulsions. Multiple emulsions (b) can be used to create a product with similar overall physicochemical properties with lower fat content in comparison with regular o/w emulsions (a). *Source*: Adapted from McClements and Demetriades, 1998.

other appropriate ingredients in the formulation (such as emulsifiers and stabilizers) are needed to obtain desirable characteristics (such as texture and organoleptic attributes) in the end product.

5.2.2 Egg Yolk Replacers

Egg yolk, as a traditional ingredient in salad dressing and mayonnaise-type food emulsions, acts as an emulsifier that decreases the interfacial tension between the oil and water phases, providing a protective coating around the emulsion droplets, which prevents aggregation. Due to the high cholesterol content in egg yolk ingredients as well as food allergy concerns, there is some demand to identify and develop appropriate and healthy alternatives to egg yolk that can provide an adequate supply of energy as well as desirable functionality.

Common ingredients that have been researched and applied to replace egg yolk in food emulsions include dairy proteins (whey protein, caseinates), vegetable proteins (pea and soya protein), phospholipids (lecithin), fat-derived emulsifiers (mono- and diglycerides, and esters of mono- and diglycerides), carbohydrate-derived emulsifiers (starch ester, sucrose ester, and polysorbates), and hydrocolloids (gum Arabic and propylene glycol alginate).

Plant and Animal Proteins Dairy proteins (including caseins, whey proteins, and their bioactive peptides) are an important category of animal proteins that have been widely utilized as emulsifiers in salad dressing products (Álvarez Cerimedo et al. 2010, Dickinson et al. 1999, Riscardo et al. 2003, Turgeon et al. 1996). Milk proteins including casein, whey, and their bioactive peptides and amino acids provide multiple health benefits and can be incorporated as value-added ingredients in a variety of food products. A number of animal, observational, and clinical studies have indicated that consumption of dairy foods may help reduce the risk of hypertension. Emerging data also suggest that specific peptides associated with casein and whey proteins may significantly lower blood pressure. Additionally, many studies provide evidence of the beneficial effects of dairy foods on body weight and fat loss (Huth et al. 2006).

Casein and α-lactalbumin (an important whey protein in bovine milk) have an isoelectric point in the range of 4.1–4.5; β-lactoglobulin (another major whey protein in bovine milk) has an isoelectric point of 5.3. The pH environment for salad dressing products varies from 3.0 to 4.5, which is close to the isoelectric point of these dairy proteins, and this may cause decreased emulsification at the oil–water interface. Selection of dairy proteins as emulsifiers in salad dressing applications must therefore be carefully made. Riscardo et al. (2003) studied the use of binary blends of egg yolk and different types of amphiphilic molecules including pea protein and sodium caseinate (which is the commercial form of caseins) in emulsion systems. Their results suggested that the rheological properties, droplet size, and physical stability of the salad dressing emulsion studied were dependent on the weight ratio of emulsifier in the binary blends. Turgeon et al. (1996) studied the stability and rheological properties of salad dressings containing peptidic fractions of whey proteins, and reported that the peptidic fractions obtained from tryptic hydrolysates produced the most stable salad dressings (over 6 months at the 1.0% and 1.5% protein level) with rheological properties similar to commercial products.

Application of animal-based ingredients in salad dressing and mayonnaise products may be of some concern for people seeking a better balance between animal-based and plant-based ingredients and products with low cholesterol levels. Plant protein extracts generally have little to no saturated fat and no cholesterol. Evidence from prospective cohort studies indicate that high consumption of plant-based foods is associated with a significantly lower risk of coronary artery disease and stroke (Hu 2003).

Soy protein is one good example of plant-based proteins that have been used extensively in food product development and may have great potential for use in salad dressing and mayonnaise products. The consumption of soy protein has been related to a variety of health benefits such as cholesterol lowering. A meta-analysis carried out by Anderson et al. (1995) suggested that consumption of soy protein (25 g/day) resulted in significant reduction in total cholesterol (9.3%), LDL cholesterol (12.9%), and triglycerides (10.5%), with a slight increase in HDL cholesterol (2.4%). In 1999, the US Food and Drug Administration (USFDA) approved a cholesterol-lowering health claim for soy proteins, which indicated that daily consumption of at least 25 g of soy protein (6.5 g soy protein per serving) as part of a diet low in saturated fats and cholesterol may help lower the risk of heart disease. Soy protein ingredients may also prevent obesity, and may have the potential to play an important role in preventing diseases such as osteoporosis and in providing protection against bowel and kidney disease (Sacks et al. 2006). They also have a good supply of the essential amino acids required by the human body when compared with other plant proteins. In addition to the major components, soybean protein also contains minor components such as isoflavones that may be beneficial for human health. About 30% of the total isoflavones are lost in the insoluble fraction during soy protein isolate production (Rickert et al. 2004). Studies carried out by Erdman and Potter (1997) suggested that consumption of 40 g of soy protein isolates containing 90 mg total isoflavones per day increased both bone mineral content and density in the lumbar spine by about 2% over a period of 6 months.

Besides soy proteins, which have been studied extensively (Diftis et al. 2005, Gao et al. 2003, Hu et al. 2003), potential applications of other plant-based proteins in dressing-type emulsions include: lupin protein (Franco et al. 1998, Papalamprou et al. 2006, Raymundo et al. 2002, Riscardo et al. 2003), pea protein (Franco et al. 2000, Raymundo et al. 2005), canola protein (Vioque et al. 2000), wheat protein (Ghoush et al. 2008), and potato protein (Calero et al. 2012). These plant proteins not only increase the nutritional value, but they also provide advantageous functional properties including good protein solubility, high emulsifying and foaming properties, as well as desirable water/fat-binding capacity and gelation property. The application of these plant proteins in foods may have valuable implications from an agricultural point of view as they help to add value to these crops.

Dietary Fiber and Resistant Starch Dietary fiber covers a class of compounds that generally include a mixture of plant carbohydrate polymers, and may be divided into two forms – insoluble dietary fiber (e.g., cellulose, hemicelluloses, and lignin) and soluble dietary fiber (e.g., β-glucans, pectin, gums, mucilages, and some hemicelluloses). Several studies have indicated that increased intake of dietary fiber can promote beneficial physiological effects, including reduced risk of coronary heart disease, diabetes, obesity, and some forms of cancer (Mann and Cummings 2009). Most commonly, dietary fiber is added into bakery products to improve freshness and modify loaf firmness. Other current applications include in jams, soups, dairy products, and meat products. Dietary fiber can impart useful functional properties to a broader range of food products by improving viscosity, texture, sensory characteristics, and shelf-life. Furthermore, food companies can take advantage of this value-added ingredient as inexpensive, non-caloric bulking agents for partial replacement of fat in food emulsions and salad dressings, as enhancers of water and oil retention, and to improve the stability of food emulsions (Elleuch et al. 2011). By applying different milling methods and fractions, the particle size of the dietary fiber can be varied, and when supplemented in food emulsions, it can impart to these food products a wide range of textures and mouthfeel. The amount and percentage of such supplementation is finite and needs to be controlled carefully during food design and processing, as improper amounts may cause undesirable change to the texture and color of the emulsion-type products.

Resistant starch (RS) is the fraction of starch that is not subjected to hydrolysis into D-glucose in the small intestine within 120 min; instead, it is fermented in the colon. RS is thus defined as a fiber component with partial or complete fermentation in the colon producing various beneficial short-chain fatty acids. Whether resistant starch should be considered as a component of dietary fiber is still a controversial issue. According to the Food and Nutrition Board of the Institute of Medicine of the National Academies, the definition of dietary fiber includes resistant starch (Institute of Medicine 2005). On the other hand, in the United Kingdom, dietary fiber includes only non-starch polysaccharides and lignin, and excludes RS (Department of Health 1991). Nevertheless, resistant starch is similar to non-starch polysaccharides based on its nutritional properties, and is similar to soluble fiber based on its physiological benefits; it does not have all the properties of soluble and insoluble fiber together (Nugent 2005). Thus, "functional fiber" might be an appropriate definition to describe resistant starch for nutritional labeling (Yue and Waring 1998). Most of the promising physiological benefits of RS in humans are closely related to their fermentation and prebiotic properties, including the improvement of glycemic and insulinemic response, improvement of blood lipid profile and bowel health, the synergistic interaction with other dietary components (e.g., dietary fibers, proteins, and lipids), increase in the absorption of micronutrients, and their potential for protection against bowel cancer (Brown 2004, Sharma et al. 2008). It appears, however, that the beneficial effect of resistant starch is more on colonic heath than on lipid and glucose metabolism (Sharma et al. 2008). Although these health benefits of resistant starch have been acknowledged for the past 30 years, they have generated a lot of interest in the last few decades in functional foods and other food applications. Functional foods containing resistant starch are mostly designed for those with special physiological or medical needs, such as individuals desiring to manage energy intake and control their weight (Brown 2004). Resistant starch has been used in breads, buns, breakfast cereals, extruded foods and snacks, pasta, noodles, biscuits, beverages, and yogurt. Examples of commercially available resistant starch ingredients include (1) raw high-amylose starch (mostly from maize) (e.g., Hylon^{R} VII and Novelose^{R} 240); (2) retrograded high-amylose starch (mostly from maize) (e.g., Novelose ^{R} 330); and (3) retrograded maltodextrins (e.g., from high-amylose starch for CrystaLean or tapioca starch, for C* Actistar) (Eliasson 2004).

Based on its properties and previous applications, RS may have potential to be used in food emulsion products such as salad dressings, although no such application studies were found in the review for this chapter. Compared with products containing traditional fiber, the fine particles and bland taste of RS in food emulsions may contribute better acceptability. RS also has desirable techno-functional properties such as swelling, viscosity increase, gel formation, and water holding capacity. Fang et al. (2012) invented a resistant starch-based emulsifier product that has high paste transparency, high freeze–thaw stability, good emulsifying property, and has been shown to have potential for use in starch-based beverages, dressings, jams, and frozen rice foods. Basman et al. (2008) found that the supplementation of RS preparations with gum resulted in a cold thickening capacity that might influence emulsion capacity and stability.

Hydrocolloids Hydrocolloids include natural and synthetic polysaccharides that are able to form gels and provide viscosity (known as *gelling agents*), or simply provide viscosity (known as *thickeners*) to food emulsion systems. In other words, hydrocolloids are stabilizers that have gelling and/or thickening effect. They influence texture and mouthfeel and can be used to control the distribution of particles in solution. Hydrocolloids are mainly long-chain, straight or branched polysaccharides that contain hydroxyl groups that can bond to water molecules. The type and number of monosaccharides and their configuration, as well as the type, number, and location of the linked groups on the polysaccharides, determine the particular properties of hydrocolloids. Using scanning electron microscopy, Tanaka and Fukuda (1976) observed that the addition of xanthan gum to French salad dressing inhibited lipid droplet fusion and extended shelf life up to 6 months.

Table 5.4 summarizes some common hydrocolloids and their unique properties and uses in salad dressings. A variety of hydrocolloids as described in the following text (including xanthan, gum arabic, propylene glycol alginate [PGA], pectin, guar gum, and cellulose) are derived from different

Table 5.4 Common hydrocolloids/polysaccharides and their use in salad dressing emulsions

Hydrocolloids/starches	Comments
Xanthan	Most widely used gum; salt, acid, and heat resistant; suspending agent; stabilizer; gelling reaction with locust bean gum and guar gum
Sodium alginate	Gels with Ca^{2+} ions; high viscosity with heat
Propylene glycol alginate	Stabilizer; emulsifier; thickener; some gelling with Ca^{2+} ions; pH tolerant
Locust bean gum	Thickener; gels with xanthan; insensitive to Ca^{2+} ions
Guar gum	Thickener; insensitive to Ca^{2+} ions; cost-effective
Gum arabic	Some emulsifier activity; thickener; stabilizer
Gum acacia	Suspending agent; forms films at interface
Starch	Thickener; retrogradation is a problem
Modified starch	Inhibits retrogradation; thickener
Microcrystalline cellulose	Adds body and mouthfeel

Source: Adapted from Ford et al., 1997. Reproduced with permission of Taylor & Francis Group.

food sources, with each having a unique chemical structure and functional properties. They can be considered for use in the production of reduced-fat dressing and mayonnaise products to improve flow behavior, texture, appearance, and, where required, organoleptic properties.

Xanthan gum is the extracellular anionic heteropolysaccharide produced by fermentation of the bacterium *Xanthomonas campestris*. Xanthan consists of pentasaccharide repeating units formed by a (1-4)-β-D-glucan backbone linked to a charged trisaccharide side chain (β-D-mannopyrannosyl-(1-4)-α-D-glucopyrannosyl-(1-2)-β-D-mannopyrannosyl-6-O-acetate) at the 3 position on alternate glucose residues (Williams and Phillips 2009). Xanthan gum exhibits pseudoplasticity and thixotropy, and xanthan gum solutions have high yield stress, making them useful for stabilizing salad dressings (Parker et al. 1995). Gum arabic is a natural exudate obtained from the stems of *Acacia senegal*. Structurally, it is a high-molecular-weight, charged heteropolysaccharide consisting of branched galactan heteropolymers. Hydrolysis results in D-galactose with lesser amounts of L-arabinose, D-glucuronic acid, and L-rhamnose, along with a small amount of 4-*O*-methyl-D-glucuronic acid (Fennema 1985, Williams and Phillips 2009). Gum arabic solutions are the least viscous of the natural food-grade polysaccharides (Fennema 1985). The structure of gum arabic comprises approximately 2% proteinaceous compounds, which are covalently linked to the polysaccharide moiety (Akiyama et al. 1984).

Propylene glycol alginate is a derivative of alginic acid with an average molecular mass ranging from 30,000 to 200,000 Daltons. It is a surface-active biopolymer that has both hydrophobic and hydrophilic groups, and could therefore cause a reduction in the surface tension at the oil–water interface (Pettitt et al. 1995, Yilmazer et al. 1991). Pectin is a naturally occurring polysaccharide that is present in the primary cell walls of almost all terrestrial plants. It is usually extracted from citric fruits and apples. Pectins are a group of heteropolysaccharides that contain at least 65% by weight of galacturonic acid-based units, which may be partially esterified with a methoxyl group. Pectins are often classified according to their degree of esterification (DE): the ones with DE of up to 50% are classified as high-methoxyl pectins (HMP), and those with DE of less than 50% DE are classified as low-methoxyl pectins (LMP) (Guimarães et al. 2008, Thakur et al. 1997).

Guar gum is a galactomannan polysaccharide that is formed by galactose and mannose molecules. It is obtained from the endosperm of the seed of *Cyamopsis tetragonolobus*. The principal backbone of guar gum is a chain of (1-4)-β-D-mannopyranosyl units, with single (1-6)-α-D-galactopyranosyl units linked to the principal chain (Casas et al. 2000, McCleary et al. 1981). Cellulose is a group of important hydrocolloids with substituted groups (the substitution is mostly done to improve its solubility). The most common type of cellulose used in reduced-fat food emulsion products are those substituted by carboxymethyl, methyl, and hydroxypropyl plus methyl groups (Ma and Boye 2013).

Most of these hydrocolloids are hydrophilic, except for gum arabic, propylene glycol alginate, and hydroxypropyl methylcellulose, which are amphiphilic and can prevent droplet aggregation by steric and/or electrostatic forces. Mixtures of hydrocolloids may act synergistically to increase viscosity or antagonistically to reduce it. Their interactions have been studied extensively in an effort to generate new functionality or to manipulate the texture and rheology of food systems, with the ultimate goal of replacing expensive polysaccharides with cheaper alternatives. The interactions between xanthan and guar gum have been studied previously (Casas et al. 2000, Tako and Nakamura 1985, Wang et al. 2002), with several types of evidence supporting the existence of intermolecular binding between xanthan and galactomannans. Sikora et al. (2008) have reviewed some interactions of different polysaccharides and their potential application in the food industry.

Several studies indicated that many hydrocolloids, including xanthan, guar gum, gum arabic, and pectin, are linked with potential health benefits on account of their role in reducing blood cholesterol levels and their prebiotic effects (Edwards and Garcia 2009). However, gums are often incorporated in foods, especially in food emulsion products, in very small amounts. For instance, guar gum is typically added to food emulsions at a level less than 1%. Health benefits of guar gum are reportedly achieved at higher levels of use (3–5%); increased amount of incorporation, on the other hand, might compromise the rheological and organoleptic properties of these food emulsions.

Besides these traditional polysaccharides, a great number of research studies have explored new sources of non-digestible polysaccharides and oligosaccharides, including those of algal origin (Goni et al. 2001, Panlasigui et al. 2003), spent brewer's yeast such as β-glucan (Worrasinchai et al. 2006), new fruits such as Chinese Jujuba (Huang et al. 2008), seeds such as flaxseed and fenugreek (Hannan et al. 2007, Stewart and Mazza 2000), leaves such as the hsian tsao leaf (Lai and Lin 2004), and nuts such as almonds (Mandalari et al. 2008). Studies on new plant and other sources of food hydrocolloids, and their potential health benefits at the concentrations at which they will be used in foods, need to be fully explored.

5.2.3 Health Ingredients

Healthy Oil Oils from marine animals and plants (such as fish oil and algae oil) are rich sources of nutritionally important omega-3 (n-3 or ω-3) long-chain polyunsaturated fatty acids (PUFA) (e.g., decosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]). Examples of sources of these bioactive compounds and their physiological effects are summarized in Table 5.5. The USFDA allowed the use of a qualified health claim for conventional foods containing EPA and DHA in relation to reduced risk of coronary heart disease (USFDA 2004). However, the high degree of unsaturation of the fatty acids (i.e., EPA and DHA) enables the n-3 PUFA-rich oil to undergo

Table 5.5 Health benefits and physiological effects of healthy oils

Health ingredients	Bioactive compounds	Health benefits and physiological effects
Fish oil	Omega-3 fatty acids (necessary for properly maintaining human and animal health)	May play a role in reducing risk of CVD Helps to improve mental function (e.g., attention deficit disorder and Alzheimer's disease) Improves visual function; anti-inflammatory effects
	DHA (decosahexaenoic acid)	A major component of brain and eye tissue
	EPA (eicosapentaenoic acid)	Anti-inflammatory properties
	ALA (α-linolenic acid)	Prevents blood clotting (cause of fatal heart attacks) and lowers low-density lipoprotein (LDL) and total cholesterol
	CLA (conjugated linolenic acid).	May improve body immune system May decrease risk of certain cancers
Olive oil	Oleic acid, phenolic constituents, and squalene	Oleic acid (a monounsaturated fatty acid) may prevent certain cancers; squalene has anticancer effects Consumption of olive oil may prevent colon and breast cancers and reduce blood pressure and low-density lipoprotein (LDL) cholesterol

Source: Adapted from Jackson and Paliyath, 2011.

rapid oxidative deterioration, which can cause problems in emulsion systems (Frankel et al. 2002). The addition of antioxidants to prevent lipid oxidation in such products is described in section 5.3.2.

Flaxseed oil contains high levels of the omega-3 fatty acid ALA (α-linolenic acid) (57%) (Oomah and Mazza 1998). This essential fatty acid may help to prevent blood clotting, which may cause fatal heart attacks (Jackson and Paliyath 2011). Olive oil is another healthy oil with a well-balanced fatty acid composition, and its nutritional characteristics have been highly appreciated. The consumption of olive oil is often linked with health benefits, including reduction in coronary heart disease and prevention of certain cancers (Jackson and Paliyath 2011, Stark and Madar 2002). Greek salad dressing is commonly formulated with virgin olive oil and lemon juice, and is a good source of phenolic compound and lipid-soluble and water-soluble vitamins (such as tocopherols, β-carotene, ascorbic acid) (Đilas et al. 2002, Jackson and Paliyath 2011, Paraskevopoulou et al. 2005). The mayonnaise and Italian salad dressing prepared with olive oil and enzymatically synthesized structured lipid from olive oil and caprylic acid showed acceptable stability and promising applications (Fomuso et al. 2001).

The oil content of salad dressings varies from 20 to 65%. Full or partial substitution of the regular oils used in salad dressings with healthier oils (such as marine oil, flaxseed oil, olive oil, etc.) could provide health benefits. In fact, food emulsions such as dressings, mayonnaise, sauces, and dips provide a good medium for the delivery of healthy oils in the diet.

Micronutrients and Others Minerals and vitamins are essential inorganic micronutrients or biochemical compounds required by the human body in small quantities. A lot of minerals, including magnesium, iron, phosphorus, potassium, sodium, and sulfur, are enzyme cofactors that control the rate of enzyme reactions and energy production within the human body. Minerals including calcium and phosphorous are also necessary for building strong bones and teeth; sodium and potassium usually function to control the level of water inside and outside cells (Anderson and Allen 1994). Vitamins are essential for proper functioning of the human body. Vitamins are generally classified as water-soluble (vitamin B_6, B_{12}, biotin, colic acid, niacin, pantothenic acid, riboflavin, thiamin, and vitamin C) or fat-soluble (vitamins A, D, E, and K). Many foods and beverages worldwide are fortified with various vitamins and minerals. The most successful examples of micronutrient fortification are fortified breads and beverages. Fortified products must be proven to be beneficial to health, safe, and must have an effective method of delivery of vitamins and minerals. The level of fortification should also be controlled carefully. For example, when vitamin A and β-carotene are consumed in excessive amounts, they can accumulate in fatty tissue.

Salad dressings fortified with soluble calcium sources comprising specific molar ratios of calcium, citrate, and malate or calcium acetate were developed by Burkes et al. (1993) as a nutritional supplement for building bones, strong teeth, and in general supplementing the daily requirement of calcium. Salad dressings are good vehicles for micronutrient fortification as they contain both an aqueous and non-aqueous phase, thus excluding the concern of insolubility of some micronutrients. However, there are several precautions that need to be considered to protect the bioactivity of these compounds. For instance, calcium supplements tend to be rather insoluble or tend to have a chalky taste or mouthfeel, which are organoleptically unacceptable. Calcium carbonate ($CaCO_3$) is a preferred source of calcium, since this calcium source leads to the greatest and most rapid initial solubilization and causes the least amount of off-flavor generation. Calcium hydroxide ($Ca(OH)_2$) and calcium oxide (CaO) are also acceptable calcium sources. The weight ratio of total acids to calcium added in the solution is typically from about 0.5 to 12. Some micronutrient blends, such as mixtures of iron and calcium, tend to interact with each other, which can affect their nutritional bioavailability.

In some instances, encapsulation may be applied to protect the bioactivity of the micronutrients and to ensure the quality of the final products. Thiamine, for example, has an extremely strong, yeasty, fishy odor, which has limited its uses in many food products. Encapsulation provides a method whereby thiamine can be included in a food matrix without negatively impacting odor and taste in both aqueous and non-aqueous food systems, and yet remain bioavailable. Encapsulation also ensures that the product has a broad range of uses, such as under extended storage conditions when temperature is high. When used in salad dressing products, some vitamins may not remain stable under the acidic pH environment, and other components may also interact with, and interfere with the absorption of, some minerals such as iron and calcium. Some vitamins – for example, vitamin C (ascorbic acid), which acts as an antioxidant – tend to decompose over time in food systems. Vitamin A and its precursor, β-carotene, and riboflavin also tended to degrade over time. Microencapsulation can be very helpful under these conditions for micronutrients that have properties incompatible with the food system or which easily degrade. Encapsulation, as proposed by Hall and Pondell (1978), involves fluidization of at least one micronutrient (which may include potassium citrate, magnesium phosphate, magnesium oxide, ferrous sulfate, gluconate, and thimine) and contacting the suspended micronutrient with a solution comprising of ethyl cellulose, distilled propylene glycol mono-ester, acetylated mono-glycerides, ethanol, etc. Further information on microencapsulation is provided in Chapter 11 in this book.

5.3 Factors Influencing Physical Characteristics of Salad Dressings and Other Food Emulsions

5.3.1 Rheological Properties

Most food emulsions including dressings and sauces exhibit viscoelastic properties. Specifically, mayonnaise and spoonable salad dressings show viscoelastic properties with a yield stress. Pourable salad dressing including Thousand Island dressing and French salad dressings are generally more viscous than elastic during flow; however, they exhibit varying degrees of thixotropy with a yield stress (Ford et al. 1997). Figure 5.2 gives the relationship between shear rates and the relevant viscosity and the corresponding behavior of food emulsions during different processes. In general, multiple senses are involved in the assessment of rheological and textural properties of food emulsions. For example, the slowness with which a food emulsion (e.g., salad dressing, ketchup, and other sauces) pours out of a bottle gives a visual assessment of the product's thickness (textural attributes) and viscosity (rheological attributes); attributes such as cling and mouthfeel also provide consumer perceptions of rheology and texture (Ford et al. 1997).

Reducing the oil content of dressing-type and mayonnaise-type emulsions can lead to a significant decrease in viscous and elastic properties,

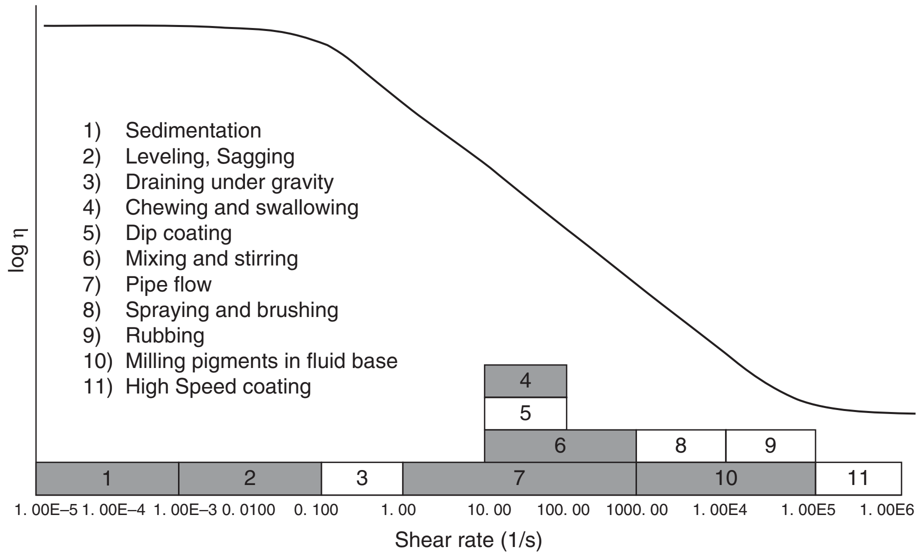


Figure 5.2 Shear rates and corresponding behaviors for different processes.

as reported extensively in previous studies (Gladwell et al. 1986, Ma and Barbosa-Cánovas 1995a, Ma and Barbosa-Canovas 1995b, Peressini et al. 1998, Wang et al. 2010), possibly because there is a less compact network formed when the fat content is decreased.

Hydrocolloids/gums and stabilizers typically have non-Newtonian rheological properties and can impart non-Newtonian characteristics to food emulsions, even when the concentration of the dispersed phase is relatively low. Different combinations of xanthan gum with gum arabic or propylene glycol alginate when applied in salad-dressing-type emulsions have shown positive effects on creaming and rheological behavior, the rate of oil droplet coalescence, and sensory properties. The effects of adding a variety of thickeners, emulsifier blends, mixed gums, fat replacers, and stabilizing agents on the rheological and textural properties of different emulsion systems have been extensively reviewed by Sikora et al. (2008).

5.3.2 Emulsion Stability

Food emulsion stability is a relative term, as food emulsions are thermodynamically unstable food products, and, given enough time, undergo phase separation. The half-life of food emulsions may range from seconds, for a product such as Italian dressing, to years, for products such as mayonnaise. Some emulsions such as mayonnaise are designed to be stable against coalescence, while others such as pourable salad dressings are formulated to give maximum stability against creaming (Ford et al. 1997). Indeed, food emulsions such as dressings and sauces with less oil and/or less gums and stabilizers can be formulated by causing controlled aggregation as aggregation leads to increased rheological properties that can provide cost-saving opportunities (i.e., stable against creaming and coalescence) (Ford et al. 1997). Despite these different requirements, all food emulsion products share a common need, which is to maintain the emulsion integrity during processing, packing, transportation, storage, consumer preparation, and consumption. Destabilization of food emulsions not only can lead to undesirable changes in appearance, but also may result in the release of flavor and have a negative impact on mouthfeel.

Three mechanisms are generally involved during the stabilization of food emulsions, that is, electrostatic interactions, steric effects, and particle stabilization. The addition of emulsifiers protects droplets from coalescence. Small emulsifiers are mostly used to impart transient stability, whereas large-molecule surfactants function best at long-term stability. The addition of gums and starches to food emulsions increases the chances of aggregation either through bridging or depletion mechanisms; they can also stabilize food emulsions against creaming by decreasing the creaming velocity when viscosity of the continuous phase is proportionally increased. Low-fat and fat-free dressings are mostly produced by the addition of various fat-mimetics and stabilizers. Emulsion stability is not regarded as a major concern when the oil content is dramatically reduced in such reduced-fat semisolid dressings and creamy dressings (i.e., pourable dressing). However, it can be a challenge to maintain low amounts of oil in a fully dispersed state in lower-viscosity pourable dressing types (i.e., oily dressing).

The changes that occur during the normal aging process of emulsions, including oxidation and hydrolysis, are also of primary importance in emulsion stability. Lipid oxidation is especially of great concern, as it may result in the development of undesirable "off-flavor" (i.e., rancidity) and potentially toxic reaction products. The mechanisms of lipid oxidation in bulk fats and in emulsified fats are significantly different. In emulsified fat products such as salad dressing and mayonnaise, the organization of the lipid molecules within the system and their interactions with other food components can have a great impact on their susceptibility to lipid oxidation.

As mentioned earlier, n-3 PUFA enriched oils when not protected can undergo rapid oxidative deterioration. Extensive studies have been carried out on the impact of various factors on the oxidative stability of oil-in-water emulsion products, including the chemical structure of lipids, oxygen concentration, interfacial properties, antioxidants, droplet characteristics (size and concentration), interactions with the aqueous-phase components (salts, sugars, polysaccharides, and proteins), and the presence of prooxidants (e.g., transition metal impurities) (McClements and Decker 2000).

The addition of antioxidants is critical for the retardation of lipid oxidation. Antioxidants commonly used to protect food emulsions against lipid oxidation generally include: (1) tocopherols, which are lipid-soluble and recognized as efficient chain-breaking antioxidants on the basis of their radical scavenging potential; (2) metal chelators, which are represented mainly by ethylenediaminetetraacetate (EDTA), which can efficiently chelate transition metals present in food emulsion matrices; (3) ascorbic acid or ascorbyl palmitate, which function as antioxidants based on several mechanisms, including singlet oxygen quenching, metal chelation, and free-radical scavenging potential (Let et al. 2007); and (4) other antioxidants, including gallic acid and propyl gallate, etc. The polarity and solubility of an antioxidant determine the actual location of the antioxidant in the food matrix, which again determines the antioxdative efficacy of the antioxidant. Thus, antioxidant mechanisms can vary significantly in different types of food emulsion matrices. Let et al. (2007) studied the oxidative stability of fish-oil-enriched salad dressing. Their results suggested that EDTA (10 and 50 $\mu g\ g^{-1}$ of dressing) was the most efficient single antioxidant, and the overall peroxide values and volatiles were reduced by about 70% and 77–86%, respectively. The combination of three antioxidants, including γ-tocopherol, EDTA, and ascorbyl palmitate, completely inhibited lipid oxidation of salad dressings during storage. On the other hand, a mixture of α-, β-, γ-, and δ-tocopherol isomers showed limited efficiency as antioxidants in fish-oil-enriched mayonnaise (Jacobsen et al. 2000, Jacobsen et al. 2001). Among these tocopherol isomers, γ- and δ-tocopherols have exhibited higher ability than α-tocopherol in inhibiting the autoxidation of 10% rapeseed oil emulsion (Wagner et al. 2004). In a fish-oil-enriched milk product (PV 0.7 $mequiv\ kg^{-1}$), Let et al. (2005) found that the antioxidative performance of ascorbyl palmitate was better than other products studied, and neither EDTA nor a combination of α-, and γ-tocopherol protected the milk emulsion from oxidation. The disparity in the results sometimes obtained in different studies suggests that the oxidation mechanisms, and the antioxidant efficacies in different food matrices, are not only affected by the solubility and polarity of antioxidants, but also by the particular environment, including the composition of the food emulsions (emulsifier and metal ions) as well as the pH.

Polysaccharides have been shown to retard lipid oxidation in some oil-in-water emulsions (Shimada et al. 1992, Shimada et al. 1994, Shimada et al. 1996), owing to (1) the increased viscosity of the aqueous phase induced by the addition of polysaccharide, which inhibits oxygen diffusion and slows down the movement of oil droplets; and (2) the metal-ion-chelating ability of polysaccharides (Paraskevopoulou et al. 2007). A study conducted by Paraskevopoulou et al. (2007) confirmed that the inclusion of polysaccharides (such as gum arabic and propylene glycol alginate) in food systems can inhibit lipid oxidation. Gum arabic, in particular, showed the highest ability to suppress oxidation, owing to its better surface-active properties.

5.3.3 Flavor

Fat plays an important role in the flavor perception of foods, including temporal profile, flavor impact, perception of flavor notes, duration of the perception, and the order of their occurrence (Hatchwell 1996, Vroom et al. 1996). The solubility and hydrophobicity of flavoring agents determine how and when these ingredients will be tasted. When fat is fully or partially replaced, the intensity of the initial taste and the fading flavor would be affected greatly. For example, reduced-fat dressings generally have a more intense initial taste and a more rapidly fading flavor, and they also tend to leave only the acidity of vinegar on the palate. When other texturizing and nutraceutical ingredients are added to food emulsions, the interactions between the flavor compounds with these added ingredients (protein and carbohydrates, in most cases) are generally different from the binding between flavors with fat. For example, the use of carbohydrate-based fat mimetics in food emulsions might have a profound influence on the final flavor profile of the products, either through flavor masking and/or flavor contribution. Starch-based and cellulose-based fat mimetics tend to decrease flavor intensity, while often adding a slight flavor of their own. This, however, could be beneficial when the intent is to mask unwanted flavor. Similarly, protein-based fat mimetics can chemically bind to some flavor compounds and cause a decrease in the intensity of the overall flavor or contribute to off-flavor (depending on the type of product) when applied in food emulsion products. Gum-based fat mimetics, on the other hand, do not generally influence the overall flavor or mask any flavors or taste (Lucca and Tepper 1994).

Besides the effect of binding and interaction, the partitioning of the volatile hydrocarbon flavor compounds is another important factor that influences the overall flavor. The partitioning of volatiles can be changed dramatically when an increased amount of hydrophilic molecules of starch, gum, fat mimetics/replacers, and other nutraceutical ingredients are added to food emulsions. This could cause a significant impact on the flavor perceived by consumers, in terms of flavor intensity perception and the flavor release rates. The production of volatiles in an oil-in-water emulsion system using purge-and-trap dynamic headspace/gas chromatography was studied by Jo and Ahn (1999). They showed that the amount of volatiles released from oil emulsions was negatively correlated with fat content; additionally, the production of volatiles was not only influenced by the polarity and partition coefficients of the volatile compounds – the characteristics of the medium in the food system also determined the overall flavor profile. Similar observation was reported by Guinard et al. (2002), who found that a reduction in fat content resulted in more intense flavor release, that is, the flavor release of garlic, pepper, and sourness decreased in the high-fat salad dressings due to the thickness of the sample in comparison with the low-fat emulsion.

5.4 Novel Food Regulations of Salad Dressing and Mayonnaise Products

According to the USFDA (2013), salad dressing must contain at least 30% vegetable oil and the equivalent of 4% liquid egg yolk. Health Canada regulations require that mayonnaise contain at least 65% vegetable oil, and that French dressing and other salad dressings contain at least 35% vegetable oil (Health Canada 2012). Commercially available reduced-fat dressings or mayonnaise usually contain 3 g of fat or less per serving. Fat-free products contain no fat or only a physiologically inconsequential amount (<0.5 g fat) per serving (Table 5.1). Although the general trend in developing dressings with reduced-oil content and plant-based dressing products with additional health ingredients responds to current market demands, existing legislation is insufficient to regulate the composition of these products. Legislative concerns, cost consideration, technical challenges during application, and (sometimes) unacceptable sensory properties of fat replacers have somewhat restricted their widespread use (Sandrou and Arvanitoyannis 2013). For example, Olestra inhibits the absorption of some vitamins and other nutrients. Although the USFDA has approved its use in food products, they also demand special labeling on food products containing Olestra, in order to inform consumers regarding the side effects associated with Olestra. While some fat replacers are currently being used in food production, others are still waiting for USFDA approval.

Regarding hydrocolloid use in salad dressings, Canada's Food and Drug Regulations provide no provisions as to the maximum permitted levels of different gums in formulated dressing products, which means that their use is governed by good manufacturing practices (GMPs) (Health Canada 2012). Recently, permission to use gum arabic modified with octenyl succinic anhydride (OSA) as an emulsifier was assessed using Health Canada guidelines, and the maximum-level-permitted use of 1% was approved in French dressing and salad dressing (Health Canada Amends 2013). No related regulations regarding the maximum levels of use for these gums in salad dressings and mayonnaise products are provided in the USFDA (2013) and European Union (EU) legislations (EEC 1992). Further information and review on this subject is clearly needed.

5.5 Processing of Salad Dressings and Other Food Emulsion Products

The following considerations are of importance during the processing of food emulsion products: (1) process type (batch or continuous); (2) selection of emulsification devices; (3) order of addition of ingredients; (4) process temperature; and (4) process shear rates.

The process type (i.e., whether a batch or a continuous process should be chosen) is determined by various factors, including run-time length, the space, the frequency of manual ingredient addition, as well as the capital issue. A batch process type is usually used when the process has a high number of product changeovers and a significant number of ingredient additions. On the other hand, processes with long run times are often designed as continuous processes that reduce labor and space requirements (Ford et al. 1997). In most cases, a coarse emulsion is first prepared by premixing the emulsion ingredients, which have been dosed separately prior to feeding the coarse emulsion into the homogenization devices for finer emulsification. A mixing tank is often used before applying the mix to the shearing device in the batch process. With the continuous process, the oil and aqueous streams are joined and sheared via inline mixers to form the coarse emulsion prior to feeding into the final emulsification device (Ford et al. 1997).

5.5.1 Homogenization Device Selection

Homogenization is a critical step in converting two immiscible liquids into a food emulsion or to reduce the droplet size in an existing emulsion. Shear, turbulence, and cavitation alone or in combination are generally involved, depending on the specific homogenization devices applied (Ma and Boye 2013). The energy imparted also varies with the targeted type of product, and the desired droplet size is of primary importance. The characteristics of different homogenization devices are discussed in the text that follows.

Rotor–Stator Mixers Rotors and stators generate the mixing energy, shear and elongational stresses, turbulence, and cavitation, which ensure mixing and particle size reduction during emulsion production (Paul et al. 2004).

The configuration of a commonly used rotor–stator mixer is shown in Figure 5.3(a). As the rotor blades pass each port in the stationary stator at very high velocity, the raw materials of the emulsion are expelled and forced into the surrounding mixture due to the high pressure in front of the blades, while the low pressure behind the blades pulls the fluid back through. As a result, a continuous and vigorous flow is generated and recirculated as more materials are drawn into the system (Utomo et al. 2009). At the same time, large particles are physically ground and hydraulically sheared. The particle size of the emulsion can be reduced to a threshold of about 4–10 μm with a rotor–stator mixer. These devices are popular in food research laboratories and in the food industry at large, since they can be used in process vessels, in-line between vessels, or in a recycle loop (Paul et al. 2004). However,

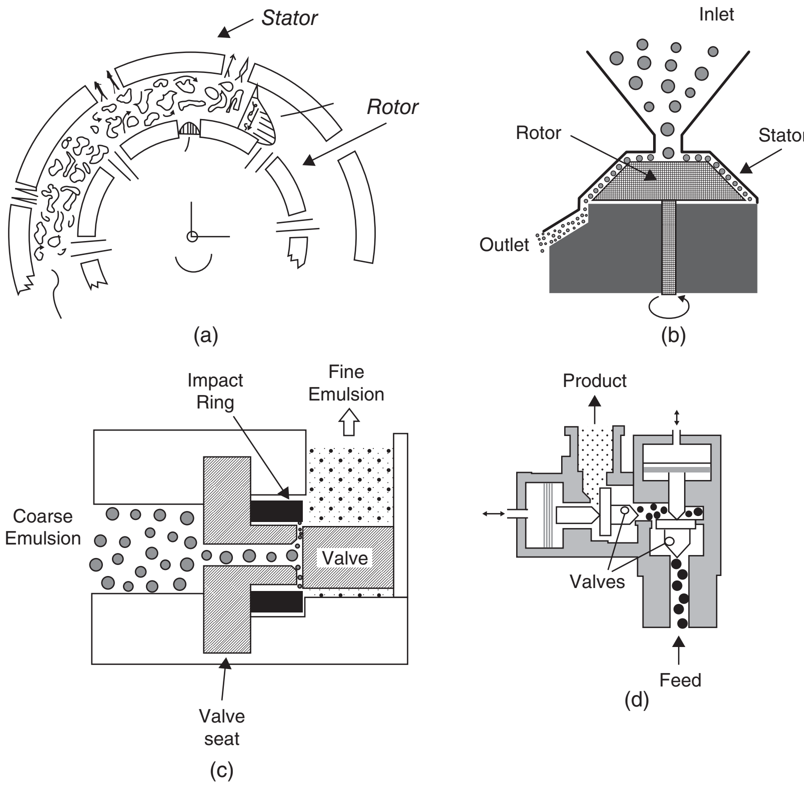


Figure 5.3 (a) Schematic representation of the hydrodynamics of a rotor–stator mixing device. *Source*: Adapted from Atiemo-Obeng VA & Calabrese RV 2004. Rotor-stator mixing devices. In: *Handbook of Industrial Mixing: Science and Practice* (Vol. 1), Paul EL, Atiemo-Obeng VA, and Kresta SM (eds). Reproduced with permission of Wiley. (b) Schematic of a colloidal mill. *Source*: Adapted from McClements 2005. Reproduced with permission of Taylor & Francis Group. (c) Schematic representation of a high-pressure one-stage valve homogenizer. *Source*: Adapted from McClements 2005. Reproduced with permission of Taylor & Francis Group. (d) Hydraulic two-stage pressure homogenizing valve (Courtesy of APC Crepaco Inc.). *Source*: Fellows 1996. *Food Processing Technology: Principles and Practice*. UK: Woodhead Publishing Series in Food Science. Reproduced with permission of Elsevier.

operation in an open vessel (e.g., Ultra Turrax and Polytron homogenizers) typically reveals the disadvantages of the system – that is, as both the viscosity of the materials and vessel size increase, it is difficult to maintain adequate flow, and this may result in unevenly distributed particle sizes. Furthermore, uneven distribution of heat within the vessel may create zones of localized heat, leading to thermal degradation. In some cases, such as in large vessels, the rotor–stator mixer must be fitted with an auxiliary impeller in order to create circulation flow.

Ultra Turrax and Polytron PT homogenizers as well as colloid mills are generally based on the rotor–stator design principle. These devices are commonly used in the manufacture of salad dressing and mayonnaise products with medium and high viscosity. They are available in a wide range of power ratings and sizes designed to handle different sample sizes. The shear rates of rotor–stator mixers generally range from 20,000 to 100,000 s^{-1}. Choosing the shear rate and the appropriate dispersing head are quite important for producing a homogeneous emulsion with a maximum reduction in particle size. The particle size of a reduced fat dressing and a mayonnaise emulsion is determined by the nature of the disruptive forces and the flow conditions (i.e., laminar, turbulent, or cavitational flow) generated inside the different types of homogenizers (McClements 2005). The three types of mixers mentioned earlier differ in their geometry, design configuration, and characteristic lengths. Stator geometries consist of slotted, disintegrating, fine emulsor, square hole, and standard emulsor. Differences in design configuration include the number of teeth, tooth-to-tooth spacing, number of stages, and percent open area. Different characteristic lengths include rotor diameter, the gap length between the rotor and stator, and the openings through which fluid jets exit the mixing head (Paul et al. 2004). The flow behavior and the recirculation pattern differ among the Ultra-Turrax, the Polytron, and the colloid mill, although these devices are all based on the same principles.

Franco et al. (1998) studied how emulsion characteristics were affected by the Ultra-Turrax homogenizer at different agitation speeds (8,000–20,500 rpm) and emulsification times (3–10 min). In general, an increase in the energy input decreased the mean droplet diameter and increased the rheological and textural parameters of the emulsions. The speed and duration used with different types of homogenizers as reported in previous studies are summarized in Table 5.6.

The colloid mill is a conical device with a hopper that directs the mixture into the grinding gap between the rotor and stator (Figure 5.3b). It is commonly used in pilot plant and industrial production, and the liquids to be homogenized are fed in the form of a coarse emulsion. The mill exploits the simple shear flow conditions associated with laminar flow. As shown in Table 5.6, various gap distances and flow rates have been used to produce emulsions with desired size distribution and physicochemical properties. The gap between the rotor and the stator (Figure 5.3b) can generally be adjusted from about 50 to 1,000 μm to change the intensity of the shear stresses, and the flow rate can be varied between about 4 and 20,000 Lh^{-1} (McClements 2005). An emulsion with a minimum droplet size of 1 μm can be produced by using colloid mill homogenizers. Since materials can be continuously fed into a colloid mill, using it makes it possible to avoid issues such as the uneven and inadequate flow that occur in Ultra-Turrax and Polytron homogenizers.

Table 5.6 Examples of equipment type and processing conditions used in the production of salad dressing and mayonnaise-type emulsions

Number	Homogenization techniques	Speed and time duration	Reference
1	Ultra Turrax T-25	8,000 rpm for 5 min	Sun et al. 2007
2	Ultra Turrax T-8 (pre-homogenization) Ultrasonic liquid processing (final homogenization)	20,000 rpm for 1 min (pre-homogenization) 20 min (final homogenization)	Álvarez Cerimedo et al. 2010
3	Two-stage high-pressure valve homogenizer	270 bar (first stage) 340 bar (second stage)	Singh et al. 2003
4	Pilot plant colloidal mill	2,830 rpm for 5 min	Riscardo et al. 2003
5	Waring blender (pre-homogenization) Colloid mill (final homogenization)	High speed for 120 s with gap setting of 0.2 mm (pre)	Stewart and Mazza 2000
6	Hand-held homogenizer (pre-homogenization) Two-stage high-pressure valve homogenizer (final homogenization)	High speed for 2 min (pre) Four times at 5,000 psi	Hu et al. 2003
7	Ultra Turrax T-50	5,000 rpm for 7 min (oil was slowly added during the first 3 min)	Dolz et al. 2008
8	Ultra Turrax T 50	6,000 rpm for 7 min	Romero et al. 2009
9	Pilot plant colloidal mill	Rotation speed at 2,830 rpm with rotor–stator gap of 1 mm for 5min	Martínez et al. 2007
10	Rotor stator turbine	6,000 rpm for 3.5 min	Moros et al. 2002
11	Ultra-Turrax T-50	5,000 and 8,000 rpm for 3–5 min	Franco et al. 1995
12	Pilot plant colloidal mill	2,830 rpm for 3, 5, or 10 min (with a rotor–stator distance of 1 mm)	Franco et al. 1995
13	Propeller-type mechanical stirrer (pre-homogenization) Ultra-Turrax T-50 (final homogenization)	8,000 rpm, 9,500 rpm, 13,500 rpm, 20,500 rpm, and 24,000 rpm	Paraskevopoulou et al. 2007
14	Ultra Turrax T-25	8,000–20,500 rpm for 3–10 min	Franco et al. 1998
15	Emulsor high-pressure homogenizer	Pressure was kept at 3–4 $\times 10^3$ kPa	Wendin and Hall 2001
16	High shear mixer (pre-homogenization) Two-stage high-pressure homogenizer (final homogenization)	2,500–10,000 psi (first stage of high-pressure homogenization); 500 psi (second stage of high-pressure homogenization)	Breitbart et al. 2000

High-Speed Blenders High-speed blenders, such as a Waring blender, turbines, and propeller-type stirrers, are common devices used in laboratories and in the food industry. The ingredients are usually agitated by a stirrer that rotates at high speed; the rapid rotation of the stirrer generates a combination of longitudinal, rotational, and radial velocity gradients in the liquids. There are different types of high-speed blenders, which vary in the design of the stirrers. Blades (which may be flat, angled/pitched, or curved), propellers, and turbines are some of the common stirrers used (Fellows 1996). High-speed blenders are able to produce emulsions of low or intermediate viscosity with a minimum droplet size of about 2 μm (McClements 2005, Sugiura et al. 2002).

High-Pressure Valve System High-pressure homogenization, often referred to as *secondary homogenization*, is a process used to produce finely dispersed emulsions. A coarse emulsion is usually formed with a high-speed blender or a rotor–stator mixer, and then its particle size is further reduced in the high-pressure system. The droplet size of emulsions is dependent on the degree of stress applied, which is affected by the inertia, shear, and expansion forces in turbulent and laminar flow during homogenization (McKenna 2003). Cavitation (i.e., collapse of air bubbles) often occurs in the emulsion system under high pressure when the materials are subjected to rapid changes in pressure (at $10{,}000–70{,}000 \times 10^3$ Pa) (McClements 2005). Examples of high-pressure valve homogenizer configurations are presented in Figure 5.3 (c, d). The system has a pump that can pull the coarse emulsion into a chamber and then force it through a narrow valve in which the sample experiences a combination of intense shear, cavitational, and turbulent flow conditions (Phipps 1985). An instantaneous drop in velocity also occurs as the samples emerge from the valve, and these extreme conditions of turbulence produce powerful shearing forces, which disrupt the droplets (Fellows 1996). Valves in the high-pressure systems have different designs, such as a one-stage spring-loaded valve (Figure 5.3c) and a two consecutive valves (Figure 5.3d). The latter is often used in the two-stage homogenization process: the droplets are broken up during the first stage, and the flocculations are disrupted during the second stage (Phipps 1985). The high-pressure homogenizer is most suitable for producing low- and intermediate-viscosity emulsions with a minimum droplet size of about 0.1 μm.

Extruders Extrusion is basically a process of shaping and restructuring food by forcing the material through a restriction or die. Extruders have been used for a variety of purposes, including cooking, forming, mixing, texturizing, and shaping food products under conditions that promote quality retention, high productivity, and low cost (Riaz 2000). Materials are fed continuously into an inlet hopper that rests on top of the barrel of the extruder, and are transported forward by the rotation of the screw in the barrel. As the extrudate reaches the die, the pressure increases. The pressure is needed in order to propel the extrudate through the die orifice; the material is shaped into the finished product upon exiting the die. A continuous extruder can be either single-screw or multi-screw in design.

The authors found very little published work on the application of extruders in the production of salad dressing and mayonnaise-type emulsions. However, the potential exists. Several factors and conditions would need to be considered carefully in setting up the processes and conditions for extrusion, in order to maintain desirable nutritional quality, texture, and flavor of the final products. Firstly, ingredients would likely need to be pre-homogenized before extrusion, since there is a major concern that oil might be lost as free oil during extrusion at the die. Secondly, the selection of extruder type and die conformation will be important in determining the quality of the final products. Different types of extruders have their own specific operating ranges, which will need to be respected in selecting operating conditions. In the case of emulsified reduced-fat/reduced-cholesterol dressing products, a twin-screw extruder would likely be an appropriate choice as it can handle viscous, oily, sticky, and wet materials. In addition, twin-screw extruders can handle fat levels of up to 25%. Thirdly, the rate at which the ingredients are introduced into the extruder, together with the extruder processing rate, will directly determine the uniformity and particle size distribution of the sample, and therefore the texture and mouthfeel of the mixed products. Fourthly, the temperature and flow rate of the thermal or cooling circulating fluid in the barrel jackets, along with the moisture and pressure control in the extruder, will be important in determining the processing conditions for the emulsified dressing products. Temperatures would need to be kept at ambient levels or <50 °C by circulating cooling water through the jackets of the extruder barrel to remove the heat generated during extrusion (Riaz 2000). The right type of extruder used under appropriate conditions could provide a promising potential approach for the production of emulsified matrices, such as salad dressings and mayonnaise products.

In general, the type of emulsification equipment used and the conditions applied have marked impacts on the microstructure and particle size distribution of the resulting dressing emulsions, and therefore they affect the overall quality, including appearance, rheology, texture, and sensory properties. The choice of a homogenization device may be driven by the formulation – for example, the volume or amount of sample, the nature of the starting materials, the desired physicochemical properties and overall quality attributes of the final products, as well as the cost and the operating context (i.e., laboratory, pilot plant, or industrial food processing). Food emulsions formulated with slow emulsifiers (e.g., large protein molecules) and high oil levels are more suited to equipment that provide sufficient residence time to allow for droplet stabilization. On the other hand, emulsion products containing fast emulsifiers (e.g., small molecule surfactants) and low fat levels are more suitable to be mixed in equipment that provide significant droplet size reduction with very little residence time in the unit (Ford et al. 1997).

5.5.2 Desired Attributes of Food Emulsion Products

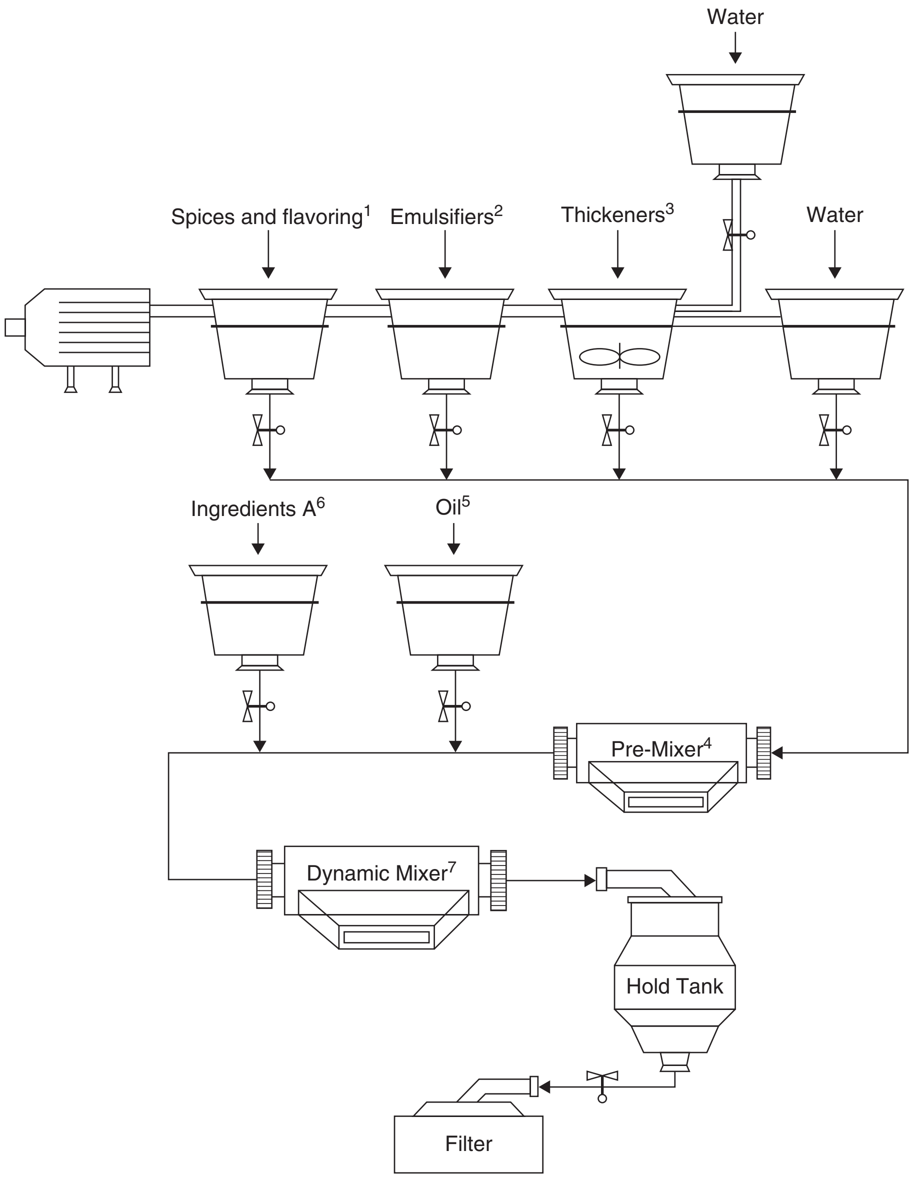
The primary objective when processing salad-dressing-type emulsions is to produce a uniform, physically stable product with desired textural attributes. With the growing trends of fortifying and supplementing food emulsions with novel and healthy ingredients, additional precautions may need to be taken to protect the bioactivity and health attributes of these bioactive components.

For salad dressing and other oil-based sauces, minimizing lipid oxidation is of primary importance during processing and packaging. For food emulsions enriched or supplemented with healthy oils such as fish and algae oil, the use of sequestrants and antioxidants are essential to prevent rancidity and to prolong the product shelf life. In addition, common methods such as processing in an inert-gas environment and the use of barrier packaging material to displace oxygen from the product may also be necessary to prevent lipid oxidation. The impact of low oxygen pressure on the kinetics of lipid oxidation in linoleic acid oil-in-water emulsions stabilized by Tween 20 was studied previously (Marcuse and Fredriksson 1968, Marcuse and Fredriksson 1969). The authors found that, at high concentrations of oxygen, the rate of oxygen diffusion was much faster than the rate of lipid oxidation; the diffusion, thus, was not limiting. Lipid oxidation was also found to be very sensitive to the speed at which

Figure 5.4 The proposed schematic diagram for the industrial production of regular salad dressings and salad dressings containing health ingredients. [1]Spices and flavorings include any or all of the followings: vinegar, citric and/or malic acids, salt, and spices (e.g., ground mustard or turmeric powder, onion or green and red peppers). Certain minerals (such as calcium and iron) could be added here in this step in micronutrients-fortified salad dressings. [2]Emulsifiers include any or all of the followings: macromolecules such as egg yolk, or plant-based proteins (soybean, lupin, and pea) and other animal-based proteins (casein and whey), small-molecule emulsifiers (such as Tween 20, Spans, or fatty acids) and phospholipids (egg or soy lecithin), etc. [3]Thickeners include any traditional gum or gum combinations or those derived from new sources (fruits and nuts, and other plant sources), pre-cooked starch, or modified starch paste. The water device above is for pre-hydration of the thickeners. [4]Pre-mixer is for premixing aqueous ingredients that have been dosed separately prior to feeding into the homogenization devices with oil for fine emulsification. Certain vitamins (such as β-carotene and riboflavin) could also be added here, together with sugars in this step in salad dressings fortified with micronutrients. [5]Oil can be regular oil including soybean, canola, and sunflower oil, or it can be partially or fully replaced by fat replacers or fat mimetics. [6]Ingredient A includes any or all of the followings: antioxidants, phytochemicals, and encapsulated micronutrients. [7]Dynamic mixer is the final emulsification device that can be chosen depending on the desirable properties of the targeted products and the nature of the starting materials.

the reaction vessel was shaken and also to the temperature in the low-oxygen system (Coupland and McClements 1996). Therefore, when the emulsification device causes an elevation in temperature, a cooling system is often necessary during the final product processing of food emulsions, especially when n-3 PUFA-rich oil is used in formulating the salad dressing products.

As shown in Figure 5.4, the components of oil should be added at the last step before homogenization, particularly when n-3 PUFA-rich oil is



used in the formulation of salad dressings, which can reduce the amount of stresses applied. Furthermore, contact between n-3 PUFA oil and potential prooxidative compounds in food emulsions is reduced during processing by adding oil in an already stabilized pre-emulsion at the final step of processing. Antioxidants should also be incorporated at the last step (Figure 5.4). Some non-polar antioxidants (such as α-tocopherol) are more effective in food emulsions because they form a protective membrane around the droplets. The free radicals responsible for initiating lipid oxidation are sometimes present in the aqueous phase, and must cross the droplet membrane before interacting with oil in the interior of the droplets (Moberger et al. 1987). In such cases, antioxidants solubilized in the membrane surrounding the droplets can be more effective than when dissolved in the interior of the droplets.

In salad dressings supplemented with minerals or vitamins (i.e., to enhance micronutrient value) that contain added sugar and polysaccharides (i.e., premix stabilizer), the latter ingredients must be added before or after the addition of the minerals. Sugars (such as sucrose, glucose, fructose), sugar alcohols (such as sorbitol), and polysaccharides (such as pectin, aligns, hydrolyzed starches, xanthan gum) can form complexes with calcium and/or act as crystallization inhibitors. In continuous processing systems (Figure 5.4), ingredients such as water, acids, calcium source, and other premix stabilizers can be constantly mixed together to form the premix solutions. Similarly, vitamins such as β-carotene and riboflavin, etc., can be added to the sugar or concentrated syrup either before or preferably after the addition of the calcium premix. An alternative method of adding the calcium is as a dry mixture of calcium carbonate, citric acid, and malic acid (Mehansho et al. 1991). While processing salad dressings fortified with micronutrients, in order to maintain the stability of the iron salts, and to keep the vitamin C from oxidizing, the supplemented food emulsions should be packaged under nitrogen, carbon dioxide, or other inert common non-oxidizing gaseous mixtures.

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6
Processing of Beverages for the Health Food Market Consumer

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6.1 Introduction

Beverages targeted for the health food market, also termed as *functional* or *nutraceutical beverages*, contain bioactive ingredients that offer health benefits. Beverages are an excellent delivery system to offer health benefits and provide a win-win proposition for both consumers and manufacturers, as they offer value-added products to consumers and higher profit margin to manufacturers. The nutraceutical beverage market was projected to grow by 52% to $87,740 million between 2011 and 2016 (BCC Research 2011). Although Europe accounts for the biggest market share among all global regions, the United States is the topmost country in terms of the sales of functional beverages (BCC Research 2011) (Figure 6.1). Some of the growth-generating factors are: increased consumer trend toward managing health through diet, higher interest of manufacturers in high-margin functional drinks due to a consistent decline in the sales of carbonated soft drinks, and growth in consumer awareness about the growing repertoire of innovative and exotic functional ingredients.

This chapter offers insights on the types of functional beverages and claims that consumers favor. Also included is a discussion on the legislative hurdles that can potentially impact the decision to include certain bioactive ingredients and/or the variety of claims that may find favor among consumers. The discussion and information in this chapter are primarily focused on non-alcoholic beverages.

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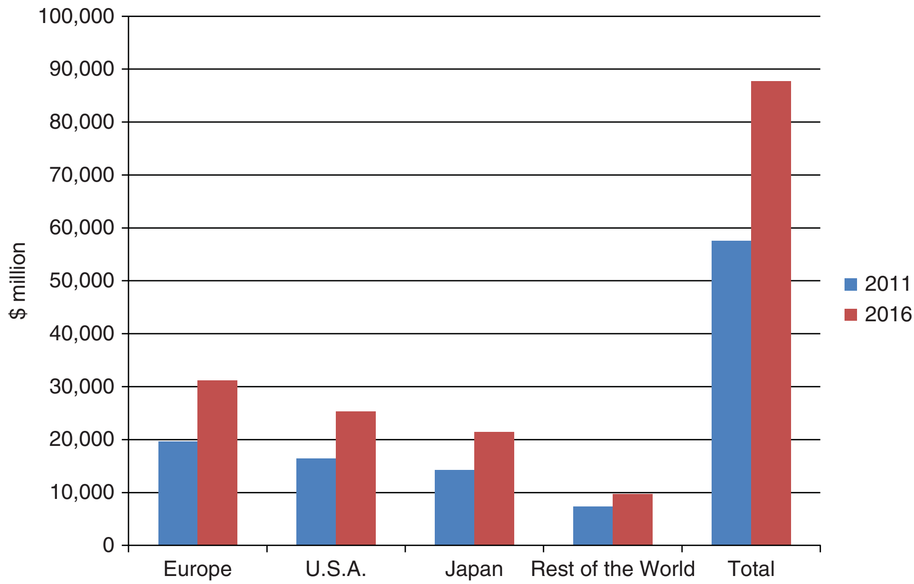

Figure 6.1 Top global functional beverage markets, 2011 and 2016. *Source*: Data from BCC Research, 2011. (For a colour version, see Plate 3).

6.2 Consumer Trends in Beverage Consumption and Functional Beverages

The first factor to be considered when formulating a functional drink is to determine the beverage type in which bioactive ingredients are included to make a functional claim. This largely depends on the following factors:

- The current consumer trend toward health that dictates their beverage choices is an important factor. For example, even though soda is the top consumed beverage in many countries – including the United States, consumers increasingly view soda and juice drinks as unhealthy due to the nutrient-deficient profile and the presence of high amounts of sugar. Alternatively, beverages that are inherently perceived as healthy – such as juices for the presence of vitamins and minerals, teas for antioxidants, and water for no calorie load – continue to find higher acceptance among consumers and hence make desirable base (beverages) for adding bioactive ingredients to make functional claims.
- Consumers also increasingly show inclination toward eating "all-natural" and "organic" food. This suggests that functional beverages that exclude artificial additives – colors, flavors, and preservatives – are likely to find more acceptance among health-conscious consumers. Indeed, adults (aged 18+) who report using functional beverages are significantly more

likely than the average to report consuming all-natural and organic foods (Mintel 2010).

- The age of the consumer is important in deciding which functional claims should be added to a beverage type. It should be noted that adults aged 18–34 are the primary consumers for non-alcoholic beverages (Mintel 2011), and the incidence and volume consumption of beverages decline by increase in age. Additionally, adults aged 18–34, compared to those aged 35+, exhibit significantly higher incidence of drinking energy drinks and sports drinks, and, among the two groups, the gap between the consumption incidence of these two drinks is much higher compared to other non-alcoholic beverages such as bottled water, soda, fruit juice and drinks, tea and ready-to-drink (RTD) tea, and RTD coffee. Therefore, formulators should be aware of the target consumer of different types of beverages, because it is likely that functional claims which may appeal to younger consumers, that is, those aged 18–34 years, may not be deemed beneficial in the 35+ age group.

6.2.1 Functional Beverage Segments

Non-carbonated functional drinks, which include sports and energy drinks and functional bottled water, not only make up the topmost segment in the market but also are slated to experience the biggest sales growth between 2011 and 2016 (Figure 6.2). Energy drinks and functional water are relatively newer beverage segments compared to more traditional beverages such as dairy-based drinks, fruit juice/drinks, tea and coffee, and sports drinks.

The unprecedented growth in *energy drinks* can be attributed to the functional benefit of the "energy boost," as well as to the "image-based marketing" tactics that associate (many) energy drinks brands with fast-paced sports and bold advertising with "I-dare-You" undertones. This is likely why energy drinks are more popular among teens and young adults aged 18–34 years – the consumer groups who also tend to live on-the-go lifestyle and are the primary consumers of any beverage type. Caffeine is the main functional ingredient in energy drinks, along with other ingredients such as taurine, guarana, ginseng, and B vitamins. Therefore, one of the challenges for formulators in making energy drinks more palatable is to mask the bitter flavor of caffeine that comes from two of its ingredients – caffeine and guarana.

Sports drinks have faced stiff competition from enhanced bottled water segment, as light users – primarily non-athletes – of sports drinks moved to enhanced bottled water for the perceived benefit of getting vitamins/minerals, without consuming too many calories. In the United States, sports drinks have also been criticized for their high calorie content and the presence of high-fructose corn syrup (HFCS). Gatorade, the biggest sports drinks brand in the world, in response to consumer criticism, replaced HFCS with regular sugar and introduced an all-natural line sweetened with stevia. The brand's rebranding also included sports drinks with protein, positioned as post-workout recovery drinks. The innovation opportunities in this segment include natural flavors, natural sweeteners, and proteins.

Functional bottled water – bottled water enhanced with vitamins/minerals and other functional claims – became popular in the United States in the early 2000s and experienced initial success due to its novelty status as well as perceived benefits of getting vitamins and minerals from a relatively low-calorie source. However, consumers soon became wise to the fact that many enhanced bottled water brands contained as much sugar as many other sugar-dense beverages such as soda. Additionally, many brands included artificial ingredients – color, flavor, and sweeteners, which did not go well with the healthy image of enhanced water. Manufacturers have duly made consumer-desired changes by reducing sugar count, formulating with the natural low-calorie sweetener stevia, and removing HFCS. One of the challenges in this segment is flavor innovation and the efficacy of vitamins and minerals at the time of consumption.

Dairy drinks make up the second biggest segment of the market. Some of the challenges in this segment are the ones that influence the whole beverage market – removal of excess sugar and cleaning up the ingredient list, that is, reduction in the use of artificial ingredients. Some challenges are unique to the dairy segment – growing trend toward veganism, lack of exciting flavors offered by other beverage segments, the inclusion of nutrients found in milk in other beverage segments (i.e., calcium, vitamin D, and protein), and increased awareness about lactose intolerance among people. Nevertheless, some areas in the segment continue to offer growth. Meal replacement drinks are an important part of this segment. Inclusion of ingredients such as grain fibers (i.e., oats, quinoa, etc.), a range of vitamins, antioxidants, and an extra dose of protein (whey, soy, or pea protein) is likely to make these beverages compatible with others in the same league, such as fruit juice smoothies. Kefir, a fermented milk drink, remains a tiny sub-segment of overall dairy; however, it has experienced consistent growth in the United States in the past 5 years. Kefir milk is said to boost immunity and digestive health. Achieving a palatable taste in kefir presents a challenge for formulators.

Fruit and vegetable juices/drinks suffered greatly with the advent of the low-carb trend in the early years of 2000, where consumers reduced the consumption of high-carb (high-sugar) beverages. One of the top reasons why consumers drink fruit juice and drinks is to get vitamins and minerals. However, enhanced bottled water became popular at the same time as the low-carb trend, and consumers embraced vitamin-enhanced water as a low-carb alternative to juice/juice drinks. Juices drinks experienced bigger decline compared to fruit juices, due to the presence of artificial ingredients and high amounts of sugar. Some brands, such as Trop50 from Tropicana, which have removed artificial ingredients and incorporated an all-natural, low-calorie sweetener (stevia), have found growth. Superfruits and smoothies contributed to the growth in the segment in the first decade of 2000. Coconut water has been another bright spot in the segment, which is perceived as healthy due to the presence of high amounts of potassium and because of its low-calorie status. In addition to being positioned as a sports drink, coconut water has found its way into smoothies as a calorie-reducing ingredient. Blends of different types of 100% fruit juices (usually includes a superfruit) and fruit and vegetable juices have found popularity in the segment. Some brands have tried to bring the energy component to juices by adding green tea extract to juices; however, the concept has not appealed to a wide consumer base. Juice and tea hybrid is also a budding concept in the segment that has shown growth potential. Innovators have multiple opportunities in the juice/drinks segments – sugar reduction by using natural low-calorie sweeteners, replacing artificial ingredients with natural ones, and keeping the segment competitive by introducing new concepts such as blends, hybrids, and carbonation.

Tea has found success among consumers, especially in developed countries, since early-to-mid 2000s due to the research studies that affirmed the presence of antioxidants in tea polyphenols. The proliferation of many types of teas – black, green, and white – in myriad flavors available in RTD format primarily contributed to the impressive growth in the RTD tea segment as consumers interpreted the beverage as an on-the-go health elixir. However, a 2010 study published by the American Chemical Society (ACS) suggested that the amount of polyphenols found in RTD teas were too low to offer any health benefits. Therefore, formulators would benefit by testing the polyphenol levels in RTD teas throughout the duration of storage on shelves. Research would be needed to ensure the stability of these nutrients to garner consumers' continued interest in RTD teas. Tea and juice hybrids offer opportunities, especially since a 2007 study has proved that the addition of citrus juices enable more of green tea's antioxidants to remain in the body after digestion (Green et al. 2007).

Coffee is a source of energy for consumers, especially those aged 35+, and is the second biggest functional beverage consumed in the United States, behind orange juice (Mintel 2010). Coffee's functional attributes include antioxidants and an energy boost. Formulators can enhance its appeal for the younger consumer segment by adding flavors such as chocolate, citrus, and others.

6.3 Taste Is the Prime Factor in Choosing Food and Beverages

While bioactive ingredients help decide the likely success of a functional drink, if it does not taste good, the beverage is unlikely to garner sustained

Beverage type	2011 (US$ millions)	2011 (% of total)	2016 (US$ millions)	2016 (% of total)	CAGR (2011–2016)
Non-carbonated drinks*	31,030	53.9	50,500	57.6	10.2
Dairy drinks	20,320	35.3	28,800	32.8	7.2
Fruits and vegetable juice and drinks	3,330	5.8	4,380	5.0	5.6
Tea and coffee	2,340	4.1	3,250	3.7	6.8
Others	600	1.0	810	0.9	6.2
Total	**57,620**	**100.0**	**87,740**	**100.0**	**8.8**

*Non-carbonated drinks include non-dairy functional beverages such as energy drinks, sports drinks, and functional bottled water.
Source: Reproduced from *Nutraceuticals: Global Markets and Processing Technologies*, with permission from BCC Research LLC.

Figure 6.2 Global nutraceutical beverage market, by segment, 2011 and 2016.

consumer interest. According to a 2012 survey by the International Food and Information Council Foundation (IFICF), taste is the topmost factor in driving food and beverage choices. Initially, consumers purchased functional beverages primarily for health benefits; however, as the functional beverages have evolved and become mainstream, consumers have come to expect more sophisticated taste profiles. In fact, a study by Mintel reports that functional beverage users rate "taste" as the topmost reason to buy functional beverages. From the consumer perspective, the end product needs to have the right balance of sweetness and intended flavor profile.

6.3.1 Sweeteners: Finding the Right Balance between Calories and Taste

The heightened emphasis, especially in developed countries, on the role of high-calorie foods and beverages in causing obesity has cautioned consumers to watch their calorie intake. Since functional beverages offer health benefits, the presence of high amounts of sugar is likely to defeat the intended positioning of providing holistic health of such a beverage. Therefore, the presence of high amounts of sugar to make a beverage palatable is likely to result in low purchase rate or rejection from consumers. For instance, in the United States – the biggest market for functional beverages – nearly half (44%) of all adults aged 18+ agree with the statement "If a functional beverage has too much sugar, even natural sugar, I am not going to drink it" (Mintel 2010). Another hurdle to functional beverage growth from the sweetener perspective could be the use of HFCS – a sweetener type highly used but increasingly rejected by consumers in the United States. Some 48% of all adults aged 18+ agree that they believe that functional beverages sweetened with HFCS are unhealthy (Mintel 2010). Additionally, consumers who are cutting back on regular-calorie beverages appear to be reluctant to move to diet beverages, as non-nutritive sweeteners (also known as artificial sweeteners) do not offer the same taste as regular sugar (sucrose), and they have come to be viewed as unhealthy by many consumers. In the United States, for example, 58% of all beverage consumers aged 18+ say that diet beverages taste worse than non-diet beverages, and 32% agree with the statement that they are drinking less diet beverages due to health concerns related to non-nutritive sweeteners such as sucralose (Splenda), aspartame (NutraSweet), etc. (Mintel 2012).

Therefore, either reducing the calorie count of functional beverages by using less of regular sugar (sucrose) or by the use of an all-natural, low-calorie sweetener such as stevia could help increase the overall health quotient of these beverages. However, elimination of regular sugar types such as sucrose, HFCS, and glucose can introduce the following major challenges for scientists.

- The removal of sugar causes a reduction in soluble solids, which reduces the perceived mouthfeel of beverages. This can result in consumer not liking the beverage due to being habitual of experiencing the mouthfeel of full sugar.
- The other issue is created by replacing regular sugar with high-intensity (low-calorie) sweeteners. The temporal character of these high-potency sweeteners is likely to be different from the temporal sweetness profile provided by sucrose, which can potentially create an imbalance between sweetness and acidity (Paquin 2009).
- The reduction in soluble solids in the absence of sucrose can result in the increased volatility of some non-polar compounds and reduce the volatility of the polar chemicals within the flavor formula (Paquin 2009). This phenomenon can potentially influence the flavor delivery negatively, reducing the enjoyment of the beverage.

6.3.2 Stevia-Based Sweeteners Could Hold the Key to Growth in All-Natural, Low-Calorie Functional Beverages

Consumers increasingly view artificial sweeteners as hazardous to health but desire all-natural, low-calorie options that taste like the regular version of the beverage. A stevia-based sweetener could play an important role in naturally sweetening functional (and other) beverages, especially as the compound has received widespread regulatory approval globally. *Stevia rebaudiana*, a plant native to Paraguay, South America, produces a number of steviol glycosides that are significantly sweeter than sucrose. Health Canada – the federal department in Canada responsible for helping Canadians improve and maintain their health – on its website (http://www.hc-sc.gc.ca) lists nine steviol glycosides that the agency has considered as food additives. These glycosides are stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, rebaudioside B, rebaudioside D, and rebaudioside F. However, two glycosides – stevioside and rebaudioside A (also known as Reb A) – are of particular interest to scientists for beverage use as these two are the major sweet constituents of the stevia plant; Reb A is more potent and more pleasant-tasting than stevioside (Jenner 1989). Additionally, the top two beverage giants Coca-Cola (in collaboration with Cargill) and PepsiCo (with Merisant) have introduced their own Reb A-based sweeteners – Truvia and PureVia, respectively. Since the United States is the biggest market for functional beverages and Coca-Cola and PepsiCo tend to be big players in the global market, scientists may want to explore the functional beverage formulations with Reb A as a sweetener.

6.3.3 Flavors Need to Offer More than Just Taste

In functional beverages, flavors need to accomplish a dual purpose – offer the overall desired flavor to the consumer as well as mask the unpleasant taste or smell that originates from the bioactive ingredient added to achieve the desired functionality. For example, some functional ingredients have unpleasant taste – soy protein has a beany aftertaste, caffeine and polyphenols taste bitter and astringent, calcium tastes chalky, and (some) B vitamins have a meaty taste. Similarly, inclusion of elements such as whey protein, fish oil, and B1 vitamin can produce bad smell in the functional beverage concoction.

Flavor application to functional beverage should be done once the parameters for the base have been determined. Determining the kind of processing beverage required is one of the most important factors before adding flavors. This allows off-notes to be masked at the forefront rather than after the complete product is manufactured. Other factors to consider in creating the beverage base before adding flavors is deciding the number of calories/serving, overall taste, bioactive ingredients, and stability, etc. (Eckert and Riker 2007).

Most shelf-stable products have low pH (<4.4) to avoid microbial contamination. This can create challenges for manufacturers as some ingredients such as Epigallocatechin gallate (EGCG), found in green tea, tends to get increasingly bitter as the pH is reduced. Another example is the inclusion of various proteins – whey, soy, and pea – in high-pH RTD beverages, which need to go through harsher processing techniques and, as a result, produce unpleasant notes such as bitter, burnt, sulfuric, nutty, beany, etc. (Eckert and Riker 2007). Therefore, beverage formulators need to work closely with flavor scientists to smooth out the taste issues in functional beverages to overcome the numerous hurdles introduced by the interaction of multiple factors – low-calorie/sugar alternatives, influence of bioactive ingredients, processing technologies to achieve the desired pH and preservation of beverage, and overall palatability of the beverage.

6.4 Regulatory Considerations with Respect to Ingredients and Claims

Functional beverages are different from other types of beverages in that they contain bioactive compounds and make claims to offer certain health benefits. Therefore, they receive scrutiny from the food and beverage regulatory bodies such as the Food and Drug Administration (FDA) in the United States, Foods for Specified Uses (FOSHU) in Japan, and the European Food Safety Authority (EFSA) in the European Union. Functional beverage formulators should closely check the regulatory guidelines for including certain bioactive ingredients and functional claims on packaging and in marketing communications. It is much more expensive to either shelve a product after the launch or retract marketing claims after the intervention of regulatory bodies. Both of these scenarios run the risk of generating consumer distrust in the brand.

The functional beverages may run into regulatory problems for the following reasons:

- Certain bioactive ingredients may not be considered safe for human consumption in certain countries or regions. Many regulatory bodies such as the FDA in the United States require pre-market approval for adding bioactive additives to conventional beverages, unless it is generally recognized as safe (GRAS) by qualified experts under the conditions of intended use.
- Even though the bioactive ingredient may be recognized as safe for consumption, the quantity of bioactive ingredient used in functional beverage may not be deemed safe by the regulatory body in certain countries or regions. For example, in 2009, the FDA in the United States floated guidance for differentiating beverages from dietary supplement after observing that some of the beverages had started using novel ingredients in excess of amounts deemed safe or approved by the FDA.
- The claim on beverages should also follow the regulatory guidelines. For example, in the United States, health claims on food/beverages are limited to claims about reducing the risk of a disease or health-related condition and do not include claims about treating, mitigating, or curing disease, which are considered drug claims.

6.4.1 Beverage Efficacy and Complete Transparency Is Essential

A functional beverage should deliver on its claims, that is, consumers should be able to feel the physiological benefit of the claim. Therefore, proper research and (human) trial are essential to offer evidence-based products to consumers. Additionally, brands should communicate clearly the number of servings that consumers need to take to experience sustained functional benefits.

6.5 Desired Functional Benefits and Bioactive Ingredients

Consumers purchase functional beverages for different reasons, which could vary from addressing a specific health concern to enjoying general benefits such as achieving and/or maintaining good health. The number of functional ingredients currently incorporated in beverages is very high, and hence it is not viable to include all of those in this chapter. A discussion of ingredients (Figure 6.3) that have contributed to the growth in the functional beverage market is included.

6.5.1 Antioxidants

Antioxidants are molecules that inhibit the oxidation of other molecules. In the food and beverage sector, consumers have come to associate antioxidants with health benefits such as immunity boost, heart disease and cancer prevention, and longevity. Since the early 2000s, the advent of superfruit juices and related products, particularly pomegranate, fueled the antioxidant movement, at least in the United States. Around the same time, a number of research studies, which were proliferated in the media and hence created high consumer awareness about their results, confirmed the presence of high amounts of antioxidants in green tea polyphenols. To consumers, tea offered a value-priced option to get antioxidants over superfruit juices, which are often priced at super-premium or premium levels. Although the scientific community has not set any definition for "superfruits," fruits that offer antioxidants and a wide variety of health benefits tend to be included in this category. Superfruits that have mainly appeared in the fruit juice segment of the beverage market include pomegranate, blueberries, açaí, noni, acerola, and mangosteen. Vitamins A, C, and E are also identified as antioxidants. Therefore, beverages incorporating these nutrients are candidates for antioxidant claims. Needless to say, the bioavailability and efficacy of the antioxidants in these beverages need to be assessed before any claims can be made.

6.5.2 Vitamins and Minerals

Vitamins and minerals command the biggest share of sales in the nutraceuticals market. One of the reasons is that consumers have high awareness of the importance of these nutrients in their diets, and these are relatively easy to get from many food sources. In the United States, 53% of all adults aged 18+ consider the presence of vitamins/minerals when they buy food and beverages; 47% report that they try to get a certain amount, or as much as possible (IFICF survey 2012). Therefore, consumers perceive beverages with essential vitamins and minerals as healthy. Formulators should assess consumer demand for certain vitamins/minerals; for example, in the past decade, there has been a lot of emphasis globally on the lack of vitamin D. The risk is particularly higher among children, young adults, and the elderly people (Lips 2010). Vitamin D is primarily found in dairy foods and beverages, and since it is fat soluble, it can be challenging to include vitamin D in clear water-based beverages. Formulators may look into including this vitamin in beverages other than dairy, such as enhanced water.

Vitamin B has also gained popularity as an energy nutrient due to the vitamin's inclusion in energy drinks as one of the energy components. Therefore, this nutrient could also be explored for its use in functional beverages other than energy drinks.

6.5.3 Protein

Even though protein is a highly desirable nutrient in the overall diet, in beverages it does not appear to have high popularity as vitamins and minerals. In the United States, more than half(54%) of all functional beverage drinkers say of soy and whey protein that "these protein types are ok, but they do not look for these (in functional beverages)" (Mintel 2010). Some of the challenges of incorporating proteins in beverages are lack of clarity, high viscosity, and unpleasant taste. These negative attributes make protein inclusion difficult in non-dairy and low-viscosity beverages such as fruit juice, sports drinks, and enhanced water. Therefore, protein extracts that are clear, neutral in taste, and can be incorporated in low-pH beverages are likely to find higher usage in functional beverages such as sports drinks.

In 2011, Archer Daniels Midland (ADM) Company introduced a clear soy protein "CLARISOY," a technology the company licensed from Burcon NutraScience Corporation, Canada. CLARISOY is claimed to be the only vegetable-based protein that offers clarity and complete nutrition in low-pH beverages. The nearly colorless powder has no off or beany (soy) flavors, is heat stable, and produces very low-viscosity solutions, per the Brucon website (http://www.burcon.ca/products/soy_protein/clarisoy.php).

Whey protein is another type used to increase the protein content of functional beverages, but has issues such as denaturation and increased turbidity in clear beverages with low pH. These hurdles can be overcome by a three-step process: (1) centrifugation as a first step to remove damaged proteins before heat treatment; (2) selection of ingredients to reduce turbidity; and (3) beverage formulation at pH 4.0 or lower (LaClair and Etzel 2010). The inclusion of whey protein isolates (WPIs) in Gatorade Recovery sports drinks, a post-workout extension of Gatorade, the biggest sports drinks brand in the world, testifies to the advances in WPI technology. Hilmer Ingredients, a California-based whey protein supplier, now offers a WPI that the company claims to offer optimal nutritional profile, solubility, and heat stability for transparent, low-pH beverages.

Proteins from two other sources, yellow dried pea and oats, have also gained some traction in recent years. Going forward, these two types of proteins may find bigger favor among consumers for two reasons: (1) many consumers, especially in developed countries, now exclude animal products from their diet; and (2) some consumers have come to view soy protein isolates (SPIs) as a not-good-for-you ingredient. Some research studies suggest that the soy could cause estrogenic and goitrogenic activity in those who consume it (Doerge and Sheehan 2002). For example, people with preexisting thyroid deficiency, a condition known as hypothyroid, could experience decreased thyroid function if soy is consumed too close to taking the replacement hormone.

6.5.4 Omega-3

Omega-3s are considered essential fatty acids (EFAs); and alpha-linoleic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are the key EFAs beneficial to humans. The omega-3 fatty acids offer a myriad of health benefits, including reducing the risk of death from heart disease, decreasing high levels of triglyceride (a risk factor for heart diseases), good prenatal health, and reducing stiffness and joint pain from rheumatoid arthritis, among others (www.webmd.com). A study published in the *British Journal of Nutrition*, 2010, suggested that the consumption of 200 mg EPA and 300 mg DHA in a convenience drink for 8 weeks increased the omega-3 index in blood by 2.43%, which is associated with 70% decrease in the risk for cardiac death (Köhler et al. 2010). Omega-3 also garners high consumer awareness, and ranks third highest functional beverage ingredient by sales (BCC Research 2011).

When used as an ingredient in beverages, omega-3 fatty acids are prone to oxidation, resulting in rancidity. The other issue with the ingredient is its instability in water-based beverages, due to large-size fat molecules (Paquin 2009). In 2012, Virun, a California-based nutra-biosciences technology company, developed the proprietary OmegaH2O® technology, which is able to deliver clear and shelf-stable omega-3 fatty acids in beverages. The sources of omega-3 are fish oil, flax seeds, algae, chia, blackcurrant, cranberry, hemp, perrilla, and pumpkins. In 2012–2013, chia seeds have become quite popular as a functional ingredient in food and beverages in the United States.

6.5.5 Probiotics

Probiotics has been defined as "a microbial preparation which contains live and/or dead cells including their metabolites which is intended to improve the microbial or enzymatic balance at mucosal surfaces or to simulate immune mechanisms" (Reuter 1997). Consuming probiotics either from food/beverages or supplements may offer relief from or prevention of many gastrointestinal (GI) disorders and diseases. The functional beverage market with probiotics has exhibited 20% growth in sales during 2009–2011 (BCC Research 2011), the second biggest after vitamins and minerals. Some of the consumers groups, children and elderly, are more prone to food-related and other GI health issues. Therefore, the aging population in Europe and the United States offers a sizable consumer base for probiotic-enhanced beverages. Probiotics are primarily incorporated in dairy-based beverages – fermented and non-fermented – including milk-based drinks, yogurt drinks, and cultured buttermilk. During 2008–2013, non-dairy products such as probiotic juice/juice drinks have also been introduced in the functional beverages market. Some of these brands include GoodBelly Probiotic Juice (United States), and ProViva and Gefilus (Europe). One of the issues with probiotics is the viability of probiotic strains that can be negatively influenced during processing and adequate dosage – that is, the presence of adequate amounts of culture from the bottling stage to consumer consumption, as the processing and storage conditions can negatively influence this attribute (Paquin, 2009).

6.5.6 Fiber

Fiber has gained high awareness among consumers for its role in heart and digestive health and satiety. In the United States, some 40% of all functional beverage users look for fiber in their beverages (Mintel 2010). Added fiber can now be found in beverages such as orange juice, enhanced water, smoothies, and yogurt drinks. New oat-based beverages, naturally rich in fiber, are also on the rise.

6.5.7 Green Tea Extract

Green tea has experienced the greatest growth among all types of tea in the last decade, primarily for its healthy profiles. Green tea contains four catechins – gallocatechin (GC), epigallocatechin (EGC), epicatechin (EC), and epigallocatechin gallate (EGCG). Catechins are high in antioxidant properties and are often known as *tea flavonoids*. These catechins are said to offer health benefits such as reducing the risk of Alzheimer's disease, certain cancers, and cardiovascular and oral diseases. Additionally, consumption of green tea catechins can bring about diet-based weight loss by enhancing the body's metabolic rate (*Nutrition Research Newsletter* 2009). Green tea extract has become popular as a natural source of caffeine in many non-alcoholic beverages other than tea. Green tea extract is naturally bitter in taste, and hence needs flavor-masking expertise.

6.5.8 Caffeine

Caffeine has found impressive popularity in energy drinks and energy shots. Nearly a quarter of all functional beverage users look for caffeine in their beverages (Mintel 2010). Caffeine is a bitter, white crystalline xanthine alkaloid that acts as a stimulant drug. It is found in varying quantities in the seeds, leaves, and fruits of some plants, where it acts as a natural pesticide that paralyzes and kills certain insects feeding on the plants, in addition to enhancing the reward memory of pollinators. It is most commonly consumed by humans in infusions extracted from the seed of the coffee plant and the leaves of the tea bush, as well as from various foods and drinks containing products derived from the kola nut. Other sources include yerba maté, guarana berries, guayusa, and the yaupon holly (https://en.wikipedia.org/wiki/Caffeine). Caffeine can be synthetic (chemically derived) or natural (from plants). The trend toward eating all-natural food promotes the use of natural caffeine as a source of stimulant in beverages. The other issue with using caffeine is its bitter taste, which should be masked for favorable consumer acceptance.

6.5.9 Ginseng

Ginseng is categorized as an adaptogenic herb, and it commands high awareness among consumers. In the United States, only 9% of all functional beverage consumers do not recognize ginseng, and 24% of functional beverage users are likely to look for this herb in beverages (Mintel 2010). This could be attributed to the herb's use in increasingly popular energy drinks and RTD tea. The use of ginseng is claimed to offer health benefits such as immunity boost, cancer prevention, and antioxidants. In addition to tea and energy drinks, the usage of the herb is increasing in other beverage types, including juice drinks and enhanced bottled water. Current scientific research suggests that the amount of ginseng present in energy drinks is low and does not produce either positive or adverse therapeutic effects (Clauson et al. 2008). Therefore, some of the issues that formulators may be interested in exploring would be the correct amount of ginseng to produce intended health benefits and the herb's interaction with other ingredients.

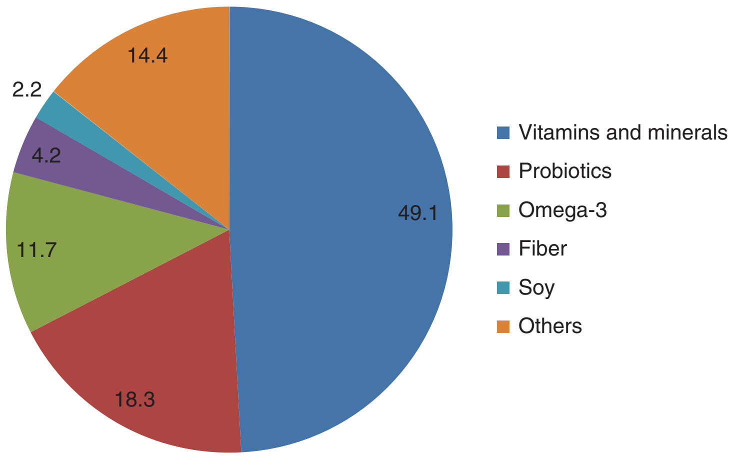

Figure 6.3 Global nutraceutical beverage market, by ingredient market share, 2011. *Source*: Data from BCC Research, 2011. (For a colour version, see Plate 4).

6.6 Health Issues Addressable through Functional Beverages

6.6.1 Weight Loss and Management

Obesity and related diseases such as diabetes and high blood pressure make up three of the top five risk factors for mortality in the world (WHO 2009) (Figure 6.4). In fact, the US population exhibits the highest rates of being overweight and obese in the world. The majority of Americans are cognizant about their weight issues and are doing something about it – some 77% of adults aged 18+ in the United States are either trying to lose or manage weight (IFICF 2012). This suggests that functional beverages that help consumers to lose or manage weight are likely to be successful.

Satiety – the feeling of fullness – is one of the important strategies for functional drinks. A number of ingredients that are now available and backed by scientific research studies offer satiety when included in food/beverages.

- Use of polydextrose – a low-calorie, highly branched-chain glucose polymer that is poorly digested in the upper GI tract and, as a result, exhibits fiber-like properties – may reduce short-term food intake (Ranawana et al. 2012).
- DSM, a New Jersey-based nutritional product company, has developed a patented oil-in-water emulsion called Fabuless™, which can be incorporated into beverage products to delay hunger. Fabuless™ fine oil droplets are made from naturally occurring dietary lipids – palm oil, coated with galactolipids from oat oil.

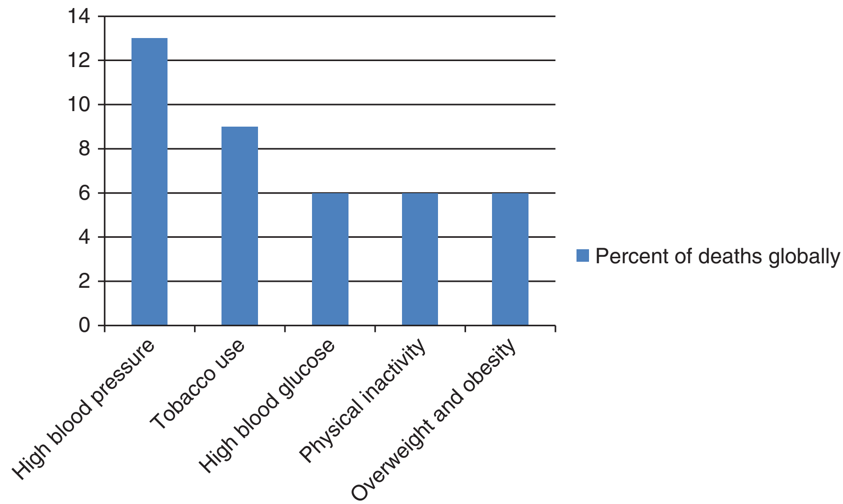

Figure 6.4 Top five global risks for mortality in the world, 2009. *Source*: Data from World Health Organization 2009.

6.6.2 Diabetes (Type 2) and Heart Health

In many countries, functional beverages may run into regulatory problems if they include the direct claim to cure diabetes or cardiovascular diseases (CVDs). Therefore, beverages targeting weight management may refer to the reduced risk of both of these diseases, as they are both linked to high body weight. Nevertheless, there have been a few research studies on certain ingredients that provide benefit to blood sugar and/or CVDs.

- In 2004, a study from UC Davis suggested that consuming plant sterol through non-fat beverages such as juices may lower LDL cholesterol – the type considered bad for heart health (Devaraj et al. 2004). In the United States, the FDA allows use of labeling health claims about the role of plant sterol or plant stanol esters in reducing the risk of coronary heart disease (CHD) for foods containing these substances.
- Pulses and grains have also been cited as beneficial ingredients in lowering diabetes and CVD risks. For example, consumption of yellow pea is associated with reduction in fasting insulin (Christopher et al. 2010).

6.6.3 Energy Boost

Energy drinks have experienced rapid sales growth since the early 2000s. In the same vein, a new product segment under the moniker of "energy shots" has emerged since 2008, which is sold as a supplement in the United States.

The phenomenal success of both energy drinks and shots is testimonial to consumers' growing need for energy boosts. While older adults (aged 35+) are more likely to derive their caffeine-induced energy boost from coffee, the younger set, that is, those aged 18–34, are more likely to gravitate toward energy drinks. Overall, more than half (51%) of all adults aged 18+ desire energy boosts from functional beverages. Caffeine remains the primary ingredient in energy drinks and shots; however, as consumers become inclined toward eating natural foods, caffeine derived from natural ingredients such as green tea may attract consumers wary of synthetic caffeine. In the United States, brands such as Campbell Soup Company's V8 V-Fusion + Energy drink and Celestial Seasonings' ENERJI™ Green Tea Energy Shots use natural green tea extract as a source of caffeine.

6.6.4 Stress Relief

The aging population in Europe and the United States is a substantial consumer base for functional beverages offering stress relief and relaxation. In the United States, 48% of all adults aged 18+ expressed interest in seeing "stress relief" claims in functional beverages (Mintel 2010). This is an upcoming beverage segment, and there is a lot of room for innovation. The main ingredient used in beverage brands (in the United States) that offer stress relief benefits are L-theanine, kava root, melatonin, and valerian roots.

6.6.5 Beauty-Enhancing Claims

A growing area in the realm of nutraceuticals is beverages that offer beauty benefits. Terms such as *cosmeceutical*, *nutra-cosmeceutical*, and *skin-gestible* have surfaced in recent years to describe products with nutrients that are quickly absorbed into bloodstream to enhance beauty. Japan is at the forefront in introducing beauty drinks; their appeal in the United States and Europe, however, has remained limited. If marketed as an alternative to the expensive and sometimes risky surgical procedures that consumers, especially women, undergo, beauty drinks could capture the attention of European and American consumers. Some of the ingredients incorporated in beauty drinks are collagen, essential vitamins, and green tea.

6.7 Beverage Processing Technology

6.7.1 Encapsulation

Nutraceutical beverages contain many ingredients that are prone to deterioration – that is, they do not offer the same potency or function after a beverage is processed and stored for the period of a beverage's shelf-stable life. Additionally, some ingredients may react and lose potency when they interact with other components in their immediate environment. Yet another issue is the smell of bioactive ingredients and their lack of compatibility with the beverage medium. Encapsulation processing technology offers solutions to these problems and more. The technology can also be used as a tool to control the retention, release profile, and targeted delivery of a number of different ingredients (Preparedfoods.com 2008). *Encapsulation (micro)* is defined as the incorporation of food ingredients, enzymes, cells, or other material within a capsule of approximately 5–300 μm in diameter (Lee 1996). Advancement in nanotechnology has enabled scientists to bring down the size of the particles to the nanoscale. The benefits of nanocapsules are twofold: (1) small particles, such as less than 100 nanometers, are optically clear; therefore, their addition to beverages produces no visual effect; (2) small-sized particles increase the bioavailability of sparsely water-soluble compounds (Huang 2012). Encapsulation is an optimal processing method used for ingredients that tend to deteriorate in the presence of oxygen or light, such as fish oil and vitamins, powdered mixes, flavors in beverages where controlled time release is desired, and targeted site delivery for ingredients such as probiotics, which must be protected until they reach the large intestine, where they offer the intended benefit (Preparedfoods.com 2008).

6.7.2 High-Pressure Processing

High-pressure processing (HPP) involves subjecting foods/beverages to elevated pressures (up to 87,000 psi), with or without the addition of heat to accomplish microbial inactivation or alter food attributes. While thermal processing can cause changes in food/beverage ingredients, HPP retains the fresh characteristics of foods by not or minimally altering the beverage ingredients. Therefore, beverages processed with HPP result in fresher taste, texture, and nutrition. Foods with high acid content (low pH), such as juices, are good options for HPP technology (http://ohioline.osu.edu/fse-fact/0001.html).

6.8 Packaging

Nutraceutical beverage packaging can serve multiple functions, as discussed in the following paragraphs.

6.8.1 Package Labeling as a Marketing Tool

Packaging is one of the best tools to communicate the functional beverage positioning with consumers. In the United States, more than 60% of all adults pay attention to the health and nutrition claims on packaging (Mintel 2013). Therefore, it is important to include the type and amounts of bioactive ingredients and their intended benefits on the packaging. The name of the bioactive ingredient included should be listed by its “common name” rather than “scientific name,” so that consumers can easily make the connection of a claim with the known benefits of ingredients.

6.8.2 Bringing Functional Innovation through Packaging

Packaging can be a medium used to offer functional novelty to consumers. For example, a new trend in functional waters is to store ingredients in the cap of the bottle, in the dry form, for release at the time of consumption. The idea behind such an innovation is that premixing with the liquid medium can deteriorate the quality and functional benefits of the ingredients, owing to external conditions such as light exposure, storage temperature, etc. Karma Wellness Water in the United States uses a proprietary cap technology where wellness ingredients are hermetically sealed between the cap and the base, and released into the water at the time of consumption (http://www.drinkkarma.com/press/materials/karmacap-technology-overview.pdf).

6.9 Other Marketing Considerations

Price is an important consideration in selling any beverage to consumers. Nutraceutical beverages, in general, are priced at a premium, likely due to the extra costs to manufacturers from the extra research efforts and proprietary ingredients. Therefore, marketing efforts should be focused on communicating the value proposition of the beverage. For example, a functional beverage positioned at weight loss benefit may underscore the possible avoidance of obesity-related diseases such as diabetes and CVDs, which are more expensive to manage.

Avoid making unreasonable claims, such as weight loss in 1 week, etc. Consumers are more likely to believe claims offering general wellness rather than extreme claims such as curing diabetes. Any claim targeting a specific malady such as heart disease should be backed by iron-clad research and regulatory approvals to avoid the regulatory intervention.

Usage occasion is another marketing tool to increase functional beverage consumption among consumers. A functional beverage brand could depict usage occasions, such as lunchtime for a meal-replacement smoothie, in advertising messages. Energy shots usually show their product’s efficacy in the afternoon hour, when many adults feel lethargic and need a strong “pick-me-up.”

As many food and beverages contain similar bioactive ingredients, dosage disclaimers should be included on the packaging, so that consumers do not overdose by consuming the same ingredient through multiple foods and beverages and/or through multiple servings of the same food and beverage and experience harmful effects. For example, energy drinks, if consumed in excess, pose the risk of caffeine poisoning. Some of the energy drinks brands, in an effort to make the beverage extra potent and differentiate from other brands, include caffeine in amounts that can be unhealthy for the consumer. This is why, in the United States, many brands choose not to disclose the ingredients or nutrition information, and are sold as dietary supplements and not as beverages. However, as energy drinks have reportedly been linked with deaths worldwide, many top brands such as Monster Energy Drink and Rockstar have made decisions to disclose the amount of caffeine on their packaging.

6.10 Conclusion

Functional beverages offer a wealth of (profit) opportunities to beverage manufacturers. However, the success of the end product depends on careful planning with respect to the consumer interest in diverse functional claims, which is greatly influenced by the current consumer trend toward health as well as their age, race, and gender. Manufacturers engaged in cutting-edge functional ingredient innovation might also need to take the onus of educating consumers without sidestepping regulatory consideration.

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7
Incorporation of Nutraceutical Ingredients in Baked Goods

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7.1 Introduction

People consume foods not only for energy intake but also for their positive health effects; as Hippocrates said, "Let food be the medicine and medicine be the food" (Sohaimy 2012). Evidences have been obtained from ancient cultures such as Egyptians, Chinese, and Sumerians, suggesting that foods can effectively be used as medicine to treat and prevent disease (Sohaimy 2012). Although there are naturally beneficial ingredients in food, incorporation of these ingredients into the food products might also be performed as an alternative approach in order to make use of their health-improving properties.

In the past century, essential nutrients that can prevent specific diseases have been a major focus of human nutrition research (Sohaimy 2012). Research results have shown that some bioactive compounds or products having nutraceutical compounds can protect consumers from cancer, hypertension, depression, heart diseases, obesity, arthritis, and diabetes. In the last 20 years, there has been a significant amount of development in the nutraceuticals market, with companies launching many types of products as food supplements and functional/healthy food products (Fulendra et al. 2012).

"Nutraceuticals" and "functional foods" are two terms used to define health-promoting foods, or components that are extracted from them (Sohaimy 2012). There are no universally agreed definitions for nutraceuticals and functional foods (Wildman and Kelly 2007). The term "nutraceutical"

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was first coined from “nutrition” and “pharmaceutical,” and defined as “a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease” (Dureja et al. 2003; Sohaimy 2012). The term is used for anything that is consumed primarily or particularly for health reasons (Wildman and Kelly 2007). This approach would suggest that a functional food could be a kind of nutraceutical product. Health Canada has its own description, describing nutraceuticals as “prepared from foods, but sold in the form of pills or powders, or in other medicinal forms not usually associated with foods”. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic diseases. With respect to this expression, nutraceuticals would be distinct from functional foods in terms of the format of the product (Wildman and Kelly 2007). In a boarder definition, nutraceuticals are defined as “the products developed from either food or dietary substance or from traditional herbal or mineral substance or their synthetic derivatives or forms thereof, which are delivered in the pharmaceutical dosage forms such as pills, tablets, capsules, liquid orals, lotions, delivery systems, or other dermal preparations, and are manufactured under strict good manufacturing practices” (Pathak 2010).

Nutraceuticals offer physiological benefits or protect against chronic diseases, while functional foods have a broad definition as foods that supply additional physiological benefits to the consumer with their usual diet beyond basic nutritional functions (Shahidi 2007; Sohaimy 2012). Some advantages of nutraceuticals have been described as follows: (1) diets containing a high amount of nutraceuticals, along with regular exercise, help reduce disease risk and stress and regulate body weight; (2) nutraceuticals protect from age-related diseases such as arthritis; and (3) nutraceuticals fulfill the consumer tendency for self-treatment and healthy life (Fulendra et al., 2012).

7.2 Bakery Products

The term “baked products” refers to a wide range of food products such as breads, cakes, cookies, etc. As defined by Cauvain and Young (2006), “bakery products are based on wheat flour and *undergo heat treatment* in the bakeries”. Since 95% of consumers in most developing countries consume cereals that have comparatively inexpensive prices, these foods are the primary vehicles for food fortifications (Akhtar and Ashgar 2011). Bakery products and cereals account for about 23% of the global functional foods market (Incles and Starling 2012).

Among baked goods, bread is the most consumed staple food product all over the world. Depending on the cereals used and the processing methods, bread alone as a fermented product might provide some benefits for human health. For instance, research has suggested potential chemopreventive (prevention of colon cancer) effects of different breads after *in vitro* fermentation

(Schlörmann et al. 2012). In the study, the chemopreventive effects of the breads (wheat, wholemeal wheat, and wholemeal rye) were assessed in colon cells after *in vitro* fermentation. Effects of fermented bread samples on gene expression, glutathione S-transferase activity and glutathione content, differentiation, growth, and apoptosis were investigated using the human colon adenoma cell line LT97. The expression of 76 genes (biotransformation, differentiation, apoptosis) was found to be significantly upregulated (1.5-fold) in LT97 cells, and the fermented bread samples significantly inhibited LT97 cell growth. In order to further improve the health-promoting properties of baked goods, another alternative has been the incorporation of nutraceuticals into bakery products (Quilez et al. 2006). Incorporation of many different nutraceuticals into baked goods has been explored. The following sections in this chapter summarize the application of bioactive ingredients in bakery formulations and their main health effects.

7.3 Nutraceuticals and Nutraceutical–Incorporated Baked Goods

7.3.1 Polyphenols

Phenolic compounds are nutraceuticals used to increase the health benefits of food. There are many studies evidencing antioxidant and other health effects of polyphenols (Sun et al. 2002; Cirico and Omaye 2006; Dubick and Stanley 2007). Some studies have shown that phenolic compounds inhibit platelet aggregation and may increase the time for coagulation, which may decrease risks relating to cardiovascular diseases (CVDs), and they may also provide protection against cerebrovascular diseases (Cirico and Omaye 2006; Dubick and Stanley 2007). Phenolic compounds are known to have antimicrobial effects (Shahidi and Naczk 2004). In addition, antiatherosclerotic, antidiabetic, and anticarcinogenic effects of these compounds have also been discussed (Shahidi and Naczk 2004).

Several investigations have been performed to study the health effects of nutraceutical-incorporated bakery products as well as their technological, sensory, and physicochemical properties. There are studies about breads enriched with phenolics such as caffeic, ferulic, syringic, and gallic acids (Han and Koh 2011), or with kiwifruit, barley flour, apple, and blackcurrant, which have high phenolic content (Holtekjolen et al. 2008; Sun-Waterhouse et al. 2009; Sun-Waterhouse et al. 2011). Another nutraceutical incorporation application for biscuit is the addition of *Emblica officianalis*, *Moringa oleifera*, *Vitis vinifera* (Reddy et al. 2005), and mango peel powder (Ajila et al. 2008) as sources of natural antioxidants. Green tea was also used for antioxidant enrichment of sponge cake (Lu et al. 2010).

7.3.2 Lycopene

Lycopene is a potent antioxidant (Agarwal and Rao 2000). *In vitro* studies have shown the antioxidant effects of lycopene, and this feature may underlie its protective effect against cancer (Bruno et al. 2007; Sohaimy 2012). Lycopene contributes to health by reducing the effects of harmful compounds such as reactive oxygen species (ROS) which harms DNA and oxidises lipids. Furthermore, it contributes to cardiovascular health and may have protective effects against neurodegenerative diseases and hypertension (Sohaimy, 2012). Prebiotic antioxidant bread that contained tomato paste as a lycopene source was prepared, and the effect of the enriched bread on immunological and antioxidative parameters were studied. The results showed a significant increase of antioxidant properties (Seidel et al. 2007).

7.3.3 Beta-carotene

Beta-carotene has been described as an antioxidant compound and, according to relevant studies, it may have a protective effect against cancer, heart diseases, macular degeneration, and ageing (Burri 1997; Naves and Moreno 1998). Research results have suggested that 4–6 mg of beta-carotene taken per day may provide physiological protection effects against several types of cancers, especially those of epithelial origin, and at the early stages of carcinogenesis (Naves and Moreno 1998). Supplementation of bread with nutraceuticals such as beta-carotene has been studied (Brufau et al. 2004; Park et al. 1997). The enrichment of muffins with beta-carotene has also been reported (Quilez et al. 2006).

7.3.4 Dietary Fibre

The World Health Organization recommends a daily fibre intake of 27–40 g (Nishida et al. 2004). Many epidemiological studies have provided evidence that fibre intake at high ratios reduces CHD risk in both males and females (Jalili et al. 2007). Another effect of fibres is to regulate blood coagulation (Jalili et al. 2007). In addition, it has been reported that dietary fibre may have protective effects against gastrointestinal cancers, increases satiety, regulates the glucose and insulin systems, and helps in the treatment of obesity and diabetes (Jalili et al. 2007; Cui and Roberts 2009). One of the significant soluble dietary fibres considered as nutraceutical is inulin, which is not absorbed or digested in the small intestine but is fermented by useful bacteria in the colon. Inulin, which functions as a prebiotic, also encourages calcium and magnesium absorption, regulates blood glucose, and reduces serum lipid and cholesterol (Ohr 2004).

In a study, the glycaemic and insulinaemic response to oat bread, oat bread with lingonberry fibre, and oat-buckwheat bread were investigated (Rokka et al. 2013). The mean glycaemic and C-peptide indices were, respectively, reported as 32 and 100 for oat bread, 47 and 119 for oat–lingonberry fibre bread, and 58 and 105 for the oat–buckwheat bread. The authors reported that buckwheat and lingonberry fibres might be considered as new alternative ingredients for low-glycaemic-index (GI) foods (Rokka et al. 2013). A study was performed to evaluate the estimated GI of cookies containing extruded wheat bran (Reyes-Pérez et al. 2013). Wheat bran was subjected to extrusion under three different temperature profiles (ranging from 60 to 140 °C) and three different (15, 23, and 31%) moisture contents. The lowest GI was obtained from the cookies prepared with extruded wheat bran (Reyes-Pérez et al. 2013).

A type of nutraceutical supplement was prepared with whole-grain corn flour with high amylose content and different types and levels of guar gum, and investigated for the possibilities of modulating glycaemia, insulinaemia, and perceived satiety (Ekström et al. 2013). The research results indicated that, by combining medium-weight guar gum and whole-grain corn flour with an elevated amylose content, the glycaemia, insulinaemia, and subjective appetite ratings, and the content of resistant starch, were improved, compared to the reference white wheat bread. Another way of enriching bread with dietary fibre is by adding plant materials to it, and *Theobroma grandiflorum* and *Auricularia auricular* have been used for this purpose (Fan et al. 2006; Salgoda et al. 2011). Dietary-fibre-incorporated cakes using watermelon rinds and sharlyn melon peels (Hanana and Ahmed 2013), Cheonnyuncho (*Opuntia humifusa*) (Kim et al. 2012), cereal bran (Lebesi 2012), apple pomace (Sudhaa et al. 2007), guar gum and oat fibre (Gularte et al. 2012), inulin (Moscatto et al. 2006; Gularte et al. 2012; Volpini-Rapina et al. 2012), barley flour (Gupta et al. 2011), yacon meal (Moscatto et al. 2006), polyols and nondigestible oligosaccharides (Ronda et al. 2005), and high-fibre cookies enriched with antioxidants (Udarbe 2004) have been reported. Apple skin (Rupasinghe et al. 2008), cocoa fibre (Martínez-Cerveraa et al. 2011), and other fibre sources (Thomason 1999; Zahn et al. 2013) have also been used to enrich the dietary fibre content of muffins.

7.3.5 Resistant Starch

Resistant starch as a prebiotic product has a positive effect on useful bacteria populations in the large intestine, and it can reduce the amount of pathogen bacteria. Many relevant studies have shown that intake of resistant starch reduces risk of colon cancer, contributes to intestine health, and helps to develop the immune system (Ohr 2004). Enrichment of cookies using resistant starch and banana starch has been studied (Aparicio-Saguilan et al. 2007). Similarly, resistant starch has been used for preparing mufins (Baixauli et al. 2008).

7.3.6 Beta-glucan

Beta-glucans are not digested in the body, but they can be fermented by intestinal microflora. Beta-glucans have an effect on improving the immune system because they support useful microflora and immune cells by binding directly to specific receptors. Furthermore, they have no cytotoxic effect and reportedly have an anticancer effect (Chan et al. 2009).

A research to evaluate beta-glucan-enriched flours, obtained from barleys with either normal or waxy starch, for their effects on GI of bread was conducted (Finocchiaro et al. 2012). Its positive consequences on glycaemia were determined, compared with the normal-starch barley: the GI of the all-wheat bread (82.8 ± 7.2) was significantly reduced (57.2 ± 7.9) when 40% of wheat flour was substituted with beta-glucan-enriched barley flour (6.0% ± 0.1 beta-glucan in the final flour blend), suggesting that the ability of beta-glucans to lower the GI was affected by the barley starch type (Finocchiaro et al. 2012). The effect of barley beta-glucans on short-term appetite and on satiety-related hormones in healthy subjects was evaluated in an investigation (Vitaglione et al. 2009). Fourteen volunteers were selected and randomly assigned to have isocaloric breakfasts, including 3% beta-glucan-enriched bread (betaGB) or control bread (CB). The betaGB gave a significantly higher reduction of hunger and increase of fullness and satiety than the CB. A 23% lower total area under the curve (AUC) (60–180) of plasma ghrelin and a 16% higher total AUC of PeptideYY (PYY) response after betaGB consumption compared to the CB, independent from insulin response, was found. It was concluded that barley beta-glucans were able to control appetite in the short term by modulating sensations and reducing energy intake (Vitaglione et al. 2009).

Hypolipaemic effects of prebiotic biscuits containing barley meal (BM) were investigated in rats (Hassan et al. 2012). Biscuits supplemented with 30% BM contained higher amounts of total dietary fibre and beta-glucan than controls. Results indicated that feeding of diets supplemented with different functional prebiotic biscuits to hypercholesterolaemic rats for 8 weeks significantly ($P \leq 0.05$) reduced serum total cholesterol, triglycerides, LDL, very-low-density lipoprotein (VLDL), ratios of total cholesterol/high-density lipoprotein (HDL) cholesterol, LDL cholesterol/HDL cholesterol, and atherogenic index, but increased HDL levels. Histopathological examination revealed that feeding of diets containing BM to the hypercholesterolaemic rats reduced the extent of lesions to heart and liver tissues. Results suggest that BM-based bakery products could be developed as a treatment for hyperlipidaemia (Hassan et al. 2012). The materials that have high fibre content such as coffee silverskin, barley hull, wheat bran, rice bran, maize, and buckwheat have been added to bread (Pourfarzad et al. 2013; Hao and Beta 2012; Sidhu et al. 1999; Hu et al. 2009; Brites et al. 2011; Lin et al. 2009). Enrichment of Taralli, a specific type of bakery product, with barley flour and its lipid stability has been investigated (Verardo et al. 2010).

7.3.7 Omega-3

Major sources of omega-3 fatty acids are fish (eicosapentaenoic acid [EPA]), walnuts, seeds (docosahexaenoic acid [DHA]), and vegetable oils (α-linolenic acid [ALA]) (DeFilippis and Sperling 2006). Intake of omega-3 fatty acids may have protective effects against hypertension (Mori 2006). Studies suggest that omega-3 fatty acids may have antiatheromatous, hypolipaemic, and antithrombotic actions, in addition to vascular regulation and also antiarrhythmic and anti-inflammatory effects (Rose and Connolly 1999). Furthermore, it has been reported that omega-3 fatty acids have protective and healing effects against arthritic disorders, diabetes mellitus, and cancer (Rose and Connolly 1999); protective effects against neurological and psychiatric diseases and efficiency in treatment of Alzheimer's disease (Mazza et al. 2007); a key role in retina health (Kaarniranta and Salminen 2009); and may provide protection against age-related macular degeneration (SanGiovanni and Chew 2005). Researchers have further shown that omega-3 fatty acids function in synergy with certain drugs, reducing their toxicity (Simopoulos 1991). Studies regarding incorporation of omega-3 fatty acids, fish oils, and flaxseed into bread formulation have been performed (Saldeen et al. 1998; Liu et al. 2001). Saldeen et al. (1998) found that intake of stable fish oil containing n-3 fatty acids increased long-chain n-3 fatty acids in plasma phospholipids. Liu et al. (2001) found that triglyceride levels were decreased and HDL cholesterol increased with the consumption of bread containing stable fish oil. Flaxseed has also been incorporated into cakes as an omega-3 source (Lee et al. 2004).

7.3.8 Spices and Herbs

Plant roots, leaves, branches, flowers, and seeds are used in various preparations purported to have beneficial effects on health such as regulation of nervous system, heart, and circulation, and curing the immune, digestive, respiratory, urinary, and musculoskeletal systems (Percival and Turner 2007). Plants such as ginger, rosemary turmeric, onion, grape seed, green tea, curcumin, strawberry, blackcurrant seed, and their extracts or powders are added to breads (Frutos and Hernández-Herrero 2005; Wang et al. 2007; Peng et al. 2010; Balestra et al. 2011; Lim et al. 2011; Korus et al. 2012; Gawlik-Dziki et al. 2013; Vitaglione et al. 2012). Antioxidative and antimy-cotic effects of extracts of turmeric were compared with antioxidant activities of BHA and BHT in butter-containing plain cakes (Lim et al. 2011). Turmeric extracts proved to be more antimycotic and antioxidative. These spice extracts were often more effective (at 1 g per kg fat) in preventing oxidation and shelf life loss of cakes than were BHA or BHT (Lean and Mohamed 1999). Turmeric (*Curcuma longa* L.) powder was used to substitute 0%, 2%, 4%, 6%, and 8% of wheat flour for making turmeric wheat breads. Breads containing turmeric powder have been reported to have good antioxidant activity, which has led some researchers to suggest that such breads could be developed as a health-promoting functional food (Lim et al. 2011). Data on *in vivo* effects would, however, be required.

Soda cracker biscuits have been prepared with the addition of a fine powder of basil at the 0.5% level and BHA (at levels of 0.01, 0.02, or 0.03% by weight of shortening) (Bassiouny et al 1990). The results showed that plant material in the form of fine powders or extracts could be used as replacements for conventional antioxidants in soda cracker biscuits without negative effects on sensory properties. Cumin seeds were dried, ground, and de-aromatized by a two-step extraction in several solvents (60–96% ethanol, acetone, diethyl ether, and petroleum ether), before analysis for composition, and used in bread-making as a source of dietary fibre. Bread with 10% spice solids contained higher amounts of dietary fibre and minerals than white loaves (Ying Chien and Potty 1996). *Nigella sativa* (black cumin) fixed oil (BCFO) was added to cookie formulations as a functional ingredient. Gradual increases in BCFO levels in cookies resulted in significant increases in total tocopherol contents, from 9.85 to 53.19 mg/kg oil. BCFO addition significantly enhanced levels of α-, β-, γ-, and δ-tocopherols, from 8.80 to 32.19, 0.96 to 3.47, 0.09 to 14.98, and 0 to 2.55 mg/kg oil, respectively. Thymoquinone contents were found to be highest in cookies containing 5.0% BCFO, at 7.25 mg/100 g (Tauseef Sultan 2012).

7.3.9 Conjugated Linoleic Acids

The most significant reported benefit of conjugated linoleic acid (CLA) is protection against cancers such as skin, stomach, colon, mammary, and liver cancers (Li and Watkins 2007). CLA also has been reported to have anti-inflammatory effects and to improve bone properties (Park 2009). Additionally, some research results suggest that CLA may improve insulin sensitivity (Li and Watkins 2007). A patent has been obtained on conjugated-linoleic-acid-fortified cookie, cake, and muffin formulations (Changaris 2012).

7.3.10 Olive Oil

Recently, many studies carried out on the health effects of olive oils have revealed a decrease in LDL cholesterol levels and LDL oxidation, an increase in HDL/total cholesterol levels (López-Miranda et al. 2010), and protection against cancer (Medeiros and Hampton 2007). Olive oil also improved glucose metabolism in normal subjects and patients with type-2 diabetes, and regulated blood pressure and endothelial functions; furthermore, some research studies suggest that olive oil may have favourable effects on obesity, reduction of platelet aggregation, and prevention of Alzheimer's disease (Medeiros and Hampton 2007; López-Miranda et al. 2010). Oxidative stability of olive oil containing focaccia, in common bakery products of many Italian regions, has been investigated (i.e., determining the amounts of *trans* fatty acids and compounds of triglyceride polymerization, oxidation, and hydrolysis). Researchers found that the overall levels of oxidation was lower than the levels found in refined oils (Delcuratolo et al. 2008; Gomes et al. 2010).

7.3.11 Probiotics

The term *probiotic* is defined by the United Nations and World Health Organization Expert Panel as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Probiotic organisms used in food must have some properties such as surviving passage through the gut, resisting gastric juices and exposure to bile, and increasing and colonising in the digestive tract, in addition to being safe, effective, and stable during storage. The most important benefit of probiotics is modulating the intestinal microbiota and/or inhibiting pathogens, and this property helps to improve the immune system (Saad et al. 2013). The lowering of faecal enzyme activity, treatment and prevention of many types of diarrhoea (e.g., antibiotic-associated, viral rotavirus, and acute diarrhoea), alleviating atopic dermatitis symptoms, constipation, and positive effects on superficial bladder cancer and cervical cancer have also been reported (Saarelaa et al. 2000). Probiotics are present not only in dairy foods but also in cereals, fruits, vegetables, legumes, and meat. Traditional fermented foods are especially good targets for the incorporation of probiotics (Rivera-Espinoza and Gallardo-Navarro 2010). Fried sourdough bread (bhatura) containing high levels of gamma-aminobutyric acid (GABA) was produced using a lactic acid bacteria (LAB) starter (*Lactococcus lactis* subsp. lactis). The results indicated the potential of LAB starter for the production of GABA-enriched bhatura (a fluffy, deep-fried, leavened bread from North India) (Bhanwar et al. 2013). To determine the influence of *Bifidobacterium bifidum* on the release of some minerals from bread using an *in vitro* enzymatic digestion process, white bread with addition of 15, 30, or 45% of bran, yeast, and rye sourdough were prepared. Bread was enzymatically digested *in vitro* without and with the addition of *Bifidobacterium bifidum* KD6. Increase in bran content resulted in decreased release of some minerals (Ca, Mg, Mn, Zn, Cu, Fe). *Bifidobacterium bifidum* KD6 enhanced the amounts of magnesium and zinc released from all types of bread (Nalepa et al. 2012). In another study, sourdough was prepared with a pure culture of *Lactobacillus plantarum* or with commercial starters containing *Lactobacillus brevis*, combined with *Saccharomyces cerevisiae* var. chevalieri (LV4), *Lactobacillus fermentum* (PL1), or *Lactobacillus fermentum* with phytase (PL3), and the GI was studied. The GI of control bread without sourdough was significantly higher than that of bread containing sourdough prepared with LV4 starter, PL1 starter, or PL3 starter, but not from bread with *L. plantarum* sourdough (Novotni et al. 2011).

7.3.12 Exopolysaccharides

Exopolysaccharides (EPSs) are known to have many benefits on health. The high viscosity of EPS solutions increases faecal volume, and this can change the transit time, faecal weight, and faecal water content. Some EPSs have immune-stimulating characteristics, similar to some polysaccharides, and these characteristics result from their stereochemistry, molecular size, and the sugar types that form them (Whitney and Howell 2013). Researchers have reported that EPSs have a lowering effect on serum cholesterol, and this probably results from their behaviour as dietary fibre. In addition, researchers report that EPSs have antitumor effects, and that they strengthen the immune system. Food viscosity affects the speed of gastric emptying. Using the EPS xanthan gum has been one way to slow glycaemic response, by delaying intestinal digestion and absorption. Highly viscous dietary fibres, especially the EPS xanthan gum, may prevent hyperuricaemia by changing digestive and metabolic processes (Farnworth et al. 2007). The performance of dextran forming *Weissella cibaria* MG1 and reuteran forming *Lactobacillus reuteri* in bread has been studied (Sandra et al. 2012), as well as the effect of added EPS-containing sourdough on wheat bread (Sandra et al. 2010).

7.3.13 Vitamins

Micronutrient malnutrition affects more than half of the world's population. Epidemiological studies show that some vitamins (folate, and vitamins E, B6, and A) play an important role in improving health and preventing diseases (Sohaimy 2012). Vitamins and vitamin derivates such as folic acid, folate, and vitamins B12, B6, B2, and E are regularly used for bread fortification (Czeizel and Merhala 1998; Dunn et al. 1999; Ranhotra et al. 2000; Omar et al. 2009; Capozzi et al. 2011). Iron- and iodine-fortified biscuits have been prepared and given to primary school children in feeding programs (van Stuijvenberg et al. 2001). Biscuits that are fortified with vitamin B12, folic acid, and vitamin C have also been reported (Boobier et al. 2007). The stability of vitamins and bioavailability and storage stability of vitamin A fortification (retinyl acetate) in cookies have been studied, and results have shown that cookies enriched with 257.85 μg of retinol could be used for vitamin A deficiency in children (Butta et al. 2007). Many other products, including whole wheat, are frequently fortified with vitamin A (Lotfi et al. 1996).

7.3.14 Minerals

Various minerals are known for their nutraceutical potentials. For instance, calcium provides protection against colon cancer (Lipkin and Newmark 1995) and improves bone health (Wildman and Kelly 2007). Potassium reduces hypertension risks and, consequently, could help in the prevention of cardiovascular diseases. In addition, copper, selenium, manganese, and zinc have specific health benefits (Wildman and Kelly 2007). Iron deficiency can cause anaemia, increased susceptibility to infection, impaired growth, muscle cramps, impaired cognitive function, defects in thermoregulation, increased risk of pregnancy complications, and increased risk of low birth weight and heart attack (Akhtar and Ashgar 2011). Studies on fortification of bread with iron (Najm et al. 2010) and iron, zinc, and iodine incorporation into biscuits have been reported (Kandhro et al. 2008). Fortification of wheat-flour tortillas with calcium lactate, calcium carbonate, or calcium citrate have been performed (Romanchik-Cerpovicz and Mckemie 2007). A study using beetroot, which has a high nitrate content that, after ingestion, can be reduced to nitrite by oral bacteria and further, endogenously, to vasoprotective nitric oxide (NO), in bread formulation has been carried out (Hobbs et al. 2012). Two separate, randomly controlled, single-blind, cross-over, postprandial studies were performed in normotensive volunteers. Ambulatory blood pressure (BP) was measured over a 24-h period following consumption of three bread products – control bread (0 g beetroot), red-beetroot-enriched bread, and white-beetroot-enriched bread. Total urinary nitrate/nitrite (NOx) was measured at baseline, and at 2, 4, and 24 h post-ingestion. Bread products enriched with 100 g red or white beetroot was determined to lower systolic and diastolic blood pressures over a period of 24 h (red-beetroot-enriched bread, $P < 0.05$), with no statistical differences between the varieties supporting the evidence for cardioprotective BP-lowering effects of dietary-nitrate-rich vegetables (Hobbs et al. 2012). An iron-rich bread (2.2 mg iron per 50 g slice) was developed using *Eragrostis tef* flour, naturally rich in iron. In the study, women consumed an average of 2.3 slices per day, providing a total of 5.0 mg iron (Bokhari et al. 2012). Bread with 10% debittered moringa seed (DBMS) and cookies with 20% DBMS grits were prepared, and results showed them to contain more iron and calcium (Ogunsina et al., 2011); incorporation of moringa seeds into baked foods may, thus, be exploited for use to counter micronutrient malnutrition.

7.3.15 Tocopherols

Almonds, safflower oil, sunflower seeds, and canola oil are the main sources for alpha-tocopherol, whereas gamma-tocopherol is mainly present in some vegetable oils such as soybean, canola, and in nuts such as walnuts, peanuts, and pecans. Vitamin E has been reported to be effective in reducing the risks of some chronic diseases such as cardiovascular disease (Harris et al. 2002), Alzheimer's disease (Williamson et al. 2002), and cancer (Klein 2005). Gamma-tocopherol is a physiological metabolite that has anti-inflammatory activity (Bruno 2007). Bakery product enrichment with α-tocopherol and the effects on LDL cholesterol have been studied (Brufau et al. 2004).

7.3.16 Folic Acid

It is quite well-known that the incidence of neutral tube defects can be reduced by folic acid intake (Czeizel and Dudas 1992; Cornel et al. 2005). Supplementation with folic acid can help protect against diseases such as anaemia, cardiovascular disease, type-2 diabetes, hypertension, other congenital defects such as heart anomalies and clefts, neuropsychiatric disorders, and cancers (Cornel et al. 2005; Sohaimy 2012). With the aim of producing pita bread with increased folate content, a study was conducted using germinated wheat flour (GWF) and wheat flour substituted with different levels of GWF (Hefni and Witthöft 2012). Folate content in both flour and bread increased, depending on the level of flour replacement with GWF. Pita bread baked with 50% sieved GWF had a folate content of 50 μg/100 g DM, compared with 30 μg/100 g DM in conventional pita bread (0% GWF). Consumption of GWF-incorporated bread would represent an increase in the average daily folate intake by 75 μg (Hefni and Witthöft 2012).

7.3.17 Proteins, Amino Acids, and Enzymes

One of the health effects of some of the proteins we consume as food is the lowering of cholesterol (Sohaimy 2012). When proteins are hydrolysed during digestion, various oligopeptides differing in amino acid composition and sequence, called bioactive peptides, may be liberated. There are also bioactive peptides that can be consumed directly with food. Bioactive peptides have significant physiological and hormone-like effects in the human body, in addition to their nutritional properties (Seyler et al. 2007). Some bioactive peptides have antihypertensive (ACE inhibitory), antioxidant, antithrombotic, hypocholesterolaemic, hypotriglyceridaemic, antiobesity, and immunomodulatory characteristics, and may help prevent cardiovascular disease (Korhonen and Pihlanto 2006; Erdmann et al. 2008). Mushroom mycelium was used to substitute 5% of wheat flour in bread; it was found that the mycelium-supplemented bread contained substantial amounts of gamma-aminobutyric acid and ergothioneine (0.23–0.86 and 0.79–2.10mg/g dry matter, respectively) (Ulziijargal et al. 2013). The effect of phytase supplementation on zinc, iron, and calcium status in growing rats fed with a diet containing high-phytate Iranian bread (Sangak) showed increased blood zinc levels in the phytase group (Shockravi et al. 2012). Gamma amino butyric acid has been reported to reduce blood pressure, and has been used for chiffon cake fortification (Lee and Lin 2008). Lupine protein is a newer protein source that has been added to bakery products such as cake (Arozarena et al. 2001).

7.3.18 Lignans

A study of the association between intake of flaxseed, the richest source of dietary lignans (a class of phytoestrogens), and breast cancer risk showed that consumption of flaxseed bread was associated with a significant reduction in breast cancer risk (Lowcock et al. 2013). Another study on the ability of isoflavone extracts from whole soy bread (made with 49% replacement of wheat flour) and two soy bread fractions (bread crumb and crust) to modulate the proliferation of human prostate cancer PC-3 cells found that both soy bread and crust extracts modified PC-3 cell proliferation compared to untreated control cells. Soy bread crust extract (10 mg/ml) reduced PC-3 cell proliferation by 15% compared to untreated control cells. Wheat bread extracts, on the other hand, increased cell proliferation at all levels tested. Although extracts from both breads possessed biological activity, only soy bread crust extract reduced PC-3 cell proliferation, implying that the presence of soy in the bread may have contributed the observed positive health effect (Zhang et al. 2003).

7.3.19 Legumes

A study was undertaken to determine whether the addition of Phase 2 (a dietary supplement derived from the common white kidney bean) would lower the GI of a commercially available high-glycaemic food (white bread) (Udani et al. 2009). Standardized GI testing was performed on white bread with and without the addition of Phase 2 in a capsule and in powder form, each in dosages of 1,500 mg, 2,000 mg, and 3,000 mg. For the capsule formulation, the 1,500-mg dose had no effect on the GI, and the 2,000-mg and 3,000-mg capsule doses caused insignificant reductions in GI. For the powder, the 1,500-mg and 2,000-mg doses caused insignificant reductions in GI, and the 3,000-mg dose had a significant effect. Phase 2 white bean extract appears to be a novel and potentially effective method for reducing the GI of existing foods without modifying their ingredient profile.

A fenugreek bread formula was produced in a commercial bakery by incorporating fenugreek flour into a standard wheat bread formula (Losso et al. 2009). Whole wheat bread was prepared using the same formula in the same bakery, and the products were tested for their nutrient composition, sensory analysis, and effect on carbohydrate metabolism for diabetic subjects. The authors reported that the area under the curve for glucose and insulin was lower in the fenugreek condition. The results showed that acceptable baked products could be prepared with added fenugreek, which could reduce insulin resistance and help with the treatment of type 2 diabetes.

7.3.20 Okara, Honey, and Sesame

An investigation on whether the combination of okara bread and appetite stimulants (honey and black sesame seeds) could improve defaecation status in humans was performed (Minari et al. 2011). The amount of total dietary fibre for the food consumption period was 7.6 g and 8 g/1,000 kcal/day in the test and control food groups, respectively. The defaecation frequency and number in the test food group was found to be significantly higher than that of the control group, resulting in the authors' conclusion that the combination of okara, black sesame seeds, and honey in bread may be an effective approach for the improvement of defaecation (Minari et al. 2011).

7.4 Conclusion

Nutraceuticals are known to have health-promoting effects through their physiological benefits and protection against chronic diseases. Diseases such as cancer, hypertension, cardiovascular diseases, obesity, and diabetes are widespread around the world, and preventive solutions including nutraceutical intake through the consumption of food in which bioactive compounds have been incorporated may be helpful. Incorporation of naturally beneficial ingredients into food products, especially staple foods such as bread, may contribute to the improvement of human nutrition and, therefore, human health. Various bioactive components with nutraceuticalpotential have been investigated for their potential positive health effects. Investigations on the incorporation of nutraceutical ingredients including phenolic compounds, lycopene, beta-carotene, dietary fibre sources, resistant starch, beta-glucan, omega-3 fatty acids, medicinal species and herbs, conjugated linoleic acids, olive oil, probiotics, exopolysaccharides, vitamins and minerals, tocopherols, folic acid, proteins, amino acids and enzymes, lignans, legumes, okara, honey, and sesame into baked products have been performed. Depending on the type of bioactive ingredient incorporated, different health effects have been reported. Preventive treatment is one of the main approaches that could be envisaged to improve the health status of different populations. Incorporation of bioactive ingredients into baked good is a promising approach. Further research in the following areas will help to expand application in the food industry:

- Development of new health-promoting baked goods with better nutritional profiles and nutraceuticals
- Studies to understand minimum effective upper levels of bioactive incorporation in baked goods
- Investigation of the best means to provide targeted delivery of bioactive ingredients
- Determination of the eficacy of diverse nutraceutical ingredients in reducing cardiovascular diseases such as reducing total cholesterol, and protecting against coronary heart disease and blood pressure.

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8
New Technologies in the Processing of Functional and Nutraceutical Cereals and Extruded Products

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8.1 Introduction

Various species of the cereal family (e.g., wheat, rice, corn, barley, oats) exist over diverse geoclimatic zones and have adapted to extremely different challenges. Cereals play a central role in feeding the world's population, since the endosperm of cereals is mainly comprised of starch, making them humanity's primary nutritional energy source. Nearly a century ago, grain foods were first recognized by the first USDA Food Guide in 1916, promoting them and other starchy foods as part of a healthy eating plan (Mobley et al. 2013). The germ of most cereal grains is rich in oil and lipid-soluble vitamins, while the outer layers have a high content of cellulose, hemi-cellulose, and minerals. Cereals are considered good sources of many bioactive components such as dietary fibers (e.g., β-glucan, lignan, inulin, arabinoxylan, and resistant starch), phenolic compounds (e.g., phenolic acids, alkylresorcinols, and flavonoids), carotenoids (e.g., lutein and zanthein), anthocyanins, vitamins (e.g., B and E), and minerals (e.g., iron, zinc, magnesium, and phosphorus) (Ragaee et al. 2013). Even food industry by-products such as bran are being recognized for their nutritional value due to these health-beneficial constituents. The recovery of food waste is rapidly expanding around the

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world, since these by-products can be utilized as functional ingredients in food product development.

A series of traditional technologies is applied to alter the characteristics of cereals and transfer them into ready-to-eat foods. For example, dry milling reduces the size of cereal seeds and mechanically separates the starchy endosperm from the rest of the kernel; extrusion mechanically breaks down raw food ingredients, transforming them into a well-cooked matrix that extends shelf-life and improves both product presentation and packaging; fermentation naturally changes product volume, destroys some undesirable components, making products safer, and can increase processing efficiency by reducing the cooking energy required (Blandino et al. 2003); and the use of enzymes improves the processing behavior or properties of cereal foods and is becoming more popular in the food industry (Poutanen 1997). New developments in processing are frequently incorporated into existing technologies to prepare functional foods to meet rising demands and changing consumer perceptions. At the same time, newer and novel technologies such as encapsulation are being explored in cereal food processing to enhance their nutritive value. A number of reviews have greatly contributed to our present understanding of these technologies, and, in this chapter, we focus on the recent improvements in the processes behind some of the new functional cereal products reported in the last 5 years. We discuss our understanding of the effects of these treatments on food nutrients, phytochemicals, and food texture.

8.2 Cereals and Their Food Applications

In general, all cereal grains have similar parts, which are present in approximately the same proportion; these include a germ, an endosperm enclosed by a nucellar epidermis, and a seed coat surrounded by a pericarp (González et al. 2013). However, the components and structure of these different parts vary among different species, resulting in distinct oral, sensory, and nutritional values. The distribution of cereal species in the diet is further restricted by geographical location, population, and price. These factors also influence the varied applications of different cereals. Wheat, rice, and corn are the most important crops, with greatest worldwide production. Wheat and rice are mainly used as food crops, whereas corn is used primarily for animal feed. Recently in the United States, which is the world's largest corn producer, most of the corn production goes toward non-food applications, such as bioethanol and sugar production, and as ingredients for paper and pharmaceutical industries. Barley, oats, and cereal by-products have gained more and more attention for food use due to their unique characteristics.

8.2.1 Wheat

Wheat is one of the first domesticated food crops and has the most stable sowing area on a global scale, occupying one-third of the total cereal sowing area, primarily in Asia, North America, and Eastern Europe. Its success depends partially on its adaptability and high yield potential, but also on its gluten protein fraction, which confers viscoelastic properties that allow dough to be processed into bread, pasta, noodles, and other food products. Modern wheat cultivars usually refer to two species: hexaploid bread wheat, *Triticum aestivum*, and tetraploid, hard or durum-type wheat, *T. turgidum durum*, used for macaroni and low-rising bread (Peng et al. 2013). Wheat has a high starch content of about 60–70% in whole grain and 65–75% in white flour, and it is often considered to be little more than a source of calories. However, the protein content in wheat is significant, usually 8–15%, with some essential amino acids being present in higher amounts than what is required nutritionally, while lysine is deficient (Shewry 2009). Recently, whole-wheat-grain flours have attracted interest due to their high nutritional and functional components, including dietary fiber, minerals, and antioxidants (Maeda 2009). At the same time, wheat products have been blamed for a number of adverse reactions in humans, including intolerances (notably coeliac disease) and allergies (respiratory and food) (Shewry 2009).

8.2.2 Rice

Rice is the main staple food for half of the world population. Almost 90% of the total rice-sowing area is in Asia, as rice has higher heat and water growing requirements than wheat. Rice is consumed mainly as a grain (whole or polished), and therefore the texture of this cooked grain is a matter of primary importance. Generally, it is easier to cook rice with lower amylose content. Starch accounts for approximately 90% of total dry matter in rice seeds, followed by a fraction of storage proteins. The essential micronutrients are almost exclusively stored in the husk, aleurone, and embryo of rice. However, they are removed during the polishing process, since the oil-rich aleurone layer turns rancid upon storage and makes rice unsuitable for consumption (Bhullar and Gruissem 2013).

8.2.3 Corn

The role of corn as a foodstuff is especially important in Africa and Central and South America. Corn kernels can be consumed off the cob, parched, boiled, fried, roasted, ground, and fermented for use in breads, porridges, gruel, cakes, and alcoholic beverages. Corn contains about 72% starch, 10% proteins, and 4% lipids; crude fiber is high in the kernel seed coat (87% of seed coat) (Inglett 1970). A typical yellow corn has many important vitamins, such as vitamin A (as provitamin A carotenoids) and vitamin E (as tocopherols) (Weber 1987), but there is a notable absence of vitamin B-12. Phosphorus, potassium, and magnesium are the most prevalent minerals found in corn. Unfortunately, even though corn kernels supply many macro- and micronutrients necessary for human metabolic needs, the amounts of some essential nutrients are poorly balanced or inadequate for consumers who rely on corn as a major food source; examples are the lack of essential amino acids lysine and tryptophan, and of ascorbic acid (vitamin C), B vitamins, iron, and iodine (Nuss and Tanumihardjo 2010).

8.2.4 Barley

Barley is the fourth most widely cultivated cereal due to its superior heat and drought tolerance. For food uses, barley grain is first abraded to produce pot or pearled barley, and may be further processed to grits, flakes, and flour. There is renewed interest throughout the world in barley because of its nutritional value. Whole barley grain consists of about 65–68% starch, 10–17% protein, 4–9% β-glucan, 2–3% free lipids, and 1.5–2.5% minerals (Quinde et al. 2004). In comparison with corn, barley contains more methionine, cysteine, lysine, and tryptophan. The contents of copper, molybdenum, and manganese are twice as much as those in corn, and five times more calcium is found in barley than in oats. Barley has the highest levels of neutral and acid detergent fiber, potassium, and vitamin A, and the lowest levels of starch and fat among the common cereals (Nikkhah 2012). Barley β-glucan exhibits health benefits including reduction of blood cholesterol and glucose, and increased weight loss by improving satiety, and therefore could indirectly help control heart disease and type-2 diabetes (Baik and Ullrich 2008). There is great potential to utilize barley in a large number of cereal-based food products as a substitute, partially or wholly, for currently used cereal grains.

8.2.5 Oats

About 80% of oats are grown in Europe and North America. The sowing area of oats is decreasing, but its food use is on the rise. Oats are distinct among cereals due to their multifunctional characteristics and nutritional profile. Recent advancement in food and nutrition has revealed the importance of its various components. Oats provide a good source of dietary fiber, especially β-glucan, minerals, and other nutrients. They have been proven to be useful in the treatment of diabetes and cardiovascular disorders (Duss and Nyberg 2004). Adding oats to normal diet of patients with hypertension significantly reduced both systolic blood pressure and diastolic blood pressure. Oats have higher lipid content than other cereals, and the lipid content is genetically controlled and highly heritable. Oat lipids have a high proportion of oleic acid, which is preferred for human nutrition. These polar lipid fractions have good emulsifying properties that are useful in food formulations, and contain antioxidants to provide stability (Peterson 2002). The use of oats in food products improves not only the nutrition but can be used therapeutically to treat various maladies (Butt et al. 2008).

8.2.6 Cereal By-products

In cereal production, the hard bran together with the germ is often removed during milling and processing, catering to consumer tastes. Unfortunately, these discarded parts are rich in a myriad of healthful phytochemicals. Wheat bran is considered a precious by-product, since it is a concentrated source of insoluble fiber, minerals, vitamin B6, thiamine, folate, vitamin E, and antioxidants such as phenolic compounds. These factors make it an important dietary element, with reported benefits of cardiovascular disease and colon cancer prevention (Stevenson et al. 2012). Similarly, phytonutrients from rice bran have shown promising disease-preventing and health-related benefits (Jariwalla 2001), while oat bran in particular is a good source of B vitamins, proteins, fats, minerals, as well as "heart-healthy" soluble fiber β-glucan (Butt et al. 2008). Wheat germ also contains significant quantities of bioactive compounds such as tocopherols, phytosterols, policosanols, carotenoids, thiamin, and riboflavin. Its principal mineral constituents are potassium, magnesium, calcium, zinc, and manganese (Brandolini and Hidalgo 2012). In order to meet consumer demand for healthier foods, food industries are striving to enhance the nutrient value of their products with such healthy cereal food supplements (Patel 2012).

8.3 Novel Technologies in the Processing of Cereal-Based Products

Food value and functional value are two important requirements for food product development. The basic food value should provide energy and satisfy physiological needs. Additionally, modern processed foods must possess functionalities beyond basic nutrition, such as convenience, wellness, and even the capacity to prevent or treat disease. In order to match these increasing demands, a series of novel processes are being developed in the traditional food industry to make functional food products. At the same time, the effects of these treatments on the nutritional value and texture of food are better understood due to improved analytical technologies.

8.3.1 Dry Milling

The cereal dry milling industrial unit is usually composed of four stages: (1) reception and cleaning of grains, (2) conditioning, (3) size reduction, and (4) classification and separation of fractions. The grinding part is the central point of milling, of which the roller mill is the most popular. By applying dry milling, the size of cereals is reduced and the starchy endosperm is mechanically separated from kernels in the traditional process.

Dry Fractionation Because of the heterogeneous distribution of various components throughout the kernel of a cereal grain, dry fractionation has been a method of choice for enriching specific nutritive components. Dry fractionation is relatively energy efficient, requires no solvent removal and recovery, and thus requires lower capital investments. So far, several methods of dry fractionation of cereals have been developed, including pearling, roller milling, and milling, followed by air classification (Liu et al. 2009). Many research studies have focused on concentration of dietary fibers through dry fractionation, since there is increasing interest in using cereal fiber-rich fractions as functional ingredients to produce nutritionally enhanced foods.

Pearling is the process of removing the outer layers of cereal grains, typically by using an abrasive dehulling device such as a barley pearler, which provides gentle surface abrasion to grains with minimum breakage of residual kernels. Depending on the extent of pearling, pearled grain products enriched in β-glucan by up to a 25% increase in concentration compared to the original grain can be produced (Zheng et al. 2000). Recently, Brouns et al. (2012) reviewed the application of this technique in the preparation of wheat aleurone, and pointed out that dry fractionation could allow for full-scale separation of aleurone cells from wheat bran, yielding a fiber-rich concentrate that potentially contains many of the whole-grain kernel bioactive compounds.

Milling disintegrates grains into fine particles. Roller milling employs a special mill that allows grains to pass between rotating rollers for grinding and flaking and then through sifters for fraction separation. Roller milling of hull-less barley was applied to generate fractions with highly variable β-glucan and arabinoxylan (AX) content (Izydorczyk et al. 2003). For high β-glucan cultivars, yields >20% (whole barley basis) of a fiber-rich fraction with β-glucan contents >15% can be readily obtained with a simple short mill flow.

Air classification separates flours based on differences in density, mass, and projected area in the direction of flow. Research has been undertaken to produce barley flours enriched in β-glucans (Srinivasan et al. 2012). The results revealed that fiber particles were selectively carried by air because of their flat shape in contrast to the near-spherical shape of non-fiber particles. Thus, the coarse fractions from air classification (corresponding to the external layer of caryopsides) were enriched in β-glucans (Ferrari et al.

2009); β-glucan concentration effectively doubled compared to that in the original grain. Furthermore, Verardo et al. (2011a) have demonstrated that the same fractions are naturally enriched in phenolic compounds, particularly flavan-3-ols.

The preceding text demonstrates that dry milling represents an excellent opportunity to produce functional food products incorporating cereal fractions enriched in dietary fibers and phenolic compounds. These fractions have enabled preparation of acceptable muffins, breads, and pasta with significantly increased dietary fiber and phenolic compound content (Verardo et al. 2011b; Izydorczyk et al. 2008; Jacobs et al. 2008).

Mycotoxin contamination of cereal and cereal-based food and feed products poses a health risk to humans and animals if not adequately controlled and managed. Recently, research has demonstrated that dry milling can be used as a potential effective method to reduce mycotoxins in cereal products (Burger et al. 2013). Reduction of mycotoxins in the milling fractions of corn flour was accomplished by a significant removal of cereal germ, hull, bran, tip cap, and some endosperm, as these tissues have a high tendency to be contaminated with mycotoxins. The lowest percentage of mycotoxin was found in the milling fractions containing the coarse granulated endosperm. Thus, it appears that, as the particle size of the endosperm decreases, mycotoxin levels increase. Also, hard or flinty endosperm kernels have lower mycotoxin contamination compared to soft hybrids. Hard kernels are known to be less susceptible to breaking and cracking after harvesting. Characterization and the manipulation of kernel characteristics and milling practices could become important strategies to reduce mycotoxin contamination in the resulting milling fractions.

Imaging Technology for Dry Milling Products Chemical imaging technology is a rapid examination technique that combines molecular spectroscopy and digital imaging, providing information on morphology, composition, structure, and concentration of a material. A modern, high-technology approach of chemical imaging with an 82,000-pixel InSb focal plane array enables assessment of unit process efficiency for milling operations. In the dry milling of wheat flour, each unit process (roller mill, purifier, sifter, etc.) produces a mixture with varying amounts of wheat endosperm and non-endosperm by-products. This novel imaging technology provides quantitation of components in the granular solid mixture by detection of near-infrared spectra. Chemical images of each intermediate product stream are taken (as shown in Figure 8.1), and then analyzed by partial least squares (PLS) chemometric software to identify individual pixels and calculate the relative amount of endosperm and non-endosperm (Wetzel et al. 2010). When relative flow rates were known for each stream, mass balance could be calculated in terms of the product (endosperm content) and the non-endosperm by-product. These mass balance profiles thus enabled the assessment of operational efficiency. Moreover, by comparing the mass balance profiles before and after

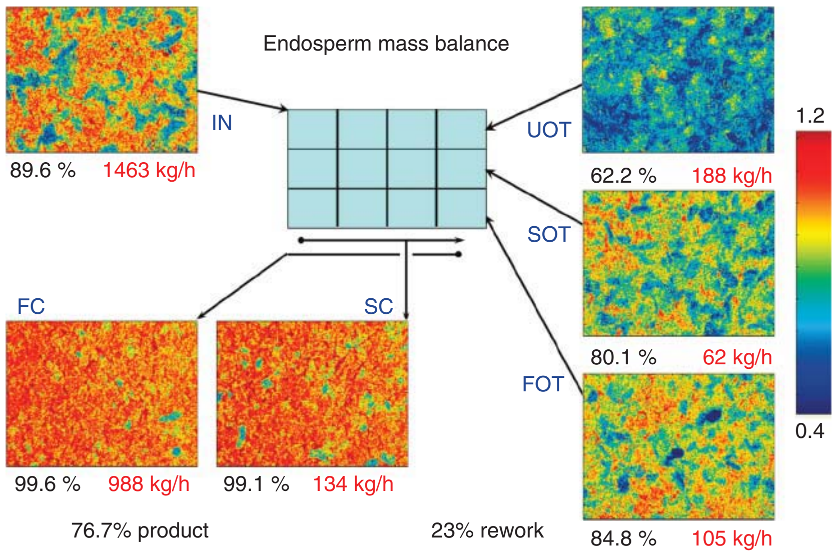

Figure 8.1 Endosperm mass balance of commercial flour mill purifier. The rectangular diagram in the center represents a purifier (left to right) with the incoming stock (IN, top left) entering, and the first cut (FC) and second cut (SC) product streams. From IN to FC, the endosperm content has been enriched from 89.6% to 99.6%, while the quantity has been reduced from 1,463 kg/h to 988 kg/h. The by-product material is divided into three streams, designated as upper overtails (UOT), second overtails (SOT), and first overtails (FOT) of varying percentage endosperm and endosperm flow. The summation of FC and SC products was 76.7%, and the combined by-product streams subject to rework was 23.3%. *Source*: Wetzel et al. 2010, Fig. 2. Reproduced with permission of Society for Applied Spectroscopy. (For a colour version, see Plate 5).

adjustment, the operational parameters could be optimized. Unlike routine near-infrared quantitative analysis methods that employ a multivariate mathematical expression derived from a large, pre-analyzed training set to determine concentration, the system employed in this study classified each pixel from its spectrum on the basis of a previously determined qualitative multivariate profile. Therefore, the devised chemical imaging analysis system would be particularly useful in the commissioning of a new mill, the replacement of machinery, or possibly the processing of differing stocks of raw material, such as a new crop wheat.

Fluorescence microscopy was recently applied to observe the tissue structures of wheat bran treated by ball milling (Craeyveld et al. 2009). The intact cell walls of wheat bran were stained with Calcofluor to appear blue, proteins were stained with acid fuchsin to appear red, and starch was unstained and appeared black. Micrographs showed that untreated wheat bran consisted mostly of large fragments of pericarp and aleurone layers attached to each other or as separate structures. After the ball-milling treatment, the samples appeared as aggregates of material in which only very few recognizable structures, including small fragments of aleurone cell walls and some protein aggregates, were observed. Microscopic analysis of ball-milled wheat bran thus showed that the cell-wall structures were completely destroyed by the ball-milling treatment. Sizes of individual recognizable cell-wall structures observed upon ball milling were in the low micrometer to high nanometer range. This implies that ball milling is able to reduce the size of the majority of the bran material to the nanoscale level. This microscopic analysis has provided important information to explain the capacity of ball milling as a dry process for the production of arabinoxylan oligosaccharides as a novel dietary fiber from wheat and rye bran. Ball-milling treatment can completely destroy cell wall structures of cereal bran, and thus can render arabinoxylan from the aleurone layer as well as the pericarp layer water-extractable, resulting in higher arabinoxylan oligosaccharide yields.

8.3.2 Extrusion

Extrusion refers to a process by which a liquid or semi-liquid product is forced through a die opening of the desired cross-section. Food extrusion has been divided into two general categories: forming and cooking. For forming applications, low-shear extruders are used to mix and form the desired product shape with minimum energy input. The products include pasta, cold-formed snacks, and other unexpended precooked pellets. The screw typically has a deep channel (20–30% of diameter) with no compression ratio, and operates at a relatively low speed (below 50 rpm). Cooking extrusion normally utilizes medium-shear and high-shear extruders, and significant energy is provided to the products through viscous heat dissipation, heat transfer through the barrel, and sometimes with steam injection. The screws run at relatively higher speeds (>100 rpm) with a shallower channel depth (Yacu 2011). The extrusion may change the content as well as the physicochemical and nutritive properties of cereal flour components, depending on the processing condition such as temperature, moisture, and pressure/shear. Investigation of the relationship between the physicochemical and nutritive properties of extruded cereal products, and extrusion conditions, will allow the development of new extrusion techniques to produce novel healthy cereal products.

Novel Nutritionally Improved Food Products by Extrusion The use of whole grains in food formulations is of growing interest, and extrusion cooking allows the preparation of precooked whole-grain products. Bhavya and Prakash (2012) studied the nutritional components and quality of several breakfast cereals (corn, wheat, ragi, and mixed millet). The results indicated that the nutritional components, such as the total protein, total dietary fiber, iron, and calcium, were present in appreciable levels in all varieties of breakfast cereals, with very low anti-nutrient content. Starch showed a high digestibility profile, which was due to the effect of excessive heat treatment during extrusion. Overall, the authors concluded that whole-grain breakfast cereals served as good breakfast food, as these are found to contribute almost a quarter of the daily nutrient requirement, and are of good nutritional quality.

Increased health awareness of consumers has led the food industry to develop fortified snacks with functional ingredients. For example, β-glucan is a commonly researched plant cell wall component that has been associated with cholesterol and glycemic response reductions when incorporated into food products. The production of extruded ready-to-eat snacks with the addition of β-glucan-rich fractions from barley was introduced (Brennan et al. 2013). Inclusion of barley β-glucan-rich fractions at 10% levels increased the total dietary fiber content and the expansion of extrudates compared to the control, which in turn resulted in a reduction in product hardness. *In vitro* digestion protocol indicated that the inclusion of 20–25% barley β-glucan-rich fractions modified the starch digestibility profile and hence the rate of glucose release during digestion, as compared to the control sample, leading to a significant reduction in potential glycemic response. Therefore, the addition of these fractions could be utilized by the food industry to manipulate the glycemic response of extruded snack products.

Recent research by Sharma et al. (2012) revealed that antioxidant activity of extruded hulled barley grit increased significantly upon extrusion and reached the highest value (36–69%) at 150°C and 20% feed moisture. The authors attributed this increase to the formation of Maillard browning pigments. This research also revealed that the rise in feed moisture and temperature increased the metal chelating activity of barley flour, but decreased the total phenolic content and total flavonoid content. Another research study showed improved bioaccessibility of phenolic acids in extruded whole-grain barley and dehulled oats, compared to the non-extruded cereal grains, as the total contents of free phenolic acids and the bioaccessibilities of bound phenolic acids were higher in the extruded diets (Hole et al. 2013). Other products, such as extruded cereal bars fortified with flaxseed meal for a balanced ratio of omega-6 to omega-3 essential fatty acids (Giacomino et al. 2013); extruded corn snacks fortified with carp mince, trout mince, and freeze-dried saithe protein to increase protein content (Shaviklo et al. 2011); and incorporation of a number of different fruits and vegetables in extruded gluten-free products to raise the level of total dietary fiber (Stojceska et al. 2010), provided further good examples of novel extruded functional foods.

Extrusion with Supercritical Carbon Dioxide Supercritical fluid extrusion is a hybrid process developed by combining extrusion and supercritical fluid technologies. It utilizes supercritical carbon dioxide as a viscosity-lowering plasticizer and expansion/foaming agent. The first application of this technology was developed for the agro-food industry, and it is well reviewed by Sauceau et al. (2011). Currently, the shelf-stable puffed rice, fortified with protein, dietary fiber, and micronutrients, is produced with the assistance of supercritical carbon dioxide. By using supercritical carbon dioxide, the expanded products have good textural qualities and are produced at lower temperatures (about 100°C) than with conventional steam-based extrusion (130–180°C). This process allows for the complete retention of all added minerals, 55–58% retention of vitamin A, and 64–76% retention of vitamin C. All essential amino acids including lysine are retained at exceptionally high levels (98.6%), and no losses were observed due to Maillard reaction or oxidation (Paraman et al. 2012). Thus, supercritical-carbon-dioxide-assisted extrusion could be an effective approach to produce cereal-grain-based, low-moisture (5–8%) expanded products fortified with protein and any cocktail of micronutrients, without compromising the end-product sensory or nutritional qualities. Another novel healthy snack has also been produced from corn starch and whey protein by extrusion with supercritical carbon dioxide (Cho and Rizvi 2010). It allowed an extrusion process at temperatures below the protein denaturation temperature, and helped to prevent the development of a hard texture due to the thermosetting property of whey protein, while at the same time yielding a uniformly expanded structure. Maximum cross-sectional expansion and most uniform internal structure were achieved at 0.3 and 0.7 wt% supercritical carbon dioxide levels, respectively.

Characterization of Extruded Products The complex nature of extruded products made using whole cereal flour, water, and various additives makes it challenging to fully understand the properties of the extruded cereal products. Interactions between the ingredients used are further affected by extrusion conditions (Kirjoranta et al. 2012). Thus, new techniques have been recently developed for better characterizations of extruded products. X-ray microtomography has been used as an effective and convenient method in the study of cellular structures of extruded cereal products, without requiring a dye or complex sample preparation (Penttilä et al. 2011). The typical 3D reconstruction and microtomography image of extruded whole-grain barley flour in Figure 8.2 clearly shows small clusters of granules and air-filled micropores inside the solid matrix. The small cluster of granules was identified as some starch granules remaining after extrusion processing. Such microtomography image has allowed a better understanding of how the structure of the extrudates may have been affected by the additives and processing conditions. The porous structure of snacks prepared from barley, wheat, and corn was also observed by X-ray microtomography (Kirjoranta et al. 2012; Chanvrier et al. 2013). The images allow correlation of the highly porous structure with extrusion conditions; this could allow extrusion processing to be optimized to produce highly expanded snack products that are crisp and

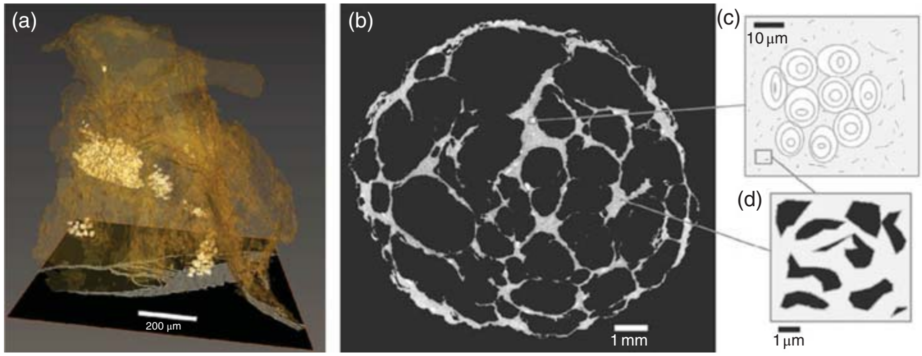

Figure 8.2 (a) 3D reconstruction of extruded whole-grain barley flour with whey protein isolate. The starch granules are rendered in white, showing their clustered distribution within the sample (voxel size 682 nm); (b) X-ray microtomography image of cross-section of extrudate; (c and d) Sketch of small cluster of granules and air-filled micropores inside the solid matrix. *Source*: Penttilä et al. 2011, Figs 6 & 8. Reproduced with permission of Springer Science+Business Media. (For a colour version, see Plate 6).

palatable. The image further revealed that the porosity of extruded cereal products decreased with the addition of oat bran or wheat bran.

A newly developed online rheometer was mounted on a twin-screw extruder in order to measure the rheological properties of extruded cereal products (Horvat et al. 2013). By applying this technology, it was possible to control the viscosity of wheat flour and corn grits at typical extrusion cooking conditions over a wide shear rate range. Flow curves for varying screw speeds and water content at a constant thermomechanical history of the starch were measured, and the temperature dependence of the apparent viscosities was also determined. Additional pressure drops that occurred at step changes of the slit height were detected. This research demonstrated the possibility of evaluating the viscoelastic properties of extruded melts at different conditions that are important to cereal and snack manufacturing.

Kaddour and Cuq (2011) reviewed the applications of near-infrared (NIR) spectroscopy in monitoring and describing physical and chemical modifications during wheat products processing. The ability of NIR spectroscopy to discriminate between the dough structure of fresh pasta produced by an extrusion process as well as to monitor changes in starch structure during extrusion cooking was introduced. However, in the last 5 years, this technology has mainly been applied in the pharmaceutical hot-melt extrusion.

8.3.3 Enzymatic Treatment

Enzymatic treatments, owing to their highly selective function and lower risk of undesirable side-reactions, have been widely used to improve nutritive value and functional properties of cereal components and products. Although the enzymatic modifications of cereal proteins, starch, and phytochemicals have been extensively reviewed, the aim of this section is to scrutinize the recent developments in the application of enzymes in cereal processing, and to summarize the knowledge generated from this research.

Protein Modification Storage proteins, including prolamin and glutelin, are the major proteins in cereal grains such as wheat, corn, and barley. Due to a large proportion of hydrophobic amino acids, cereal storage proteins are insoluble in aqueous solutions and easily form aggregates that interfere with functional properties. For the food industry, cereal storage proteins are normally produced as the by-products of food processing such as starch extraction and barley brewing. Utilization of these inexpensive by-products in food formulations requires modification techniques to improve their functional characteristics. Enzymatic modification of cereal proteins has been the focus of a large amount of recent research. Enzymatic hydrolysis and deamidation are the most widely used enzyme-based methods to improve cereal protein functionality and bioactivity. Therefore, these two methods will be addressed in this section.

Protein Hydrolysis Three main changes occur during the enzymatic hydrolysis of food proteins: (i) peptide bonds are broken down, lowering the molecular weight of proteins, (ii) protein surface properties are influenced by greater exposure of the naturally hidden hydrophobic residues, and (iii) some microchemical changes occur, such as deamidation of glutamine (Gln) and asparagine (Asn) residues that add extra charges to the protein (Dimitrijev-Dwyer et al. 2012). Many active sequences have been identified from cereal proteins with antihypertensive (Thewissen et al. 2011), antidiabetic (Lacroix and Li-Chan 2012; Velarde-Salcedo et al. 2013), hypercholesterolemic (Cavazos and de Mejia 2013), antioxidant (Bamdad et al. 2011; Cui et al. 2011; Bamdad and Chen 2013), immunomodulatory (Cavazos and de Mejia 2013), and lunasin-like anticancer activities (Maldonado-Cervantes et al. 2010).

Cereal proteins are sulfur-rich and contain repetitive sequences with a high content of proline (Pro) and Gln, making them a challenging substrate for the endoprotease enzymes (Tatham and Shewry 1995; Bamdad et al. 2011). Several pre-treatments have been used to increase the susceptibility of the protein to enzymes (Cui et al. 2011; Zhang et al. 2012). Cui et al. (2011) employed extrusion prior to pancreatin hydrolysis and observed a significant increase in the degree of hydrolysis as well as conformational changes. Zhang et al. (2012) heated wheat gluten at 120°C for 5 min before alcalase treatment. This heat treatment led to an increase of the degree of hydrolysis from 20% to 30% within 1 h of hydrolysis. Meanwhile, the percentage of the fraction with molecular weight (M_w) <1 kDa in the obtained hydrolysate increased from 17.6% to 30.7%. These treatments were associated with protein conformational changes (α-helix to β-turn and interruption). The altered structure further makes the active sites in wheat gluten protein more accessible to the enzymes.

Some antihypertensive peptides inhibit the angiotensin-I-converting enzyme (ACE) by competitive binding. Peptide sequences with hydrophobic residues at C-terminal have shown higher ACE inhibitory activity (Murray and FitzGerald 2007). Many antihypertensive peptides also possess Pro at their C-terminal position, and some of them contain arginine (Arg) or lysine (Lys) at the C-terminal. Gliadin may be a potential source of ACE inhibitory peptides, since gliadin polypeptides are composed of two distinct domains, with the central domain high in Pro and Gln, and the terminal domain high in hydrophobic residues. Lys and Arg residues are present in gliadin polypeptide structure as well. Thewissen et al. (2011) evaluated the ACE inhibitory activity of gliadin peptide fractions after purification based on Pro content. The terminal-domain-related peptides obtained from the two-step hydrolysis of gliadin with trypsin and thermolysin exhibited very high ACE inhibitory activity. Further purification using affinity chromatography resulted in a fraction with the lowest IC_{50} values of 0.02 mg/ml.

Protein hydrolysates are able to suppress oxidation reactions in a variety of ways. Peptides can inactivate reactive oxygen species (ROS), scavenge free radicals, chelate the pro-oxidant transition metal ions, as well as form physical barriers to separate ROS from food ingredients (Bamdad et al. 2011). Peptides containing hydrophobic residues have shown strong effects in retarding oxidation reactions. Barley hordein is rich in hydrophobic amino acids and Pro (Shewry and Halford 2002; Bamdad et al. 2011). In a recent study, the antioxidant capacity of hordein hydrolysates and their fractions (separated based on M_w and hydrophobicity) were evaluated, and the relationship between various antioxidant mechanisms and structural features of hordein peptides was investigated. Large-sized peptides (>10 kDa) demonstrated strong free-radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radicals, which was related to the highly exposed hydrophobic regions of the peptides obtained from partial hydrolysis of barley hordein. In the small-sized peptide fraction, the predominance of ionized carboxyl groups due to partial deamidation of Gln to glutamic acid (Glu) residues resulted in high metal chelation capacity. Peptide sequencing results revealed that intermittent Pro and Gln in the sequence of barley hordein and the pentapeptide QPYPQ might be considered as the particular structural motif responsible for high antioxidant effects of barley hordein hydrolysates (Shewry and Halford 2002; Bamdad and Chen 2013).

Enzymatic Deamidation Transglutaminase (TG), protein glutaminase (PG), and peptidoglutaminase are enzymes that catalyze deamidation reactions. Although the reaction mechanisms of these enzymes are the same, they exhibit different specificity toward the substrates. Transglutaminase as a transferase enzyme catalyzes the formation of an isopeptide bond between the carboxyl group of a gutamyl and primary amino group of a lysyl side chain. Protein glutaminase and peptidoglutaminase only attack glutamine residues, but not asparagine residues, preferably in proteins and short peptides, respectively (Chobert et al. 1996). From the chemical point of view, the loss of one amide group and replacement of a hydroxyl group adds more electrostatic charges to the protein molecule, which will result in partial protein unfolding and less aggregation. Structural evaluation of deamidated gliadin by Wong et al. (2012) has revealed a remarkable increase in β-sheet and unordered structures of gliadin upon deamidation. Partial rearrangement of protein combined with more exposed hydrophobic regions facilitated the adsorption of protein to the oil/water interface (Wong et al. 2012). Enzymatic deamidation of rice glutelin also resulted in transformation of α-helix to random coil structures and β-turns, which conferred more flexibility to the extended conformation of the deamidated protein (Liu et al. 2011). Enzymatic deamidation has been applied to modify cereal proteins for improved functionalities, such as increased solubility and emulsifying capacity (Zhao et al. 2011). In addition to the functional benefits, deamidation may improve the bioactivity of the modified protein. Enhanced iron-chelating capacity (Li and Zhao 2012) and higher antioxidant capacity (free-radical scavenging and reducing power) (Zhang et al. 2011) were reported for cereal proteins from different resources. Several investigations have shown that deamidation can either increase or decrease the immunoreactivity of the gluten, depending on the reaction condition and the extent of peptide bond scission (Kanerva et al. 2011). The possible challenge for the application of deamidated cereal proteins, particularly wheat gluten, in food formulation is the accuracy of immunoassay methods in gluten quantification. The immunoassays may not detect the deamidated gluten, due to the significant decrease in polypeptide length (Kanerva et al. 2011).

Carbohydrate Modification Starch and dietary fiber are the major components of carbohydrates in cereal grains. Starch is the main storage carbohydrate, whereas dietary fibers are the structural components of cereal bran. Resistant starch is the health-promoting fraction of starch with low glycemic index, which performs similar physiological functions as dietary fibers (Tharanathan 2005). High-amylose starch, particularly from corn, is the most important source of the native resistant starch (Tomasik and Horton 2012). Although resistant starch can be produced by physical methods (heat treatment of gelatinized starch under pressure), the enzymatic approach is more common, in which starch is first digested with α-amylase, gelatinized, and then debranched using pullulanase or isoamylase (Tomasik and Horton 2012). Incorporation of resistant starch into food formulations elevates the amount of fermentable carbohydrates without much influence on the sensorial attributes of the food product (Tharanathan 2005). Additionally, resistant starch has shown inhibitory activity toward α-amylase. Therefore, resistant starch not only improves the microbial flora of the gut, but also hinders starch digestion, which decreases the glycemic index of resistant-starch-containing foods (Bustos et al. 2012).

Dietary fiber is mostly found in the cell wall polysaccharides of cereal grains with arabinoxylan (AX), β-glucan, cellulose, and lignin as the major components (Lebesi and Tzia 2012). Dietary fiber is conventionally classified in two categories according to its water solubility: insoluble dietary fiber and soluble dietary fiber. From a nutritional point of view, it is recommended to increase the intake of dietary fiber, particularly of soluble dietary fiber. Conversion of barley and wheat insoluble dietary fiber into soluble dietary fiber has recently been achieved by enzymatic hydrolysis, using enzymes produced by *Trichoderma* species, which are efficient in generating chitinases, glucanases, xylanases, and cellulases (Napolitano et al. 2006). Various studies have demonstrated that it is possible to triple the amount of soluble dietary fiber without a marked decrease of total dietary fiber (Vitaglione et al. 2008). The enzymatic treatment also causes the release of the phenolic compounds that are linked to the polysaccharide chains. In particular, 40% of the total ferulic acid present in wheat dietary fiber was found in the soluble fraction after enzymatic treatment using optimized hydrolysis conditions (Napolitano et al. 2006). This increases the free phenolic concentration, and, in turn, the phenol compound bioavailability. Further investigation showed that the converted soluble dietary fiber could generate a gut microbial fermentation that supported bifidobacteria and lactobacilli (Napolitano et al. 2009). The concurrent increase in free ferulic acid could also result in a higher plasma ferulic acid concentration, which could be one of the reasons for the health benefits reported for dietary fiber in cardiovascular diseases (Vitaglione et al. 2008; Napolitano et al. 2009).

8.3.4 Fermentation

Fermentation is one of the oldest processes used by humans to improve shelf life and sensorial quality of cereal products. The preservative role of fermentation depends on chemical conversion of cereal components, specifically carbohydrates, to alcohol, acid, and carbon dioxide. Fermentative activities of microorganisms have been classified, based on the end-products, into four main categories: alcoholic, lactic acid, acetic acid, and alkali fermentation (Caplice and Fitzgerald 1999; Blandino et al. 2003). The first three classes of fermentation are the most popular processes in cereal-based fermented foods. Although most traditional fermented foods are obtained by using indigenous microflora, recent advances in microbiology and food processing have provided the opportunity to utilize fermentative microorganisms as starter cultures, and the ability to trigger and carefully control the fermentation process, thus eventually improving the nutritional and health-enhancing properties of the final product.

Probiotics and Prebiotics According to the Joint FAO/WHO Working Group, probiotics are "live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host" (Anonymous 2002). These microorganisms improve the balance of intestinal microbiota by changing the intestinal pH and generating antimicrobial compounds such as bacteriocins, organic acids, and hydrogen peroxide (Leroy and De Vuyst 2004; Gaenzle 2009; Lamsal and Faubion 2009; Moroni et al. 2009). The main bacterial cultures presented as probiotics are the lactobacilli, bifidobacteria, and eubacteria genera (Lamsal and Faubion 2009; Gobbetti et al. 2010). Cereals and pseudo-cereals (amaranth and buckwheat) have been used as fermentation media for probiotic strains of lactic acid bacteria. Lactic acid production and culture density studies have demonstrated the potential of these substrates in supporting probiotic growth and metabolism (Kockova et al. 2013). Probiotic starter cultures possess proteolytic activity, enabling them to either synthesize or release bioactive peptides from the cereal proteins. Certain species of lactobacilli are highly proteolytic and liberate bioactive peptides with a wide range of bioactivities (e.g., antihypertensive, antioxidant, antimicrobial, and opioid), depending on the bacteria and protein substrate (Gobbetti et al. 2010).

The positive contribution of these bacteria in intestinal health is highly affected by the presence of prebiotics. Prebiotics are a wide class of oligosaccharides that are indigestible but fermentable by intestinal microflora (Gaenzle 2009; Lamsal and Faubion 2009; Gobbetti et al. 2010). Structural characteristics of these oligosaccharides protect them from digestion by mammalian enzymes in the stomach and small intestine, while saccharolytic bacteria are able to ferment them in the large intestine (Delcour et al. 2012). Acid production following fermentation significantly reduces the pathogenic bacteria and shifts the balance toward valuable probiotics (Lamsal and Faubion 2009). Prebiotic oligosaccharides can be released from arabinoxylan, the major dietary fiber component of wheat bran. Accordingly, bran-enriched bread is a suitable source of prebiotic oligosaccharides; however, a strong taste and the undesirable texture of bran particles restrict their use in food formulations. Processing bran using fermentation and/or enzyme treatment prior to bread making has improved bread volume and crumb softness, and enhanced the concentration of soluble fiber and biologically active components (Delcour et al. 2012; Katina et al. 2007).

Effect of Fermentation on Nutritional Quality Cereal grain, particularly the outer layers of cell wall and bran, contain a wide range of phytochemicals, including phenolic compounds, folate, flavonoids, and phytoestrogens

(Dordevic et al. 2010; Gupta et al. 2010). However, most of these phenolic compounds are covalently bound to the indigestible polysaccharides of plant cell wall, and thus have low bioaccessibility (Anson et al. 2011; Delcour et al. 2012). Fermentation improves both the level of phenolic compound bioaccessibility and their antioxidant capacity. Ferulic acid, the major phenolic compound in wheat grain, showed significantly greater bioaccessibility in an *in vitro* model of the human colon following a combinatory bioprocessing involving yeast fermentation and cell-wall hydrolyzing by enzyme treatment (Anson et al. 2009). Similarly, an *in vivo* study, conducted by the same research team, confirmed the higher concentration of ferulic acid in plasma, after ingestion of the bioprocessed bread, which was correlated to the Trolox-equivalent antioxidant capacity of plasma. The effect of fermentation on folate has been the subject of several studies. Results have shown enhancement of folate delivery to blood circulation as a result of yeast fermentation of cereals (Poutanen et al. 2009). Thiamine and riboflavin content reportedly decreased during baking, but yeast fermentation prior to baking elevated the total retention of thiamine in fermented bread (Batifoulier et al. 2005; Poutanen et al. 2009). However, losses have been reported for vitamin E, tocopherol, and tocotrienol content due to oxidation during sourdough preparation and bread baking (Poutanen et al. 2009).

Fermentation in Gluten-Free Cereal Products In celiac disease, the inflammatory immune response is triggered upon ingestion of prolamin proteins from wheat, barley, and rye. During digestion, a family of peptides rich in Pro and Gln are released. These peptides bind to the antigen-presenting cells, which results in antibody production (Demin et al. 2013; Dubois et al. 2010; Moroni et al. 2009; Zannini et al. 2012). Over the last decades, various gluten-free formulations with more focus on bread dough have been evaluated, and several methods were proposed to overcome some technological and nutritional issues. Some formulations, for example, are mainly based on starch; these products suffer from fast staling and low nutritional value. Non-cereal protein additives such as skim milk powder or egg proteins are added to the formulation to improve the nutritional quality and rheological properties of the bread. Modification of protein digestion through proteolysis seems to be the most effective method to reduce the allergenicity of the gluten. However, this would compromise the viscoelastic properties of the protein network, and the approach (i.e., to reduce allergenicity *in vivo*) is not well demonstrated. Preliminary proteolysis in this case, in some studies, was performed by proteases originated from the wheat or rye flour (Moroni et al. 2009). These proteinases are activated in the low-pH condition and hydrolyze the gluten into peptides. Further hydrolysis of large peptides into short peptides and amino acids was performed by intracellular peptidases that are released by lactic acid bacteria. Although the degree of hydrolysis of various gliadin fractions (α-, β-, γ-, ω-gliadins) is very strain-specific, several studies conducted by Di Cagno et al. (Di Cagno et al. 2002; Di Cagno et al. 2006) proved that prolonged fermentation of a mixture of wheat and non-toxic flours with certain proteolytic strains of lactic acid bacteria (e.g., *Lb. alimentarius*15M, *Lb. brevis* 14G, *Lb. sanfranciscensis* 7A, and *Lb. hilgardii* 51B) are able to efficiently hydrolyze the highly toxic (33-mer) fragment of gliadin. Selected strains of lactic acid bacteria (LAB) reportedly reduced the allergenicity of gluten in rye flour (De Angelis et al. 2006).

8.3.5 Microparticles and Nanoparticles Based on Cereal Proteins for Nutraceutical Delivery

In the last few years, encapsulation techniques using cereal proteins have been developed as nutraceutical delivery systems to create novel functional foods. Aside from being a vital macronutrient in food, proteins possess unique functional properties, including their ability to form gels, films, and emulsions, offering the possibility of developing delivery systems for both hydrophilic and lipophilic bioactive compounds (Chen et al. 2006a). In past decades, zein and gliadin have been prepared into microparticles and nanoparticles, incorporating unsaturated fatty acids, vitamins, minerals, essential oils, antioxidants, herb extracts, as well as bioactive protein/peptides (Duclairoir et al. 2002; Duclairoir et al. 2003; Jin et al. 2008; Luo et al. 2010; Kajal and Misra 2011; Gomez-Estaca et al. 2012; Luo et al. 2012; Wu et al. 2012; Zou et al. 2012; Zou and Gu 2013). Hydrophilic compounds are released from the protein matrix by diffusion, whereas lipophilic compounds are released mainly by enzymatic degradation of the protein matrix in the gastrointestinal (GI) tract (Chen and Subirade 2009). This section will focus on our recent progress in the development of nutraceutical delivery systems based on barley proteins, of which hordein and glutelin are the two major fractions.

Barley Protein Microparticles The emulsifying–stabilization process has been applied to prepare microparticles using barley protein as the wall material. This process normally involves an initial step to form emulsions in which the protein wall material acts as a stabilizer for the core lipid. In the second step, the protein wall materials are solidified by adding a cross-linking reagent (e.g., glutaraldehyde, transglutaminase), or by coacervating with oppositely charged polymers. These stabilized microparticles can then be converted into free-flowing powders using a spray-drying technique (Subirade and Chen 2008). Interestingly, well-suspended solid microparticles, rather than emulsions, are formed from barley protein immediately after high-pressure treatment (Wang et al. 2011a). This phenomenon is different from that observed for the emulsion systems stabilized by globular proteins (whey and soy protein), where the emulsions only form soluble aggregates via surface

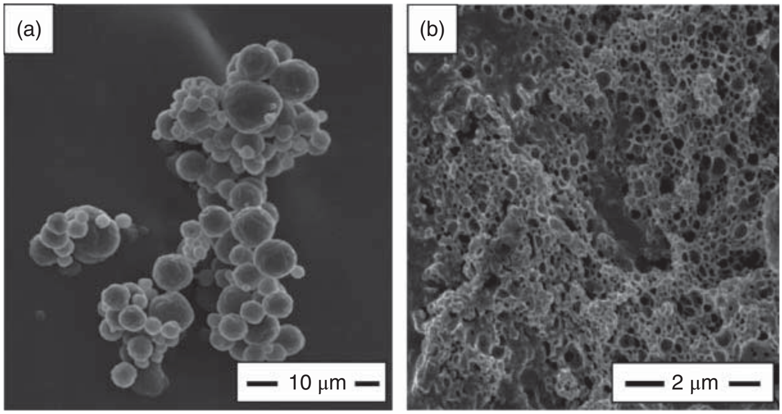

Figure 8.3 (a) Surface and (b) interior morphology of spray-dried barley protein microparticles by scanning electron microscopy (SEM). *Source*: Wang et al. 2011s. Reproduced with permission of Elsevier.

hydrophobic interactions after high-pressure treatment (Beaulieu et al. 2002; Floury et al. 2002), due to the hydrophobic nature of their molecular structures that are enriched with non-polar amino acids (~35–38%). This unique behavior is quite favorable from an industry point of view for the mass production of micro-encapsulations. Processing can be simplified by removing the cross-linking or the coacervation processing, and toxic or expensive cross-linking reagents are not necessary. Scanning electron microscope (SEM) photographs of spray-dried barley protein microparticles are shown in Figure 8.3. These particles demonstrated a spherical shape, with diameters ranging from 3 to 5 μm, and possessed dense, crack-free, and smooth surfaces (Figure 8.3a). The small pores (Figure 8.3b) inside the microparticles indicate that oil droplets were well separated within the protein micron-matrix. The prepared barley protein microparticles exhibited high encapsulation efficiency (92.9–97.0%) for selected nutraceutical models, including β-carotene and fish oil, with a payload of around 50%. The oxidative stability test results indicated that barley-protein-microencapsulated fish oil had low oxidative levels after 8 weeks of accelerated storage under dry conditions and in suspension (Wang et al. 2011b). This suggests that barley protein microcapsules may be suitable for liquid/semi-liquid food applications. The stability of the barley protein microparticles was then tested in two food products, including a fat-free milk and a yogurt. The peroxide value (PV) of the encapsulated fish oil was measured weekly for milk and yogurt at 4 and 5 weeks, respectively, corresponding to their average shelf life. As shown in Figure 8.4, the PV of encapsulated fish oil remained low ($PV < 10$ meq peroxide/kg oil) in both milk and yogurt during storage.

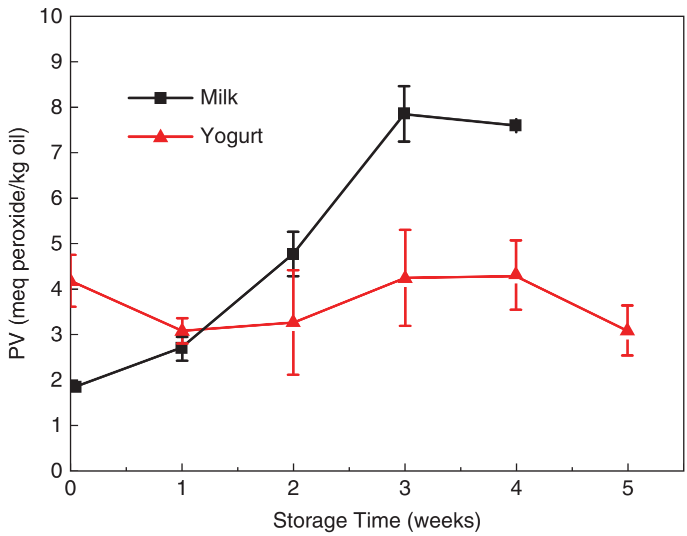

Figure 8.4 Peroxide value (PV) changes for encapsulated fish oil in barley protein microcapsules in two food formulations (milk and yogurt). *Source*: Wang et al. 2011b. Reproduced with permission of Elsevier.

The fish oil microcapsules were especially stable in yogurt, with PV levels below 5 meq peroxide/kg oil even after 5 weeks. It has been recommended that PV levels should not exceed 30 meq peroxide/kg oil in an edible food product (Naohiro and Shun 2006). Thus, both the accelerated oxidation experiment and the stability test in food formulations suggest that barley protein is an excellent candidate coating material to encapsulate fish oil and protect it from oxidation.

The release properties of barley protein microparticles were investigated in the simulated gastrointestinal tract using β-carotene as a model nutraceutical. In the simulated gastric fluid with pepsin, β-carotene was slowly released from barley protein microparticles, and less than 5% β-carotene was detected in the release medium after 2 h of the test. In the simulated intestinal fluid with pancreatin, β-carotene was steadily released from the microparticles at almost zero-order release kinetics in the first 2 h. Over time, the release curve leveled off gradually, until after 6 h when 91.6% of the β-carotene had been released. The nature of these barley protein microparticles to retard β-carotene release in the simulated gastric fluid for 2 h increases the likelihood of bioactive compounds reaching the intestine for absorption in an intact and active condition. Also, the near-zero-order release kinetics of β-carotene in the simulated intestinal tract in the first 2 h would enhance their absorption in the small intestine.

The degraded barley protein microparticles were then observed using transmission electron microscopy (TEM), after samples had been incubated in simulated gastrointestinal tract fluid with digestive enzymes (Wang et al. 2011a). Nanoparticles with average sizes between 20 and 30 nm predominated as a result of microparticle bulk matrix degradation when incubated in the simulated gastric fluid for 30 min (Figure 8.5a). After 1 h of incubation, bulk matrices disappeared, with mono-dispersed nanoparticles remaining in the release medium (Figure 8.5b). In order to test whether these nanoparticles could be transferred into the simulated intestinal tract without aggregation, their stability was further studied in pH 7.4 buffer without pancreatin. The liberated nanoparticles were still well-dispersed in pH 7.4 buffer within 30 min, as observed by TEM. Some aggregation did occur after 2 h of incubation in pH 7.4 buffer; however, most of the particles exhibited a size of 50–250 nm (Figure 8.5c). Interestingly, in the simulated

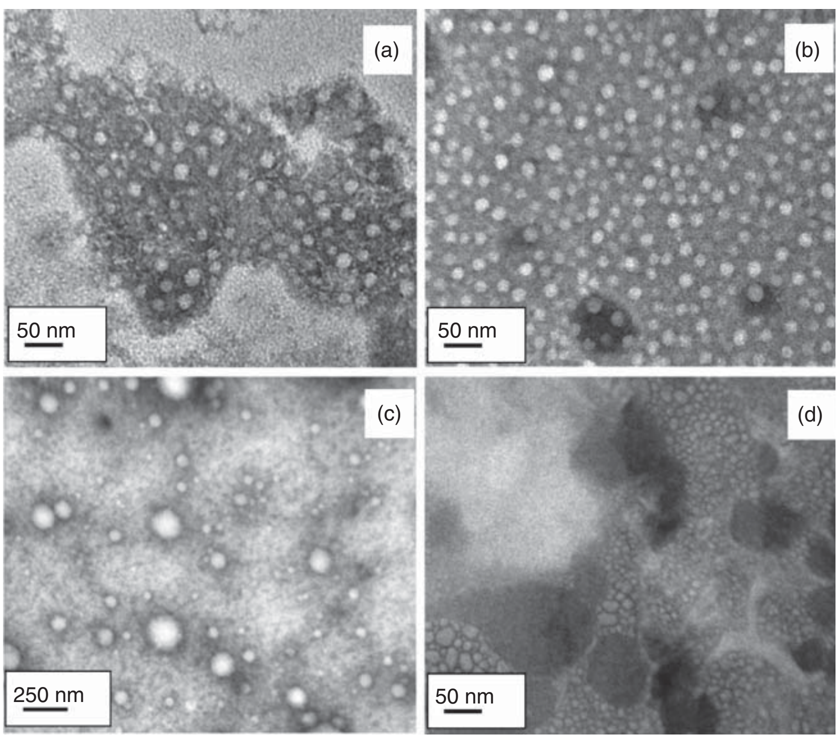

Figure 8.5 Morphology changes of barley protein microparticles in the simulated gastrointestinal tract by transmission electron microscopy (TEM): nanoparticles observed after incubating microparticles in the simulated gastric fluid with pepsin (a) for 30 min and (b) for 1 h, and nanoparticles incubated in the simulated intestinal fluid (c) without pancreatin for 2 h, and (d) with pancreatin for 6 h. *Source*: Wang et al. 2011s. Reproduced with permission of Elsevier.

intestinal fluid with pancreatin, the microparticles were degraded within 6 h of incubation, leaving well-dispersed nanoemulsions in the SIF medium. Figure 8.5d shows emulsions released from nanoparticles. This phenomenon of obtaining nanoparticles from the degradation of a protein matrix has never been reported previously. This unique degradation behavior of barley protein microparticles is likely related to the special structure of the protein layer directly coating the nanoparticles. This protein resists pepsin degradation and stabilizes incorporated oil droplets. When transferred into simulated intestinal fluid, this protein layer was hydrolyzed by pancreatin to release the nanoemulsions containing β-carotene. Two main reasons may explain at least part of this interesting property of degradation resistance. First, proteins with high proline content were identified in this coating layer, which are generally more resistant to degradation by digestive enzymes (Simpson 2001). Second, the majority of pepsin-labile hydrophobic amino acid groups in the protein chains were likely buried inside the matrix, leaving hydrophilic groups outside. Thus, the protein layer coating the oil droplet represented a less vulnerable substrate to pepsin digestion (Morr and Ha 1993; Chen and Subirade 2006b). These nanoparticles were then degraded by pancreatin, which is a mixture of several digestive enzymes produced by the exocrine cells of the pancreas (amylase, lipase, and proteases). These enzymes could breakdown the protein–lipid and protein–protein interactions, and therefore interrupt protein aggregation structures in the micro-particle and nanoparticle matrices. Thus, nanoemulsions incorporating β-carotene were gradually released during the 6 h of the test. These results suggest that these uniquely structured microparticles may provide a new strategy for the nutraceutical industries to develop intestinal-targeted delivery systems for lipophilic bioactive compounds. Additionally, they can be prepared by a simple and convenient process without the addition of organic solvents or surfactants.

Barley Protein Nanoparticles Nanoencapsulation of nutraceuticals is emerging as a promising approach for delivering health-promoting substances to wide populations without harming the sensory quality of food, while providing benefits of protection and improved bioavailability. Nanoparticles can dramatically prolong the residence time of bioactive formulations by decreasing the influence of intestinal clearance mechanisms and by increasing the surface area to interact with the biological support. In addition, some of them are small enough to cross the epithelial lining of the gut and be readily taken up by cells, allowing efficient delivery of active compounds to target sites in the body. More recently, the new technology developed for barley protein microparticles has been adapted to prepare barley protein nanoparticles of 90–200 nm (Yang et al. 2014). Interesting degradation profiles were also observed in the simulated gastrointestinal tract as even smaller particles (20–50 nm) were formed as a result of pepsin degradation of barley protein nanoparticle matrices. These smaller nanoparticles provided sufficient protection of the model nutrient in the simulated gastric fluid. After 1 hour of incubation in simulated intestinal fluid, the liberated nanoparticles were further digested into nano-sized lipid droplets, indicating that the barley protein solid coating was further degraded by pancreatin. As an example, studies have reported that β-carotene is likely to be absorbed through the small intestine in the form of mixed micelles and/or vessels (Yang et al. 2014). Barley protein nanoparticles may have the potential to improve the adsorption of β-carotene in the small intestine. Both the cytotoxicity and the cell uptake of barley-protein-based nanoparticles were evaluated on a Caco-2 intestinal cell line. Barley protein nanoparticles were biocompatible and had low toxicity even at high concentrations. In addition, they can be effectively internalized and accumulated in the Caco-2 cell cytoplasm. Thus, these barley protein nanoparticles have strong potential to be used as delivery systems of bioactive compounds to improve their bioavailability. Also, this study provides meaningful justification for further *in vivo* studies to evaluate the safety and efficacy of barley protein nanoparticles as a delivery system (Yang et al. 2014).

8.4 Future Prospects

Cereal grains represent a vital and dynamic commodity that plays an important role in nourishing an ever increasing world population. They are the most important staple food in most countries. Intense competition among food companies to address ever-evolving and sophisticated consumer expectations is driving the development of health-promoting cereal foods and ingredients. Due to their unique nutritional nature, whole-grain-based food products and foods fortified with phytochemicals are likely to continue to be the subject of much research attention. These new foods will not only improve the nutrition profile of the diet, but will also potentially provide benefits such as the prevention and treatment of some diseases. To realize this opportunity, new processing technologies are needed, as well as efforts to improve existing technologies and processing efficiency. Methods would be required that address both the separation and concentration of nutritional components in the raw materials, minimize negative processing effects on functional properties, and achieve a controlled release or targeted delivery of bioactive compounds. Several practical questions must further be considered before new ingredients are added into traditional cereal products, such as: Will the new ingredient(s) affect taste, aroma, texture, or appearance of original foods? Will they degrade, oxidize, or react with other ingredients and finally limit or lose their bioactivity after cooking? Finally, since the food industry is rigorously and strictly regulated, *in vitro* and *in vivo* studies to assess the bioavailability and toxicology of bioactive components, including their digested products and regulation of the intake of these functional components, will be required.

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9

Novel Approaches to Enhance the Functionality of Fermented Foods

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9.1 Introduction

Fermented foods have a long and culturally diverse history. Spanning several thousands of years, fermentation is a processing technology initially utilized as a food preservative method by employing microbial activity on a range of metabolites. Common microflora associated with fermentation in the food industry include *Lactobacillus spp*., *Bifidobacterium spp*., *Streptococcus spp*., *Saccharomyces spp*., *Rhizopus spp*., *Aspergillus spp*., *Mucor spp*., and *Actinomucor spp*. The ecology of fermented food products is invariably dependent on the water activity, pH, salt concentration, temperature, and substrate composition. Fermentation has also been applied in foods to produce desirable biochemical changes such as conversion of carbohydrates, protein and essential amino acid enhancement, removal of antinutrients, improvement of organoleptic properties (i.e., taste, flavour, aroma, texture) and shelf-life extension through the use of indigenous microflora present in the food substrate or the addition of starter cultures (Amoa-Awua et al. 1997; Blandino et al. 2003; Ávila et al. 2009). To achieve this, strain-specific manipulation, optimization of processing conditions (i.e. temperature, substrate, time) and fermentation process engineering are employed singly or in combination (Knorr, 1998). Recent studies indicate that the artisanal

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and industrial application of novel approaches to probiotic, prebiotic, symbiotic and other microfloral systems have resulted in the enhancement of the functional attributes of food products and ingredients (Roberfroid, 2000; Charalampopoulos et al. 2002; Puuponen- Pimiä et al. 2002; Stanton et al. 2005; Arihara, 2006; Rodríguez et al. 2006). A comparative study by Borresen et al. (2012) depicted the increasing awareness and concomitant scientific studies related to "fermentation and health" between 1959 and 2009 that were cited in PubMed Central. Common fermented foods consumed include fermented milk products (e.g., cultured milk and/or buttermilk, sour cream, cheese, yogurt), fermented vegetables (sauerkraut, pickles and olives), alcoholic beverages (beer, wine, hard cider) and fermented meats and fish. Fermented foods have enhanced preservation, nutritional value, functionality, organoleptic properties and economic value. Table 9.1 summarizes common fermented food products found globally as reported by the Food and Agricultural Organization (FAO) of the United Nations. This chapter aims to identify and describe some novel approaches to fermented functional foods, and how these techniques enhance the functionality of such foods. In so doing, we aim to address the technologies associated with nutraceutical production of fermented foods and the associated health benefits and functions of fermented food products.

9.2 Starter Culture for Fermented Food Production

Traditional fermentation techniques use bacterial, fungal (either as single- or multiple-strain) or mixed microflora to produce the desired attributes in fermented food products. Holzapfel (2002) reported that several fermented food groups applied single-strain cultures, compared to the fewer groups that used multiple-strain (sour dough, wine, dry sausage, dairy products), and mixed strain cultures (sour dough, dairy products) in industrial food production. Over the past 50 years, the growth in industrial food production utilizing fermentation has promoted the optimization and engineering of strain-specific microflora as well as the environmental and processing conditions required to achieve and enhance the quality, shelf-life and functional properties of fermented food products. Recently, the design and use of functional starter cultures in the fermentation industry has been explored (Hansen, E.B., 2002; Leroy and De Vuyst, 2004).

9.2.1 Bacterial Fermentation of Foods

Some bacterial strains are selected for their enhancement of organoleptic properties, whereas others are used during fermentation for their health and nutraceutical functions. Common genera of bacteria used in industrial

Table 9.1 Examples of fermented food products found globally

Region and name of fermented products	Type of product
Indian Sub-continent	
Achar, tandal achar, garam nimboo achar	Pickled fruit and vegetables
gundruk, lemon pickle, lime pickle, mango pickle	Fermented dried vegetable
South East Asia	
Asinan, burong manga, dalok, jeruk, kiam-chai, kiam-cheyi, kong-chai, naw-maidong, pak-siam-dong, paw-tsay, phak-dong, phonlami-dong, sajur asin, sambal tempojak, santol, si-sek-chai, sunki, tang-chai, tempoyak, vanilla	Pickled fruit and vegetables
Bai-ming, leppet-so, miang	Fermented tea leaves
Nata de coco, nata de pina	Fermented fruit juice
East Asia	
Bossam-kimchi, chonggak-kimchi, dan moogi, dongchimi, kachdoo kigactuki, kakduggi, kimchi, mootsanji, muchung-kimchi, oigee, oiji, oiso baegi, tongbaechu-kimchi, tongkimchi, totkal kimchi	Fermented in brine
Cha-ts-ai, hiroshimana, jangagee, nara senkei, narazuke, nozawana, nukamiso-zuke, omizuke, pow tsai, red in snow, seokbakji, shiozuke, szechwan cabbage, tai-tan-tsoi, takana, takuan, tsa tzai, tsu, umeboshi, wasabi-zuke, yen tsai	Pickled fruit and vegetables
Hot pepper sauce	
Africa	
Fruit vinegar	Vinegar
Hot pepper sauce	
Lamoun makbouss, mauoloh, msir, mslalla, olive	Pickled fruit and vegetables
Oilseeds, ogili, ogiri, hibiscus seed	Fermented fruit, vegetable seeds
Wines	Fermented fruits
Americas	
Cucumber pickles, dill pickles, olives, sauerkraut	Pickled fruit and vegetables
Lupin seed, oilseeds	Pickled oilseed
Vanilla, wines	Fermented fruit and vegetable
Middle East	
Kushuk	Fermented fruit and vegetables
Lamoun makbouss, mekhalel, olies, torshi, tursu	Pickled fruit and vegetables
Wines	Fermented fruits
Europe and World	
Mushrooms, yeast	Moulds
Olives, sauerkohl, sauerruben	Pickled fruit and vegetables
Grape vinegar, wine vinegar	Vinegar
Wines, citron	Fermented fruits

Source: Food and Agriculture Organization of the United Nations, 2011, Marshall E and Mejia D, Traditional fermented food and beverages for improved livelihoods. FAO Diversification Booklet 21. Reproduced with permission.

fermentation include *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Bacillus*, *Streptococcus*, *Staphyloccocus*, *Acetobacter*, *Micrococcus*, *Brevibacterium*, *Propionibacterium* and *Leuconostoc* (Knorr, 1998; Charalampopoulos et al. 2002; Holzapfel, 2002; Ross et al. 2002; Blandino et al. 2003; Leroy and De Vuyst, 2004; Hutchkins, 2006; Borresen et al. 2012). Leroy and De Vuyst (2004) reviewed functional starter cultures and their advantages in the food industry. These were categorized in terms of food preservation, and organoleptic, technological and nutritional benefits (Table 9.2). Their work identified specific strains of *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Oenococcus*, *Pediococcus* and *Streptococcus* that were responsible for functionally enhancing attributes imparted to fermented food products during industrial production.

9.2.2 Fungal Fermentation of Foods

Alcoholic beverages (e.g., wine, beer, traditional fermented drinks and hard cider), breads (sourdough), fermented soy products (e.g., soy sauce, tempeh and miso) and mould-ripened cheeses are examples of classes of fermented products that are produced through the metabolic action of yeasts and moulds. The genera of yeasts and moulds used in fermented food production include *Saccharomyces*, *Candida*, *Rhizopus*, *Aspergillus*, *Penicilium* and *Monsacus*. Several species and subspecies of selected fungal microflora have been identified as responsible for functional attributes such as preservation, production of anti-microbial agents, improved organoleptic properties including flavour and texture enhancement, modification in nutritional properties and probiotic effects in many traditional fermented food products (de Vos, 2001; Holzapfel, 2002; Jespersen, 2003; Jespersen et al. 2005; Nout and Kiers, 2005; van der Aa Kühlea et al. 2005; Hutchkins, 2006; Obodai and Dodd, 2006; Nip, 2007; Stringini et al. 2009; Padonou et al. 2010; Ryan et al. 2011). Identified fungal species include *Saccharomyces cerevisiae*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Mucor spp*., *Aspergillus niger*, *Candida krusei*, *Candida tropicalis*, *Pichia fermentans*, *Pichia galeiformis*, *Torulaspora delbrueckii*, *Saccharomyces pombe* and *Trichosporon asahii*, to mention a few.

9.3 Functionality of Fermented Foods

The functional food industry is a growing segment of the consumer market that is driven by the perception of, as well as clinically proven studies on, health and wellness benefits of foods rich in nutritional components. The present trajectory of this market segment is partly focused on probiotic and prebiotic food products, the development of readily available dietary

Table 9.2 Starter cultures and corresponding functional enhancements in fermented foods

Enhancement	Functionality	Lactic acid bacteria[a]
Food preservation	Bacteriocin production	
	Dairy products	*L. lactis subsp. lactis* *Enterococcus spp*
	Fermented meats	*Lb. curvatus, Lb. sakei, P. acidilactici, E. faecium*
	Fermented olives	*L. plantarum*
	Fermented vegetables	*L. lactis*
Organoleptic	Production of exopolysaccharides	*Several lactobacilli and streptococci*
	Production of amylase	*Several lactobacilli*
	Aroma generation	*Several strains*
	Enhanced sweetness	
	Homoalanine-fermenting starters	*L. lactis*[b]
	Galactose-positive/glucose-negative starters	*Lb. delbrueckii subsp. bulgaricus, S. thermophilus*
	Malolactic fermentation	*O. oeni*
Technological	Bacteriophage resistance	Several strains
	Prevention of overacidification in yogurt	Lactose-negative *Lb. delbrueckii subsp. bulgaricus*
	Autolysing starters	
	Phage-mediated	*L. lactis subsp. lactis*
	Bacteriocin-induced	*L. lactis*
Nutritional	Production of nutraceuticals	
	Low-calorie sugars (e.g., sorbitol, mannitol)	*Lb. plantarum, L. lactis*
	Production of oligosaccharides	*L. lactis*
	Production of B-group vitamins (e.g., folic acid)	*Lb. delbrueckii subsp. bulgaricus, S. thermophilus*
	Release of bioactive peptides	Several strains
	Reduction of toxic and anti-nutritional compounds	
	Production of L(+)-lactic acid isomer	L(+) lactic acid producing strains
	Removal of lactose and galactose	*S. thermophilus*
	Removal of raffinose in soy	Several strains
	Reduction of phytic acid content, amylase inhibitors and polyphenolic compounds	*Lb. plantarum, Lb. acidophilus*
	Decreased production of biogenic amines	*E. faecalis*

[a]E, Enterococcus, L, Lactococcus, Lb, Lactobacillus, O, Oenococcus, P, Pediococcus, S, Streptoccus;
[b]Recombinant Strain

Source: Reprinted from Leroy and de Vuyst, Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science* & *Technology*, **15**, 67–78, Copyright 2004, with permission from Elsevier.

supplementation as well as the growing "smart" food market within the agri-health and agri-innovation domains. Research into the functional attributes of fermented foods include the identification of health functions, organoleptic enhancement, nutritional modification, and shelf-life extension, as a result of metabolic changes due to microbial activity or a combination of microbial selection, metabolic engineering and optimization of process controls to achieve the desired benefits.

9.3.1 Shelf-life Extension

Biopreservation of fermented foods depends on the biological activities of microflora that produce a range of metabolites to suppress the growth, proliferation and survival of food spoilage microorganisms and food-borne pathogens. Antimicrobial metabolites produced by lactic acid bacteria include organic acids such as lactic and propionic acids, ethanol from the heterofermentative pathway, hydrogen peroxide produced during aerobic growth, diacetyl generated from excess pyruvate from citrate metabolism, reuterin, reuterocylclin, and Class I (lantibiotics, e.g., nisin and lactin 3147), Class II (small non-modified peptides classified as Class IIa and Class IIb), Class III (large heat-labile proteins, e.g., Helvetin J and enterolysin) and Class IV bacteriocins (Ross et al. 2002; Caplice and Fitzgerald, 1999; Garneau et al. 2002). Bacteriocins have been identified as peptides produced in the ribosomes of lactic acid bacteria that possess antimicrobial activity against other Gram-positive bacteria (Garneau et al., 2002; Foulquié Moreno et al., 2006). *Streptococcus macedonicus* ACA-DC 198 was discovered to produce the food-grade lantibiotic, macedocin, which has inhibitory functions against some lactic acid bacteria and *Clostridium tyrobutyricum*, under optimal conditions of temperature and pH corresponding to that during cheese making (Van den Berghe et al., 2006). Garneau et al. (2002) have reported on the applications in food preservation of the two-peptide bacteriocins enterocin, Lacticin 3147, Lactocin 705 and Plantaricin S in food products, including meat, dairy and vegetable products. In other studies, enterococci were found to function as bacteriocin-producing microbes against spoilage and pathogenic bacteria such as *Listeria*, in addition to having some probiotic functions (Foulquié Moreno et al. 2006). Several organic acids, including acetic, caroic, formic, propionic and butyric acids, have been identified to have anti-mould effects on bread and sourdoughs containing lactobacilli starter cultures. *Lactobacillus fermentum* was found to significantly delay mould growth during storage of bread, and hence extend shelf-life through the production of fermentocin B, a bacteriocin (Fazeli et al. 2004). Recently, *Bacillus subtilis* was discovered to enhance the shelf-life at 96 h of fermentation in "Ogiri," a Nigerian fermented condiment, due to the hydrolysis and biochemical metabolism resulting in the formation of high concentrations of the organic acids acetic acid, butyric acid, citric acid and formic acid (Ojinnaka et al. 2013). Similarly, inhibitory functions pertaining to lowering the pH of acid-fermented vegetables, fish and meat, bread and noodles to below pH 4 through acid production as well as bacteriocin production are preservative functions exhibited by some lactic acid bacteria such as *Leuconostoc*, *Streptococcus*, *Lactobacillus*, *Pediococcus*, and yeasts and moulds including *Saccharomyces*, *Candida*, *Mucor and Rhizopus* species in many Asian and African fermented foods (Chelule et al. 2010; Rhee et al. 2011).

9.3.2 Health Functions

"*Let food be thy medicine and medicine thy food*"
Hippocrates.

Functional food fermentation results in the production of various bioactive molecules that impart health benefits to consumers. Notably, probiotic and antioxidant functions have been identified to be enhanced in foods containing specific microflora. The severity and extent of most human diseases is significantly impacted by oxidative damage of cells, including but not limited to cancer, atherosclerosis, arthritis and emphysema. Antioxidant activity may be used to help reduce oxidative damage in human cells. Fermented soy broth containing *Acetobacter spp*., *Lactobacillus spp*., *Saccharomyces spp*., *and Streptomyces spp*. has been documented to show increased scavenging effect on, 1,1-diphenyl-2-picrylhydrazl (DPPH) radicals and superoxide anion radicals than plain soybean broth (Yang et al. 2000). Black bean koji, solid-state fermented by *Aspergillus awamori* for a period of 3 days at 30 °C, contained high amounts of total phenolics and anthocynanins content, in addition to exhibiting high Fe^{2+}-chelating ability and 2,2-diphenyl-1-picrylhydrazl (DPPH) radical scavenging effect (Lee et al. 2007). Similar studies by Lee et al. (2008) showed enhanced antioxidant activity in black bean fermented in solid state using multiple filamentous fungi culture, and the enhanced activity occurred possibly due to the corresponding increase in total phenolic and anthocyanin contents. Fermentation also enhanced the total phenolic and flavonoid content as well as antioxidant activity of black soybean extract obtained by solid-state fermentation of black soybeans with *Bacillus subtilis* BCRC 14715 (Juan and Chou, 2010). Recently, fermentation has also been attributed to the increase of the bioactive potential of cereal products (Đorđević et al. 2010). The antioxidant activity of Chinese-traditional okara (i.e., DPPH radical scavenging activity and reducing power) was substantially improved after 24 h of fermentation using *Bacilus subtilis* B2 (Zhu et al. 2008). In other work, the development of ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity followed the progress of proteolysis with

Lactococcus lactis, *Lactobacillus jensenii*, *Leuconostoc cremoris A* and *Leuconostoc cremoris B* in milk whey fermentation (Virtanen et al. 2007). Compared to milk and soymilk, both milk-kefir and soymilk-kefir showed significantly higher antimutagenic activity and scavenging radical activity in studies by Liu et al. (2005). Also, Japanese-style fermented soy sauce has been reported to contain an angiotensin I-converting enzyme inhibitor having antihypertensive effect, in addition to exhibiting anticarcinogenic effect, by performing informative clinical trials on mice in a review of the functional effects of soy sauce and its components (Kataoka, 2005).

Probiotics have been designated GRAS and are known to steadily exert beneficial health effects to the host when consumed in sufficient amounts. Many fermented foods, such as yogurt, kefir, cheese, acidophilus milk, sauerkraut and some sausages, serve as adequate vehicles for the containment and delivery of selected probiotic microorganisms to the host (Heller, 2001). Lactic acid bacteria, *Bacillus spp*., yeasts (*Saccharomyces spp*.) and filamentous fungi (*Aspergillus spp*.) are the most commonly used microorganisms in probiotic preparations. The functional effects of probiotic strains have been studied. *Saccharomyces boulardii* was found to enhance the protection against enteric bacterial pathogens, possibly due to anti-inflammatory actions and effects on immunity in fermented rice bran (Ryan et al. 2011). In other studies, *Saccharomyces spp*. have been reported to have probiotic effects in the treatment of acute infantile gastroenteritis and diarrhoea, inhibit infections with *Candida albicans* and modulate the host immune response by stimulating secretory Immunoglobulin A (sIgA) production and the phagocytic system (Jespersen, 2003). Recently, clinical studies have investigated the potential of probiotic bacteria to ameliorate the pathological features of allergic diseases (Toh et al. 2012). Table 9.3 shows the prophylactic responses of selected probiotic microflora.

9.3.3 Enhancement of Organoleptic Properties

The organoleptic properties of fermented foods are mostly dependent on the metabolites of biochemical activities of starter cultures on the sugars and amino acids within a food substrate. These compounds, which are either primary or secondary metabolites, include esters, aldehydes, alcohols, carbonyls, terpenes, lactones, organic acids and pyrazines, and are products of carbohydrate fermentation, lipolysis and proteolysis. The lactic acid bacteria, *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, were found to contribute to the production of acceptable-flavour compounds during the fermentation of *sikhae* and *kimchi* (Rhee et al. 2011). Predominant starter cultures responsible for the synthesis of flavour compounds in fermented dairy products include *Lactococcus lactis*, *Lactobacillus spp*., *Streptococcus thermophilus*, *Leuconostoc mensenteroides* and mesophilic lactobacilli, in addition to other selective cultures for targeted flavour development

Table 9.3 Prophylactic responses of selected probiotic microflora

Microflora	Prophylactic response
Lactobacillus rhamnosus	Adhesion displacement of several pathogens Enhanced cellular immunity in healthy adults in controlled trials
Lactobacillus acidophilus	Significant decrease of diarrhoea in patients receiving pelvic radiation Decreased polyps, adenomas and colon cancer in experimental animals Prevented urinogenital infection with subsequent exposure to three uropathogens *E. coli*, *L. pneumoniae*, *P. aeruginosa* Lowered serum cholesterol levels
Lactobacillus acidophilus NCFM	Modulator intestinal visceral pain Induction of opioid and cannabinoid receptors
Lactobacillus acidophilus La5	*Helicobacter pylori* inhibition
Lactobacillus plantarum	Reduced incidence of diarrhoea in day-care centres when administered to only half the children Effective in reducing inflammation in inflammatory bowel syndrome/disease Reduced pain and constipation of irritable bowel syndrome Reduced bloating, flatulence and pain in irritable bowel syndrome in controlled trials Positive effect on immunity in HIV+ children Inhibition of food pathogens
Lactobacillus reuteri	Shortened the duration of acute gastroenteritis Shortened acute diarrhoea Stimulation of nervous system development
Lactobacillus reuteri ATCC 55730	Production of CD4-positive T-lymphoctes
Lactobacillus salivarus	Suppressed and eradicated *H. pylori* in tissue cultures and animal models by lactic acid secretion Colon cancer reduction in mice
Lactobacillus paracasei	Relief of hypotension and diuresis Relief of allergenic rhinitis
Lactobacillus buchneri P2	Serum cholesterol reduction
Lactobacillus casei	Reduction of bladder cancer
Bifidobacterium species	Modulation of cellular cycle, cell proliferation Reduced incidence of neonatal necrotizing enterocolitis
Bifidobacterium longum	Antitumor effect Decrease of the risk of development symptoms related to metabolic syndrome
Bifidobacterium longum BL1	Serum cholesterol reduction
Bifidobacterium breve	Modulation of the immune system
Bifidobacterium animalis BB12	Prevention and treatment of diarrhoea

(*continued overleaf*)

Table 9.3 (*continued*)

Microflora	Prophylactic response
Bifidobacterium infantis 35624	Relieve abdominal pain
Bacteroides species	Chronic colitis, gastritis and arthritis
Bacteroides fragilis	Modulation of bone mineralization and blood coagulation
	Decrease of pro-inflammatory molecules, increase of anti-inflammatory molecules, decrease neutrophil infiltration, decrease of epithelial cell hyperplasia
Saccharomyces boulardii	Reduced occurrence of *C. difficile* diarrhoea
	Effects on *C. difficile* and *K. oxytoca* resulted in decreased risk and/or shortened duration of antibiotic-associated diarrhoea
	Shortened the duration of acute gastroenteritis
Saccharomyces cerevisae Lyo	Reduced diarrhoea

Source: Information derived from Mahasneh and Abbas, 2010; van Hylckama Vleig et al. 2011; Parvez et al. 2006.

(Smit et al. 2005). Microbial exopolysaccharides (EPS) produced by lactic acid bacteria are known to function as biothickeners to give the desired mouthfeel and texture to yogurt, skim milk powder and whey by increasing viscosity and firmness, reducing susceptibility to syneresis and optimization of the rheological properties (Hati et al. 2013). African indigenous fermented foods were reported to have increased levels of alcohols and esters when fermented with *Saccharomyces cerevisiae* (Jespersen, 2003). In other studies, yeasts and moulds, used as amylolytic fermentation starters, contributed to the texture and flavour of Asian indigenous fermented foods (e.g., pancakes, rice wine, breads, palm wines, jnard, kombucha, miso, soy sauces) by the production of folic acid for sponginess of batter products, and flavour compounds including esters, fusel alcohols and acids (Aidoo et al. 2006). Enterococci also contribute to the ripening and aroma development in fermented vegetables, sausages and cheeses due to their proteolytic and esterolytic activities, resulting in the production of flavour volatiles (Foulquié Moreno et al. 2006). The superficial inoculation of *Penicillum camamberti* on fermented sausages resulted in increased proteolysis and lipolysis, with a concomitant increase in the concentration of volatile compounds, free amino acids and free fatty acids, and an overall improvement in the “ripened” odour and flavour (Bruna et al. 2003). Similarly, dry-fermented sausages fermented with *Mucor racemosus* showed increased proteolytic and lipolytic activity with increased organic acid and volatile compound concentrations (Bruna et al. 2000). Improved starch stability and gelatinization in fermented weaned foods have also been reported (Jespersen, 2003). The contribution of microbes to the flavour, aroma, texture and mouthfeel of fermented food products have been studied extensively (Soccol et al. 2007).

9.3.4 Nutritional Modification

The fermentation process enhances the nutritional profile and affects the bioavailability of minerals, vitamins as well as other nutrients, and is perceived as a viable route for biological enrichment of vitamins and amino acids in several fermented food products. In recent studies, malting and fermentation was reported to significantly improve protein digestibility and reduced tannins and phytates in red sorghum and white sorghum and millet (Onyango et al. 2013). Anti-nutrients were reduced by 20–33% within 3 days, whereas protein digestibility for the sorghum cereals was 97.4% (red sorghum) and 98.3% (white sorghum) after 24 h of combined malting and fermentation. In cereal products, fermentation by *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Lactobacillus casei* and *Lactobacillus plantarum* were found to increase the B-vitamins thiamin, robiflavin and niacin, and vitamin C contents, and also reduce the amount of anti-nutrients, notably phytates, tannins, trypsin inhibitors and some polyphenols (Jespersen, 2003), leading to improved bioavailability of minerals. Fermentation of cassava has also been demonstrated to reduce the cyanogenic toxin content (Chelule et al. 2010; van Hylckama Vlieg et al. 2011) via lactic acid bacteria fermentation. Fermentation of finger millet flour using endogenous grain microflora showed a significant reduction in anti-nutrient components at the end of 24 h, with a simultaneous increase in mineral extractability, soluble protein, *in vitro* protein and starch digestibility (Antony and Chandra, 1998). Although the solubility of zinc was not improved, fermentation increased the total zinc content and decreased the phytic acid content during the soaking, fermentation and germination of brown rice (Liang et al. 2008). Holzapfel (2002) identified strains of lactic acid bacteria that contributed to (1) increasing the nutritional value of plant foods possibly through the reduction of protease and amylase inhibitors, polyphenols and lectin-related hemagglutinin activities, (2) lowering the levels of proteinase inhibitors in cereal products, thereby increasing the availability of essential amino acids such as lysine, leucine and isoleucine, (3) reducing phytic acid and tannin levels, (4) partial fermentation of oligosaccharides and (5) inactivation of natural toxins such as linamarin and lotaustralin. The nutritional benefits listed here provide only a few examples of the extensive reviews conducted on fermented foods and nutritional enhancement.

9.4 Novel Approaches to Enhancing the Functionality of Fermented Foods

Over the past decade, a number of approaches have been designed to enhance the functionality of fermented foods through microbial and substrate modulation, process engineering, metabolic engineering and strain-specific manipulation. The development of these processes has been made necessary due to the increasing consumer demand for innovative ingredients and fermented functional foods. We identify and report on novel approaches relating to genomics, metabolic engineering, microencapsulation and substrate manipulation to enhance the functionality of fermented foods.

9.4.1 Genomics

Genomics research and its application provide genome sequencing information of fermenting microbes and the metabolic and physiological capabilities of microbes that assist in tailoring relevant characteristics of fermented foods; selective starter cultures have been using metagenomic, metatranscriptonomic and proteomic approaches (de Vos 2001; Smid and Hugenholtz, 2010; van Hijum et al. 2013). Recent research has enabled the characterization of the genomic sequence of lactic acid bacteria including *Lactococcus lactis 1403*, *Streptococcus thermophilus*, *Lactobacillus delbruekii susp. bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus johnsonii*, *Lactobacillus acidophilus*, *Lactobacillus gaserii*, *Oenococcus oeni* and *Leuconostoc mesenteroides* (Renault, 2002). Genomics has been applied in selecting functional starter cultures, modification by introducing new genes or modifying their metabolic functions for improving food fermentations and product functionalities, such as the development of flavour, taste, and texture. Examples of genomics applications for fermented foods are listed in Table 9.4.

Due to the complexity of most food substrates, it is technically challenging to study the interactions in mixed-culture fermentation using genomic approaches. One approach is to study the interactions at the level of substrates, the exchange of metabolites and growth factors or inhibiting compounds between the mixed cultures (Sieuwerts et al. 2008). Recently, a strong correlation in the adaptability of sourdough fermentation environment was reported via investigative genome sequencing of two strains of *Lactobacillus sanfranciscensis*, obtained 18 years apart (van Hijum et al. 2013). Their work also indicates that comparative genomic sequencing of functionally equivalent ecospecies is likely to result in similar fermented products with the application of identified functionally equivalent microbes. The generation of antioxidant functional milk during fermentation by lactobacilli is reported to be strain specific, with seven strains showing a high correlation between proteolytic activity and antioxidant activity during fermentation,

Table 9.4 Genomic application in fermented foods by selected microbes

Microbial species	Fermented food application	Genome size (Mb)
Fungi		
Saccharomyces cerevisiae	Beverage and bread production	12.068
Kluyveromyces lactis	Food enzymes and dairy fermentations	12
Aspergillus niger	Food enzymes and plant fermentations	30
Bacteria		
Bacillus subtilis	Food enzymes and soy fermentations	4.214
Lactococcus lactis	Cheese and other dairy fermentations	2.365
Lactobacillus plantarum	Various fermentation and intestinal isolate	3.308
Bifidobacterium longum	Intestinal isolate and probiotic activity	2.257
Lactobacillus acidophilus	Intestinal isolate and probiotic activity	1.9
Streptococcus thermophilus	Yogurt and cheese fermentations	1.8
Lactobacillus bulgaricus	Yogurt fermentations	2.3

Source: Reprinted from de Vos, Advances in genomics for microbial food fermentations and safety. *Current Opinion in Biotechnology*, **12**, 493–498, Copyright 2001, with permission from Elsevier.

whereas four strains showed significant antioxidant activity on hydrolysis of casein with their cell-fee extract (Ramesh et al. 2012). The application of molecular techniques to identify individual microflora species responsible for cheese ripening is effective in structuring a defined targeted approach to strain selection and overall cheese quality (Beresford et al. 2001). In sausage fermentation, the use of microbial strains with antioxidant properties due to catalase or superoxide dismutase (e.g., *Staphylococcus carnosus*), bacteriocin-producing strains (e.g., *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus curvatus* and *Leuconostoc mesenteroides*) and aroma compound gram-positive, catalase-positive cocci (e.g., *Staphylococcus xylosus* and *Staphylococcus carnosus*) are known to impart industrial and nutritional functionality (Leroy et al. 2006). Genomic data has also been used for strain-specific selection of bacteriocinogenic strains of lactic acid bacteria for biopreservative functionality of fermented food products (Stiles and Hastings, 1991; Hugas, 1998; O'Sullivan et al. 2002; Urso et al. 2006; Ravyts et al. 2008; Tomé et al. 2008), as well as for the improvement of microbial cultures to withstand processing stresses (Lian et al. 2002; Ross et al. 2005; Saarela et al. 2006).

9.4.2 Metabolic Engineering

Genetic and physiological engineering are approaches that can be applied to optimize the production of desirable metabolites by regulating the genetic and regulatory responses of selected microflora. This process involves knowledge of metabolic pathways and genetic information in order to reroute the metabolic pathways. Recent work elucidates the metabolic engineering of lactic acid bacteria for the production of nutraceuticals (Hugenholtz et al. 2002). Kleerebezemab et al. (2000) reported on enhancing *Lactococcus lactis* for the production of diacetyl (flavour component in dairy products generating the butter aroma), alanine (food sweetener) and exopolysaccharide (for enhanced texture and mouthfeel) compounds. Enhanced diacetyl production was achieved by controlling the redox balance during fermentation via cofactor (NAD^+) engineering in combination with inactivation or overexpression of other enzymatic steps. The complete conversion of homo-L-lactate-fermentation to homo-L-alanine-fermentation was achieved by rerouting of the pyruvate flux via the overexpression of L-alanine-dehydrogenase encoded by the *alaD* gene derived from *Bacillus sphearicus*. The authors propose an increase in the metabolic flux toward the biosynthesis of nucleotide sugars as a means of genetic engineering to increase the production of EPSs). In other related work, the overexpression of *Zymomonas mobilis* pyruvate decarboxylase to catalyse the conversion of pyruvate to acetaldehyde, and the expression of nicotinamide adenine dinucleotide phosphate (NADH) oxidase leading to increased pyruvate availability, was found to enhance acetaldehyde production in *Lactococcus lactis* (Bongers et al. 2005). Increased diacetyl production in *Lactococcus lactis* under aerobic conditions was achieved with a combination of NADH oxidase overproduction with α-acetolactate (α-AL) decarboxylase inactivation (Hugenholtz et al. 2000). By overexpressing the EPS gene cluster of *Lactococcus lactis* NIZO B40, there was a significantly reduced growth rate and lower final optical density with concomitant threefold increase in the carbon flux toward EPS production (Boels et al. 2003). In other related work, engineering of the lactococcal folate biosynthesis pathway through overexpression of the folate biosynthetic gene in *Lactococcus lactis*, individually or in combination with *Lactococcus lactis* strain NZ9000, using the nisin-controlled expression (NICE) system, resulted in up to threefold increased folate production and higher release of folate into the environment (Kleerebezem et al. 2002). Sybesma et al. (2003) also report on the increased production and retention of folate by *Lactococcus lactis* via overexpression of the *folKE* gene and the combined overexpression of the *folKE* and *folC* encoding genes. Overexpression of all four lactococcal riboflavin biosynthetic genes and isolation of spontaneous mutants to the riboflavin analogue roseoflavin has also been reported to result in substantial overproduction of riboflavin in *L. lactis* (Burgess et al. 2004). Through a successful combination of mutagenesis engineering and metabolic engineering of the folate and riboflavin biosynthetic pathways, simultaneous overproduction of folate and riboflavin was achieved in *L. lactis* by exposure to the riboflavin analogue roseoflavin and overexpression of the folkE gene encoding the biprotein amino-4-hydroxy-6-hydroxymethylduhydropteridine pyrophosphokinase and guanosine-5'-triphosphate (GTP) cyclohydrolase I (Sybesma et al. 2004). The increased bioproduction of the sugar alcohols sorbitol, xylitol and mannitol by bacteria and yeasts via enzymatic heterologous expression, coexpression and overexpression has been reviewed (Akinterinwa et al. 2008). By overexpressing the two sorbitol-6-phosphate dehydrogenase genes, and reversing the catabolic pathway for sorbitol utilization, high levels of sorbitol production (25% for growing cells and 65% for resting cells) was produced from fructose-6-phosphate in *Lactobacillus plantarum* (Ladero et al. 2007). Similarly, increased sorbitol synthesis was achieved in *Lactobacillus casei* by the integration of a D-sorbitol-6-phosphate dehydrogenase-encoding gene (gutF) in the chromosomal lactose operon BL232, followed by inactivation of the L-lactate dehydrogenase gene in BL232 (Nissen et al. 2005). High yields of mannitol production in *Lactococcus lactis* strains was achieved by deletion of the *mtlA* and *mtlF* genes involved in the transport of mannitol, from the genome FI9630 by double-crossover recombination (Gaspar et al. 2004). Also, metabolic engineering of *Streptococcus thermophilus* was found to increase acetaldehyde via the overexpression of the glyA gene in serine hydroxyl-methyltransferase (Chaves et al. 2002) and EPS production by a combined overexpression of the *galU* and *pgmA* genes (Levander et al. 2002). In other related studies, metabolic engineering of lactic acid bacteria was not sufficient to divert the significant carbon flow to ethanol, and hence the need for more research into the metabolism of Lactobacillus strains (Nichols et al. 2003; Liu et al. 2006).

9.4.3 Microencapsulation

Over the years, functional fermented foods have been recognized as carriers of selected strains recognized to impart health functions to the consumer. However, harsh processing and delivery conditions can reduce the efficacy of the food–health function imparted by such microbes. Microencapsulation is an innovative yet relatively new functional food concept (Heidebach et al. 2012) that protects bioactive molecules and bioactive living cells from the adverse effects of processing and storage conditions, as well as the deleterious transport conditions in the gastrointestinal tract. Application of innovative encapsulation techniques is important in promoting cell viability, protecting functionality and facilitating site-specific targeted release. A variety of encapsulation matrices and technologies have been identified, including emulsification, coacervation, spray drying, spray cooling, freeze drying, fluid bed coating, extrusion, and liposomal and cyclodextrin encapsulation (Anal and Singh, 2007; de Vos et al. 2010, Rokka and Rantamäki, 2010), and the microencapsulation of probiotic cells for cheese, yogurt and other food applications has been recently reviewed (Heidebach et al. 2012). Although microencapsulation with sodium alginate by dropping method did not significantly affect the adherence of *L. acidophilus* ATCC 43121, the strain was protected from heat and acid treatments, with the corresponding functionality of the strain not adversely affected (Kim et al. 2008). *Lactobacillus paracasei* and *Bifidobacterium lactis* showed improved survival rates after incubation under simulated gastrointestinal conditions when microencapsulated via rennet-gelation of milk proteins (Heidebach et al. 2009). *Lactobacillus acidophilus* encapsulated in an alginate–prebiotic matrix was also found to have increased probiotic viability and activity after fermentation and 8 weeks of storage when exposed to simulated gastric conditions (Nazzaro et al. 2009). Similarly, microencapsulation of three lactic acid bacteria strains using whey cheese matrix was effective in improving survival rates during transit in simulated gastrointestinal systems (Madureira et al. 2011). Eight strains of probiotic bacteria, microencapsulated in alginate matrix, showed better survival than free cells at the low pH of 2, high bile concentrations and heat treatment of up to 65 °C (Ding and Shah, 2007). In other related work, the survival of calcium-induced, alginate-starch-encapsulated probiotic bacteria, *Lactobacillus acidophilus* and *Bifidobacterium lactis*, in yogurt was enhanced during storage with textural differences (Kailasapathy, 2006). Adhikari et al. (2003) also report texture changes in probiotic yogurt samples containing live cells of *Bifidobacterium longum* microencapsulated with κ-carrageenan, although there was no decline in the cell enumeration after 30 days under refrigerated conditions. Further studies have investigated and reported on the increased viability of microencapsulated probiotic culture without a significant effect on the sensory properties of frozen and freeze-dried dairy products when encapsulation was combined with other treatments, including the incorporation of resistant starch, cryoprotectants and prebiotics (Capela et al. 2006; Homayouni et al. 2008). Further applications of microencapsulation in the functional food and nutraceutical sectors are provided elsewhere in this book.

9.4.4 Optimization of Substrate and Fermentation Conditions

The optimization of fermentation conditions and medium constituents such as substrate composition, temperature, time and pH are important in standardizing the production of metabolites of fermentation, which ultimately affect the overall functionality, safety and quality of fermented foods. At optimum conditions (temperature of 36.74 °C, pH of 5.02 and fermentation time of 17 h), maximum production of ethanol from sago starch was achieved using simultaneous saccharification and fermentation with glycoamylase (AMG) and *Zymomonas mobilis* ZM4 (Ratnam et al. 2003). Similarly, maximum ethanol production was achieved with a solid state fermentation

(SSF) process with co-immobilized AMG and *Z. mobilis* cells MTCC 92 at 32.4 °C, pH 4.93 and fermentation time 17 h (Bandaru et al. 2006). In another study, maximum citric acid production was obtained from the submerged fermentation of Palmyra jiggery using *Aspergillus niger* MTCC 281 under optimal conditions of pH 5.35, temperature 29.76 °C, fermentation time at 5.7 days with 221.66 g of substrate per litre, 0.479 g of ammonium nitrate per litre and 2.33 g of potassium ferrocynanide per litre (Ambati and Ayyanna, 2001). Fermentation conditions in mango wine production using *Saccharomyces cerevisiae* (*var. bayanus*) has been explored (Sudheer Kumar et al. 2009), and temperature 22.5 °C, pH 3.8 and inoculum size 11.9% were reported to be optimum for up to 10% v/v ethanol, increase in glycerol concentration up to 6.94 g/L and minimized volatile acidity to 0.29 g/L. Moreover, higher yield of L-glutamic acid, a flavour compound, was achieved by optimizing fermentation conditions (pH, temperature and time) using *Micrococcus glutamic* and *Pseudomonas reptilivora* (Sunitha et al. 1998). Substrate and fermentation condition manipulation has also been applied to achieving increased yield of bacteriocin production of the *Lactococcus* species (Li et al. 2002; Cheigh et al. 2002). Recently, there has been a preference for the utilisation of SSF over submerged fermentation (SmF), propelled by its advantages (Table 9.5) with advancement in bioreactor design for optimum production of fermentation metabolites including flavours, enzymes and organic acids in the food and biotechnology industries (Durand, 2003; Raghavarao et al. 2003; Suryanarayan, 2003; Hölker and Lenz, 2005; Couto and Sanromán, 2006; Singhania et al. 2009; Longo and Sanromán, 2010).

Table 9.5 Advantages and disadvantages of solid-state fermentation (SSF) over submerged fermentation (SmF)

Advantages	Disadvantages
Higher productivity	Difficulties in scale-up
Better oxygen circulation	Difficult control of process parameters (pH, heat, moisture, nutrient conditions)
Low-cost media	Problems with heat build-up
Less effort in downstream processing	Higher-impurity product, increasing recovery product costs
Reduced energy and cost requirements	
Simple technology	
Scarce operational problems	
Resembles the natural habitat for several microorganisms	

Source: Reprinted with modification from Couto and Sanromán, Application of solid-state fermentation to food industry - A review. *Journal of Food Engineering*, **76**: 291–302, Copyright 2006, with permission from Elsevier.

9.5 Conclusion

The increase in consumer awareness of functional foods is recognized globally as the driving force of innovation in the development of foods rich in bioactive compounds and bioactive living cells. The identification and adaptation of novel processes are being researched and applied into the artisanal and industrial fermentation of foods. In this chapter, four such processes have been identified: genomics, metabolic engineering, microencapsulation and optimization of the substrate and fermentation conditions. These innovative processes have been shown to optimize the production of metabolites and enhance the functionality of fermented foods, as well as assist in overcoming the hurdles of production and processing.

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10
Impact of Processing on the Bioactivity of Functional and Nutraceutical Ingredients in Foods

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10.1 Introduction

Bioactive compounds are naturally occurring chemical compounds found in, or derived from, plant or animal sources that exert a particular health or wellness benefit. Bioactive phytochemicals such as polyphenols are often found in plant foods as secondary metabolites. They are well-recognized antioxidants that are linked to the reduction of the development and progression of life-style diseases.

Recent studies have shown the importance of bioactive phytochemicals in the diet. However, these compounds can be changed during processing and storage. which may increase or decrease the level of bioactivity. Most plant foods in the typical North American diet are subjected to processing, which can modify their composition and bioactivities, and so it is important to know how their bioactive phytochemicals are affected. This chapter will discuss the effects of various processing methods, both thermal and non-thermal, on

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phytochemicals and their bioactivities in fruits, vegetables, and other plant foods, while attempting to explain why these changes take place.

Many phytochemicals have shown high antioxidant activity in both *in vitro* and *in vivo* studies. This is due to their electron-rich structures with oxidizable double bonds and hydroxyl groups, which can prevent oxidative damage and scavenge free radicals through a variety of mechanisms (Kalt 2005). There has been a significant link between the regular intake of phytochemicals (e.g., polyphenols, carotenoids, phytosterols) and the prevention of life-style-related diseases, such as cancer, obesity, diabetes, and cardiovascular disease (Gresele et al. 2011).

The antioxidant profile of a plant food has been recognized to represent an important parameter in predicting the impact a food has on human health. There is increasing evidence suggesting that compounds with high antioxidant activity play a major role in explaining the benefits of fruit and vegetable consumption. Since most fresh fruits and vegetables are processed due to their short shelf-life, however, all processing methods are expected to alter the content of bioactive compounds (Nicoli et al. 1999). Food processing that includes physical damage such as maceration, exposure to elevated temperatures, and separation techniques can result in oxidation, thermal degradation, and leaching of bioactive compounds in processed foods (Kalt 2005). Thermal processing is generally thought of as the most destructive to antioxidants, and this has fostered a belief among many consumers that raw vegetables are nutritionally superior to their cooked counterparts. However, recent literature provides evidence that this may not always be the case (Pellegrini et al. 2010; Cao et al. 2011; Mazzeo et al. 2011).

This chapter will discuss how thermal processes such as boiling, steaming, microwaving, roasting, and drying, as well as non-thermal processes such as freezing, high hydrostatic pressure, irradiation, fermentation, pulsed electric fields, and reverse osmosis affect the concentration of bioactive compounds in various functional foods. It will also attempt to explain why these changes occur.

10.2 Thermal Processing

Depending on the product, different production steps are required to produce a processed food product to ensure food safety, to maintain high quality, and to extend the shelf-life. A typical processing method is heat treatment, which can be applied at any stage of the processing operation. Heat is generally used to develop the desired flavors, aroma, and color components; modify the food structure; or preserve the food by heat-induced inactivation of microorganisms, toxins, and enzymes. These heat-treatment regimens often lead to a loss of bioactive compounds, which are essential for the human diet. Therefore, fruit and vegetable products that are a major source of phytochemicals should be protected during processing and packaging (Wang and Bohn 2012).

10.2.1 Boiling

Boiling is a cooking method in which food is cooked in water that has been brought up to its boiling point (100 °C or 212 °F). The length of time for this process varies greatly. Boiling normally decreases the concentration of water-soluble compounds such as ascorbic acid. However, bioactive phenolic compounds that do not possess the same water solubility are less likely to leach out into the processing water. Also, ascorbic acid and similar compounds that are heat-unstable may also result in additional loss at high temperatures. Table 10.1 shows the effect of boiling on various vegetables.

The retention of bioactive compounds during boiling depends on the boiling time, the size and shape of the food pieces being processed (total surface area), the initial concentrations of compounds in the various plant foods, the analytical methods used to determine the bioactivity, and the experimental conditions of the different assays (such as substrates, temperatures, light, oxygen, and metals). For example, in some fruits and vegetables, vitamin C represents only a minor part of total antioxidant capacity (TAC) when measured with ORAC, TEAC, and FRAP assays.

10.2.2 Steaming

Steaming is one of the common processing steps in fruit and vegetable processing. In general, it has been shown to cause an overall increase in antioxidant capacity, and in particular an increase in phenolic compounds has been seen (Mazzeo et al. 2011). This can be explained by the inactivation of oxidative enzymes that have the ability to oxidize phenolic compounds. Steaming also relies on the non-direct contact of the products with water, which prevents the solubilization of water-soluble compounds such as ascorbic acid and anthocyanins. As seen in Table 10.1, steaming can be regarded as a better cooking method (when compared with boiling) for increasing the bioactivity of vegetables.

10.2.3 Microwaving

The effects of microwave on bioactive compounds have not been consistent. It has been shown to cause significant phytochemical degradation in some cases, including the loss of flavonoids and phenolic acids (Pellegrini et al. 2010). The high frequencies used in this processing method can alter the peel barrier of fruits and vegetables, which leads to antioxidant loss (Danesi and Bordoni2008). However, shorter processing times can lead to smaller losses in bioactivity, and, in some cases, the antioxidant capacity of the samples are not affected (Turkmen et al. 2005). This result suggests that moderate heat treatments might be considered a useful tool in improving the bioactivity of

Table 10.1 Phytochemical compounds of various raw and cooked vegetables[a]

Phenol compounds (mg/100 g)	Raw	Boiled	Microwaved	Basket-steamed	Oven-steamed
Broccoli					
Caffeic acid	6.6 ± 1.1b	2.2 ± 0.4c	2.9 ± 0.2c	4.0 ± 0.1c	9.1 ± 1.3a
Coumaric acid	11.2 ± 0.1b	3.1 ± 0.0c	1.5 ± 0.1d	3.7 ± 0.1c	12.5 ± 0.9a
Sinapic acid	27.3 ± 0.3b	14.8 ± 0.0c	13.6 ± 0.7c	23.4 ± 0.5b	38.7 ± 3.4a
Chlorogenic acid	20.2 ± 0.5c	88.9 ± 2.8b	5.7 ± 0.2c	19.1 ± 0.7c	135.2 ± 12.2a
Ferulic acid	4.3 ± 0.1a	0.8 ± 0.0b	1.2 ± 0.2b	1.8 ± 0.3b	4.6 ± 0.9a
Quercetin	23.5 ± 0.1b	10.0 ± 0.1cd	5.7 ± 0.3d	12.2 ± 0.3c	31.6 ± 4.1a
Kaempferol	18.4 ± 0.5b	8.4 ± 0.5d	6.0 ± 0.6e	10.4 ± 0.3c	31.6 ± 0.0a
Total phenol compounds	111.4 ± 0.8b	128.2 ± 3.6b	36.7 ± 1.5d	74.6 ± 1.0c	263.3 ± 20.1a
Brussels sprouts					
Caffeic acid	8.5 ± 1.2a	5.8 ± 0.6b	9.9 ± 0.4a	5.3 ± 0.0b	1.6 ± 0.6c
Coumaric acid	5.1 ± 0.3cd	5.4 ± 0.1c	6.9 ± 0.2b	18.1 ± 0.2a	4.5 ± 0.4d
Sinapic acid	18.2 ± 1.3c	38.3 ± 0.9a	32.7 ± 0.9b	30.7 ± 0.7b	3.6 ± 0.2d
Chlorogenic acid	11.2 ± 0.3d	29.3 ± 0.6c	67.0 ± 2.4a	38.3 ± 6.0b	14.6 ± 0.3d
Ferulic acid	1.3 ± 0.1c	1.9 ± 0.0c	2.3 ± 0.1c	8.0 ± 0.1a	4.7 ± 0.1b
Quercetin	12.9 ± 0.3d	23.3 ± 0.3c	25.5 ± 1.0b	25.0 ± 0.4b	36.4 ± 0.1a
Kaempferol	5.6 ± 0.0b	7.9 ± 0.3a	5.0 ± 0.1b	7.0 ± 0.5c	4.7 ± 0.6b
Luteolin	2.3 ± 0.5b	1.1 ± 0.1c	3.6 ± 0.3a	2.6 ± 0.1b	0.4 ± 0.0d
Naringenin	25.5 ± 0.6a	21.2 ± 0.8b	19.7 ± 1.9b	7.0 ± 0.5c	4.3 ± 0.5d
Total phenol compounds	90.7 ± 3.5d	134.3 ± 3.7c	172.4 ± 6.6a	142.0 ± 5.4b	74.9 ± 0.2e
Cauliflower					
Caffeic acid	16.5 ± 4.3a	0.8 ± 0.1c	8.2 ± 1.3b	6.7 ± 0.9b	11.7 ± 1.0ab
Coumaric acid	5.9 ± 1.3ab	6.7 ± 0.0a	4.5 ± 0.0b	4.9 ± 0.4b	4.9 ± 0.1b
Sinapic acid	4.7 ± 0.4bc	4.0 ± 0.1c	5.4 ± 0.1a	5.3 ± 0.3ab	4.9 ± 0.0ab
Chlorogenic acid	21.2 ± 1.7a	12.6 ± 0.1c	11.0 ± 0.3cd	9.5 ± 0.2d	16.5 ± 0.3b
Ferulic acid	1.5 ± 0.5a	0.8 ± 0.0b	1.1 ± 0.0ab	0.7 ± 0.0b	0.9 ± 0.0b
Quercetin	3.6 ± 0.6ab	3.4 ± 0.7ab	3.6 ± 0.0ab	4.4 ± 0.1a	3.2 ± 0.0b
Kaempferol	4.7 ± 0.2ab	2.7 ± 0.0c	6.3 ± 0.2a	4.6 ± 1.4ab	3.5 ± 0.1bc
Luteolin	2.0 ± 0.4b	1.7 ± 0.1b	2.1 ± 0.1b	4.2 ± 0.9a	3.8 ± 0.0a
Total phenol compounds	59.9 ± 9.5a	32.7 ± 0.8c	42.2 ± 2.0bc	40.4 ± 0.6bc	49.3 ± 0.5ab

[a]Values are presented as mean value ± SD ($n = 3$) and expressed on dry weight basis. Means in rows followed by different letters different significantly ($P \leq 0.05$). All compounds were identified by pure standards, unless differently reported.

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some functional foods. Table 10.1 summarizes the effects of microwaving on various vegetables.

As with boiling, the preservation of bioactivity during microwaving is dependent on multiple factors, including processing time, strength of the heat treatment, surface area of the samples, and the analytical methods used to determine antioxidant activity.

10.2.4 Drying

The drying process causes oxidation of bioactive compounds, which results in a decrease in the TAC (Bryngelsson et al. 2002). Longer drying times at lower temperatures may promote a decrease in antioxidant capacity. It is also theorized that there may be an accumulation of Maillard reaction-derived melanoidins that have some antioxidant properties at high temperatures. The detrimental effect of lower temperatures on the food could be due to a combination of low temperatures and longer drying times, which causes additional degradation reactions (Vega-Gálvez et al. 2009).

10.2.5 Roasting

Overall, a decline in antioxidant capacity after roasting was reported. This decrease is caused by a loss of phenolic compounds, which can be explained by the oxidation of these compounds to their corresponding quinones, as well as the further polymerization of these molecules to form insoluble quinones. Epimerization of molecules into their less bioavailable forms [ex: (-)-catechin to (+)-catechin)] has also been seen during roasting, which would explain decreased bioactivity. The roasting process is responsible for triggering the Maillard reaction, which can cause changes in the phenolic profile of a sample, resulting in a lowered antioxidant capacity (Jolić et al. 2011).

10.3 Non-Thermal Processing

Modern food processing techniques aim at preserving native bioactive compounds in the raw food as much as possible. Novel non-thermal processing treatments have been developed with the goal of preserving and protecting food nutrients and native bioactive ingredients. With these technologies, the advantages of heat treatments (i.e., microorganism and enzyme inactivation) can also be achieved non-thermally, while still protecting the organoleptic and nutritional qualities of the product (Wang et al. 2012).

10.3.1 Freezing

When plant foods are commercially frozen, they are first blanched to inactivate enzymes that are responsible for causing off-colors, off-flavors, and softening during frozen storage. Typical commercial blanching conditions are 90–95 °C for 1–10 min, and are usually achieved by exposure to hot water or steam followed by a rapid cooling procedure (plunged into iced or cold water to halt the cooking process). Typically, several months of low-temperature storage can elapse before purchase, possibly followed by several months of in-home storage. Any changes in bioactive compounds seen during the freezing process are mainly due to the thermal degradation that takes place during the blanching stage, as well as the leaching of nutrients into the blanching medium similar to those seen during the boiling process (Danesi and Bordoni2008). Frozen storage itself does not have a significant effect on the bioactivity of the products.

10.3.2 High Pressure

High-pressure processing (HPP) is a non-thermal technique that subjects foods to 100–1,000 MPa using water as a pressure-transmitting medium at room or mild temperatures (also referred to as high hydrostatic pressure [HHP]). Food treated in this way has been shown to keep its original freshness, flavor, taste, and color to the greatest extent, since smaller molecules such as volatile compounds, pigments, vitamins, and other compounds connected with the sensory and nutraceutical qualities are unaffected.

During HPP, individual phenolic compounds, total phenols, and the bioactivity of a product often decrease. The reason behind this decline is the higher residual activity of polyphenol oxidase (PPO) and peroxidase (POD), two enzymes that can catalyze the oxidation of phenols. For example, these enzymes are capable of oxidizing (+)-catechin, which causes degradation and, in turn, leads to the formation of brown polymers (Lopez-Serrano and Barceloä 2002).

As this process is conducted at room temperature or slightly above, there is no formation of the unwanted compounds that usually accompany heat treatments. Heat-triggered chemical reactions such as the Maillard reaction can cause changes in bioactive compounds, and therefore lower antioxidant capacity.

10.3.3 Irradiation

Food irradiation is the controlled application of ionizing radiation such as x-rays, gamma rays, or electron beams to improve a product's hygiene and safety, as well as to reduce its microbial load and extend its shelf-life. This processing method has been shown not to affect the organoleptic qualities of a product.

Studies have shown that irradiation has the ability to increase the determined phenolic acid concentration and total phenolic content when applied to various spices (Variyar and Bandyopadhyay 1998, Mali et al. 2011). This increase is attributed to tannin depolymerization, which produces smaller-molecular-weight phenolic compounds such as gallic and tannic acids. Some spices that contain higher concentrations of condensed tannins showed no significant qualitative changes in the distribution of phenolic acids. Condensed tannins are more resistant to gamma irradiation, whereas hydrolysable tannins are more susceptible to degradation. Another explanation for enhanced antioxidant activity is a possible increase in enzyme activity (such as phenylalanine ammonia-lyase and peroxidase) or a possible increase in the extractability of phenolics from the plant tissues due to the dissolution of cell wall polysaccharides.

10.3.4 Fermentation

There has been limited information on the effect of fermentation on bioactive compounds. Grape fermentation has been seen to result in large losses of phenolic compounds in wine, measured by the Folin-Ciocalteu method. The reduction in antioxidant capacity after winemaking was explained by the loss of vitamin C, phenolic compounds, and total anthocyanins (Klorotek et al. 2005).

An important factor affecting the content of phenolic compounds in grapes, pomace, juice, must, and wine is their concentrations in the various fractions of the grape. Intact grapes have significantly higher concentrations of phenolic compounds. Some reports showed a significantly lower amount of phenolic compounds in red grape juice than in red wine must. This is because the must is in contact with the skins of the grape for a longer period of time, and is therefore able to extract more bioactive compounds from the skins of the grape. In terms of antioxidant activity, the pomace has the highest percentage, followed by the final red wine and the fermenting grape must. Even after contact with the fermenting wine, grape pomace has a high content of phenols with potential antioxidant activity (Yildirim et al. 2005).

The separation of phytochemicals seen in grapes can also be found in other fruits and vegetables, such as apples. This is a challenge during the processing of apple juice, due to the fact that there are many bioactive compounds lost when the juice fraction is separated from the skin.

It is hypothesized that the alcohol generated during fermentation may contribute to antioxidant activity of the finished product in some way, although the mechanism of action is unclear (Pèrez-Gregorio et al. 2011).

10.3.5 Pulsed Electric Field

Pulsed electric field (PEF) treatments of liquid samples are gaining attention as an alternative method to pasteurization. The electric fields that are applied create an electrical potential across the membranes of biological cells, which cause structural damage and, eventually, cell death. This means that PEF can be used for the non-thermal inactivation of microorganisms. Studies have shown that neither phenolic content nor antioxidant capacity are affected by field intensities of 1, 3, and 5 kV/cm (n = 30 pulses). The fact that TAC was not affected suggests that PEF treatments do not contribute to the formation of radicals (Schilling et al. 2007). When this method is applied industrially, ascorbic acid is often added to the products before processing. The addition of ascorbic acid may be part of the reason why phenolic compounds are not affected by the various electric fields.

When applied to samples of orange juice, PEF showed no significant difference between the radical scavenging capacity (measured with the DPPH method) of the untreated juice and the treated juice. This suggests that PEF is more effective than any heat treatment, including low-temperature and high-temperature pasteurizations, in preserving bioactive compounds and the radical scavenging ability of orange juice (Sanchez-Moreno et al. 2005).

10.3.6 Reverse Osmosis

Reverse osmosis (RO) is a concentration technique that is commonly used on juice products. This process permits the separation of water from the juice solids, but is limited by high osmotic pressures. For this reason, it is usually used as a pre-concentration technique that permits concentration values of about 30 g TSS (total suspended solids)/100 g, corresponding to osmotic pressures of about 50 bar. RO has been shown to produce a small decrease in total antioxidant activity, which can be attributed to the high pressure employed during the process. However, this small decrease still yields a product of much higher quality than conventional thermal processing. This is probably because RO takes place at room temperature, which means that there is no formation of unwanted compounds such as products derived from heat treatments (Cassano et al. 2003).

10.4 Conclusion

There is no clear trend for how thermal processing methods affect the bioactivity of functional foods. Boiling generally tends to decrease the antioxidant activity of plant foods, due to the high temperature and the presence of water, which allows for the solubilization and leaching of polar compounds.

The literature suggests that boiling is one of the worst conventional cooking practices regarding the retention of bioactive compounds. Steaming had some detrimental effects, but in most cases increased the concentration of some antioxidants. The important difference between boiling and steaming is that there is no direct food to water contact during steaming, which prevents loss by solubilization. In some studies, the microwaving process has been found to be detrimental to foods in terms of antioxidant capacity, whereas others suggest that it has no effect. Drying and roasting can also be categorized as damaging to the bioactivity of plant foods, due to the high temperatures used. This heat can cause epimerization, oxidation, and other chemical reactions of the bioactive compounds that have negative impacts on the bioactivities and concentrations of antioxidants.

The other processing methods discussed are just as inconclusive. The freezing of plant foods has little to no effect on the composition of the food itself. Any changes found in commercially frozen products are most likely due to the blanching step that occurs before the product is frozen. HPP showed some small increases in antioxidative compounds, and overall was considered a good way to retain bioactive compounds with antioxidant activity. Irradiation increased the extractability of some phytochemicals due to the depolymerization of tannins into smaller bioactive molecules. During fermentation, bioactive compounds tend to decrease, due to chemical reactions that take place during the process. Both PEF treatments and RO showed no significant changes in total antioxidant activity and proved to be suitable processing methods for producing a quality product with high-nutritional value.

It is important to note that there are too many variations among many studies on the same food items, and this needs to be taken into account. The dissimilarities in plant secondary metabolites can be explained by variations in genetics, time of harvest, the growing conditions of the food (climate, location, temperature, fertility, diseases, pest exposure, etc.), and the methods of analysis – all of which will have an impact on the final antioxidant activity (Maršić et al. 2010).

Differences among these studies regarding increases and decreases of different compounds could also be due to the fluctuations in processing time, the size and shape of the food pieces being processed (total surface area), the initial concentrations of the compounds in the various functional foods, the analytical methods used, or the experimental conditions of the different assays (such as substrates, temperatures, light, oxygen, and metals). Other major variables during the processing of plant foods that may affect their bioactivities are endogenous enzyme levels, water activity, oxygen pressure, and thermal and mechanical energy.

The analytical method used is also an important variable to consider. Various assays measure different types of compounds. The Folin-Ciocalteu method measures total phenols, oxygen radical absorbance capacity (ORAC), and trolox equivalent antioxidant capacity (TEAC); assays measure lipophilic and hydrophilic antioxidant capacities; and high-pressure liquid chromatography (HPLC) quantifies individual compounds. These differences are why one analytical method cannot be compared to another. The experimental conditions of these assays can influence the outcome as well, such as the concentration of substrates, temperature, and exposure to light, oxygen, or metals.

In summary, the antioxidant activity of a plant food either stays the same, increases, or decreases during or after processing, depending on various factors. An increase in antioxidant capacity may sometimes be due to the transformation of larger molecules into small phenolics, or due to an increase in the extractability of these compounds. A decrease in the antioxidant activity of a plant food can be due to a loss of bioactive compounds, or due to the formation of new compounds having pro-oxidant activity (Nicoli et al. 1999). Therefore, we have to consider the effects of processing on the bioactivity of functional foods and ingredient case by case.

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11

Encapsulation and Controlled Release Techniques for Administration and Delivery of Bioactive Components in the Health Food Sector

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11.1 Introduction: Health Food Sector

Based on published data, it is now accepted that diet can have beneficial physiological and psychological effects, beyond the well-established nutritional effects, by modulating specific target functions and responses in the body (WHO/FAO 2002). Hence, diet not only promotes optimal development and health, but also plays an important role in disease prevention by eliminating or reducing the risk of certain lifestyle-related chronic diseases. Thus, to meet the optimal diet composition, promote health, and reduce modern lifestyle-related diseases, the concept of functional foods has been developed and adopted in the health food sector of food and nutraceutical industries.

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Consumers are aggressively demanding more from their food products, over and above traditional expectations. They want foods to sustain long life, promote wellness and energy, improve digestive health, manage weight, and support immunity. They want to counter the effects of their busy lifestyles with foods that taste great and are ultra-convenient. To meet consumer demand, and to comply with regulatory pressure, food and nutraceutical products are incorporating functional bioactive ingredients derived from milk, fruits, vegetables, cereals, herbs, and spices.

Functional food is a natural or processed food that contains known biologically active compounds which, when in defined quantitative and qualitative amounts, provide clinically proven and documented health benefits, and thus are important sources in the prevention of chronic diseases of the modern age (Betoret et al. 2011). Clearly, all foods are functional, since they provide taste, aroma, or nutritive value. Within the last decade, however, the term "functional" as it applies to food has adopted a different perspective – that of providing additional physiological benefits beyond that of meeting basic nutritional needs (Hasler 1998).

In the health food sector, a number of industries are extracting and concentrating naturally occurring bioactive molecules to create drug-like substances that are known as *nutraceuticals*.

Bioactive substances are naturally present in plants and falls into the group of phytochemicals that includes phenolic compounds (including flavonoids, phytoestrogens), glucosinolates, and carotenoids. There is a growing body of evidence suggesting that phytochemicals may have protective roles against a variety of chronic diseases such as cancer and cardiovascular diseases (Johnson 2003).

Development of new functional foods or nutraceuticals is a challenging process. For example, when bioactive substances are extracted, concentrated, and added back into foods or made into a nutraceutical, one of the foremost challenges is to protect the stability and bioactivity of the isolated component. New techniques are required to try and prevent the deterioration of physiologically active compounds. Microencapsulation is increasingly being used as a specific technical tool in the manufacture of functional foods and nutraceuticals to prevent the deterioration of physiologically active components (whether they be microbial or non-microbial products), to improve their bioavailability, and to reduce their interaction with the other components of the foods into which they are incorporated.

The focus of this chapter is to review the use of microencapsulation techniques and applications in the development of functional foods and nutraceuticals, including live microorganisms and non-microbial bioactive substances. The review will cover functional foods from plant and animal sources.

11.2 Microencapsulation Technologies Applicable to Bioactive Functional Ingredients and Foods

In the development of functional foods and nutraceuticals, food manufacturers are facing a number of challenges, since the relevant biomolecules are unstable with regard to a number of processing and storage parameters such as heat, oxidation, pH, water activity, as well as poor absorption and reduced bioavailability in the gastrointestinal (GI) tract. In addition, issues such as reduced shelf-life and unsuitability for controlled release in their natural, isolated, or concentrated forms also have to be addressed. The biomolecules when consumed with functional foods or as nutraceuticals should be absorbed and released into the GI tract, transported through the gut–blood barrier for improved bioavailability, and remain stable for targeted delivery within the GI tract. Encapsulation has become a significant tool in biotechnology and has great potential for protecting and delivering bioactive compounds in functional foods and nutraceuticals.

11.2.1 Encapsulation Procedures

The most common methods of encapsulation of functional ingredients are spray-drying, spray-chilling, fluidised bed coating, freeze-drying, emulsification and extrusion, coacervation, inclusion complexation, liposome encapsulation, and compression coating (Table 11.1). The common wall materials used in encapsulation are shown in Table 11.1.

Spray-drying Microencapsulation by spray-drying is applied to both bioactive food molecules and, recently, for living probiotics (deVos et al. 2010). The goal of spray-drying is to obtain an emulsion of the core bioactive material within the chosen matrix material. The prepared dispersion or emulsion is atomised in a vessel with a nozzle or spinning wheel, and the solvent is evaporated by contact with hot air or gas. The resulting particles are collected after their fall to the bottom. The relative ease and the low cost are the main reasons for the broad application of spray-drying in industrial settings. This technology has disadvantages for certain bioactive molecules and living organisms. During atomisation, bioactive molecules as well as probiotics can be exposed to high temperatures and hydration, which can cause loss of bioactivity and viability in some of them (Kailasapathy 2002).

Spray-chilling In spray-chilling, a molten matrix with low melting point containing the bioactive substance is atomised through a pneumatic nozzle into

Table 11.1 Techniques and wall materials for encapsulating functional bioactive ingredients

Techniques	Encapsulation method	Type of encapsulating/ coating materials
Chemical	Emulsification and extrusion	Hydrocolloids (e.g., alginate)
	Coacervation, phase separation	Water-soluble polymers (e.g., whey protein)
	Molecular inclusion, complexation	Cyclic carbohydrates (e.g., β-cyclodextrin)
	Co-crystallisation	Supersaturated sucrose solution
	Liposome encapsulation	Liposomes
Mechanical/ physical	Spray-drying	Water-soluble polymers (e.g., gum arabic, whey protein, and starches)
	Spray-coating, congealing	Waxes, fatty acids, gluten, casein, and cellulose
	Spray-chilling, spray-cooling (spinning disk, centrifugal co-extrusion)	Vegetable oils, light waxes, and lipid materials
	Fluidised bed encapsulation, air suspension	Water-soluble and insoluble polymers, lipids, and waxes
	Extrusion	Water-soluble and insoluble polymers
	Compression coating	Hydrocolloids with binders (e.g. sodium alginate with hydroxypropyl cellulose)

Source: Adapted from Kailasapathy, K. (2009) CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 4, No. 033: 1–19.

a vessel. This technology is, in principle, opposite to spray-drying – instead of evaporating, the dispersion containing the bioactive material is cooled to allow immobilisation. Cold air is injected into the vessel to enable solidification of the gel particle. The liquid droplet solidifies and entraps the bioactive substance. This technology is rarely used to microencapsulate live probiotic organisms, however, it is more suitable for encapsulation of vitamins, fatty acids, antioxidants, yeasts, and enzymes.

Fluidised Bed Coating The fluid bed encapsulation process consists of spraying a coating solution into a fluidised bed of solid particles. The bioactive food components or microorganisms are suspended in air, and the matrix molecules are sprayed onto them. After several cycles of wetting and drying, a continuous film is formed. The solid particles are suspended in a temperature- and humidity-controlled chamber of high-velocity air where the coating material is atomised. Figure 11.1 illustrates fluidised bed coating methods. In Figure 11.1, three fluid bed coating technologies are presented, principally differing in the type of air fluidisation employed, and the site in the vessel where the coating

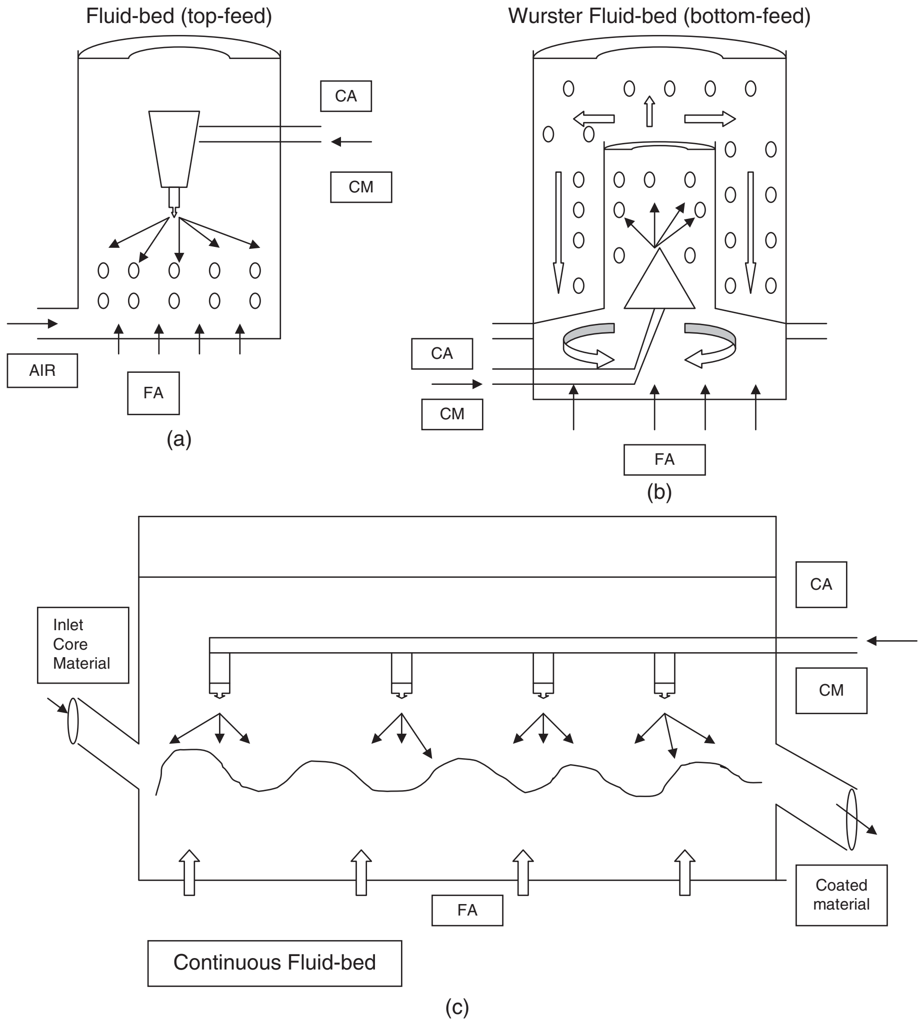

Figure 11.1 Fluid bed encapsulation techniques (FA – fluidised air; C – controlled air; CM – coating material).

material is sprayed (top spray and bottom spray). In the continuous-spray system, there are several nozzles of top spray arranged for continuous coating operation. The choice for matrix molecules is broader than for traditional spray-drying. It includes lipids, proteins, gluten, casein, cellulose, and derivatives.

Freeze-drying Freeze-drying is performed at low temperatures under vacuum, avoiding water-phase transition and oxidation. The obtained dried mixture must be ground, and the final particles are of wide size distribution and with low surface area. This technology is less frequently used, compared with other encapsulation techniques, as it is very expensive. Addition of cryoprotectants allows reduction of cell death during freeze-drying of cultures such as probiotic bacterial encapsulates, and stabilises them during storage. For example, trehalose has been used as a protective coating (De Castro et al. 2000).

Emulsions and Extrusions Emulsion encapsulation involves dispersing the compound of interest (e.g., essential oils, flavours, omega-3 fatty acids, and antioxidants in an aqueous solution of a "film-forming" polymer, usually a carbohydrate), which, upon drying (usually spray-drying), would produce a polymer matrix containing the bioactive component. Encapsulant wall materials for this purpose include gum arabic, maltodextrin, modified starches, and celluloses (Gibbs et al. 1999). In case of encapsulation of bacterial cells, a small volume of the cell–polymer suspension is added to a large volume of a vegetable oil (continuous phase), such as soybean oil, sunflower oil, canola oil, or corn oil (Figure 11.2). The mixture is homogenised to form a water-in-oil emulsion. A polymerising solution such as calcium chloride is then added to enable diffusion gelling to form discrete gel particles. In internal gelling, addition of an oil-soluble acid, such as acetic acid, reduces the alginate pH from 7.5 to approximately 6.5, enabling initiation of gelation with Ca^{2+} (Poncelet et al. 1993).

The mechanism of calcium-induced alginate gel formation occurs due to the orderly alignment of the alginate polymers, which interact with divalent cations such as calcium, where calcium ions occupy the space between two alginate polymers – similar to an egg placed inside an egg box, and is known as "egg box" gelling mechanism (Smidsrod and Skjak-Braek 1990). For encapsulation in an emulsion, an emulsifier and a surfactant are needed. Emulsifiers such as Tween 80 can reduce the surface tension between oil and water inter-phases, and also prevent spheres from coalescing before breaking up the emulsion. A surfactant such as sodium lauryl sulphate is used to lower the surface tension in the coating matrix, in order to reduce the size of the beads produced. Coating of the alginate beads can also be carried out to improve their protective properties. The beads are simply dipped in a solution containing a cationic polymer such as chitosan or poly-L-Lysine (Figure 11.2).

Extrusion essentially involves preparing a hydrocolloid solution or dispersion and adding the bioactive substance or microbial cells, and extruding the suspension through a syringe needle in the form of droplets to free-fall into a polymerising or hardening or gel setting bath (Figure 11.2). The size and shape of the beads depend on the diameter of the needle and the distance of free-fall, respectively. This method is popular due to its ease, simplicity, low-cost, and gentle gelling conditions ensuring high retention of microbial cell viability. However, this technique can be difficult to scale up for industrial production. The common polymer used to produce encapsulation matrix by extrusion technique is alginate (Krasaekoopt et al. 2003).

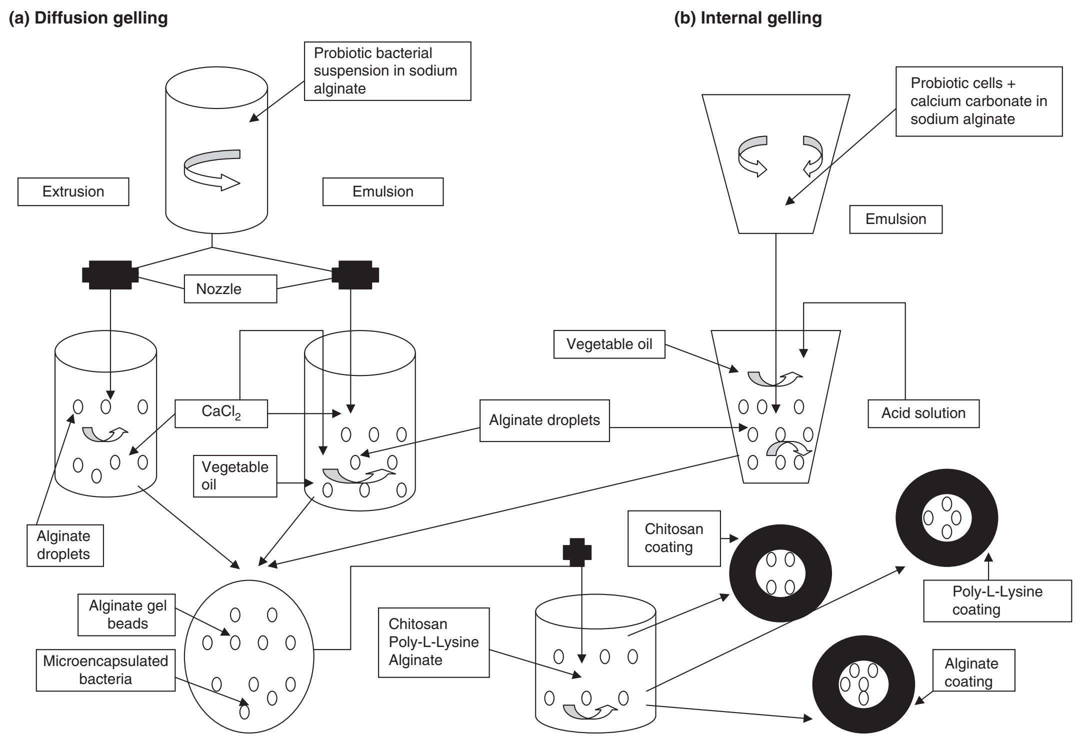

Figure 11.2 Techniques to microentrap probiotic bacteria in alginate beads (gel particle technology).

The common methods that are used to produce alginate beads that immobilise probiotic bacteria are shown in Figure 11.2. These techniques include extrusion and emulsion. The gelling types include diffusion gelling or setting and internal gelling or setting. In the diffusion gelling, a soluble cation such as calcium chloride is used and allowed to diffuse into alginate beads during polymerisation into gel particles. In the internal or bulk gelling, calcium is released from the system under controlled conditions at room temperature. It involves a combination of alginate and a less soluble calcium salt (e.g., calcium carbonate). The calcium ions are released from within the system when an acid solution is added. The internally released calcium reacts with alginate to form gel beads.

Gel Particles Technology Encapsulation in a biodegradable polymer matrix is the most widely used microencapsulation technology for probiotics (Champagne et al. 1994). The technology basically consists of producing solid matrices that contain the bacterial cells. In hydrogelling, polymers are used in the presence of monovalent or divalent cations to microentrap probiotic bacterial cells in hydrogel beads. The polymerisation is induced by the interaction of cations with the hydropolymers, resulting in the production of solid matrices that immobilise the cells.

Food-grade polymers such as alginate, chitosan, carboxymethyl cellulose, carrageenan, gelatine, starch, and pectin are mainly employed using various microencapsulation techniques. In certain cases, a filler material is used that does not play a role in the cation-induced gelling, but helps to strengthen the solid matrix of the gel beads. For example, Hi-Maize™ starch (a cross-linked resistant starch) has been used as a filler material in calcium-induced (encapsulant) hydrogelling to entrap probiotic bacteria (Sultana et al. 2000).

The polymers used in forming gel beads are essentially known as *hydrocolloids*, and are commercially available in powder form. They contain individual dry particles, which should first be thoroughly dispersed by agitation in a medium, usually water. Sufficient time should be allowed to hydrate the particles. Mechanical effects and sometimes heating help to solubilise the particles to individual molecules. Gelling involves texturisation obtained by either cooling the system or polymerisation with cations (Figure 11.3). Factors affecting gelling and the formation of gel beads depend on the length of the polymers and their capacity to form links. Long linear polymers tend to gel better than medium-size or branched polymers, while polymers that form links and junction zones can form better gels.

Liposome Technology Liposomes are produced by the association of amphiphilic compounds (mainly phospholipids) into bilayer structures. A *liposome* is defined as a structure composed of lipid bilayers that enclose a number of aqueous or liquid compartments. Using various techniques such as solvent evaporation, ultrasonication, and microfluidisation, the bilayer

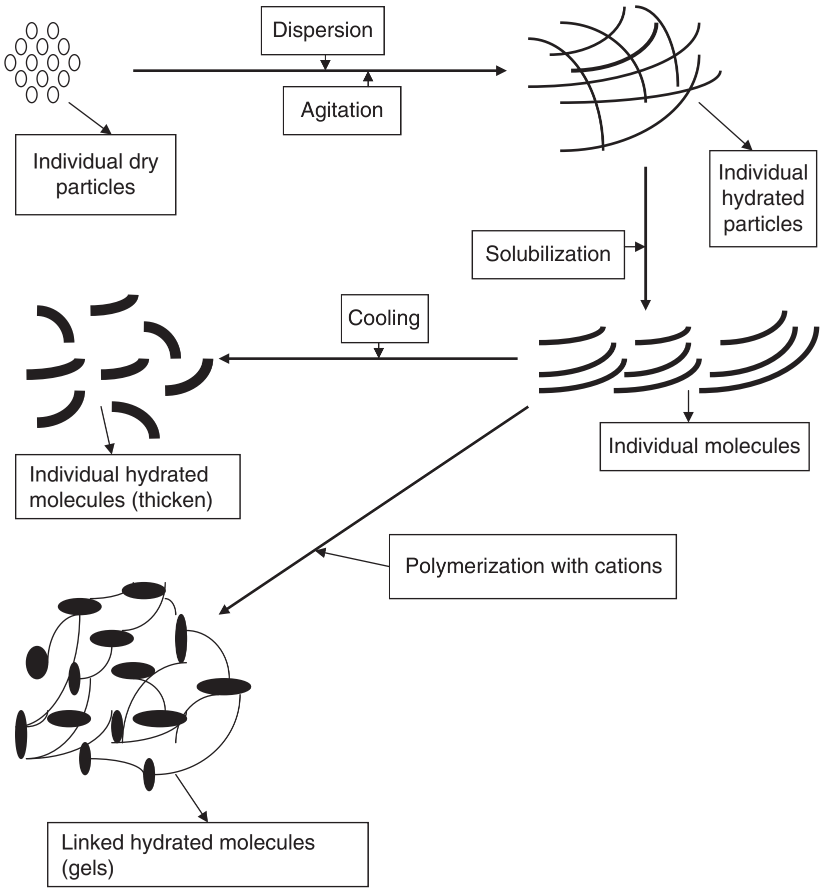

Figure 11.3 Dispersion, solubilisation, and gelling of polymers used in microencapsulation.

forms spherical core–shell structures (vesicles) (Gibbs et al. 1999). During the formation process, hydrophilic molecules in the external aqueous phase become entrapped in the liquid regions and core, while hydrophobic material may be incorporated in the bilayer membranes. Release of the entrapped substance can be either a gradual process resulting from diffusion through the membranes, or almost instantaneous following membrane disruption caused by changes in pH or temperature. Liposomes are mainly used for delivery of lipids or water-soluble materials such as omega-3 fatty acids, yeasts, and enzymes (Hawker 1995).

Molecular Inclusion and Complexation Another elegant encapsulation method involves using cyclodextrins that can envelop molecular structures by forming molecular inclusion complexes. Aromatic compounds (essential oils), colourings, and some vitamins (A, E, and K) are stabilised by forming an inclusion complex with β-cyclodextrin, which acts as a "molecule cage" to encapsulate the bioactive ingredients. β-cyclodextrins are cyclic carbohydrates (enzymatically modified starch granules) having their hydrophilic moieties oriented outward, and their lipophilic interior provides a suitable environment to solubilise organic compounds. The molecular dimensions of the inner hydrophobic cavity of β-cyclodextrin allow total or partial inclusion of a wide range of aroma compounds.

Coacervation Coacervation is a more advanced version of emulsion encapsulation. When a solution of bioactive component is mixed with a matrix molecule of an opposite charge, a complex is formed. The size of the capsule and its characteristics can be varied by changing the pH, the ion concentration, the ratio of the matrix molecule, and the type of matrix (de Vos et al. 2010). There are two methods of coacervation: a simple polymer coating and a complex polymer coating. The simple polymer coating requires the use of a polymer that is initially soluble but can be rendered insoluble after changes in pH and/or temperature (especially for proteins and ionic polysaccharides). Simple coacervation begins as a regular emulsion with the polymer adsorbed at the interphase between the emulsified phase and the solvent. Subsequently, temperature or pH is adjusted, so that the polymer becomes insoluble in the solvent and forms a separate phase (coacervate) that coats the emulsified phase. For complex coacervation, two or more types of polymers are used. It consists of mixing two polymers (one with negative charge and another with positive charge) that form an insoluble complex on the surface of the emulsified droplet. For example, essential oils have been microencapsulated by using coacervation using a mixture of whey protein and gum arabic as the coating polymers. In this method, first an emulsion of the essential oil in water is prepared with whey protein. Subsequently, a concentrated solution of gum arabic is incorporated into the emulsion at a pH at which both whey protein and gum arabic are negatively charged. The pH is then reduced to a value where the whey protein has a net positive charge and gum arabic has a net negative charge, inducing the complexation of these polymers and their coacervation (phase separation) (Weinbreck et al. 2004).

11.2.2 Encapsulation and Controlled Release of Non-microbial Functional Foods

Encapsulation and Controlled Release of Antioxidants and Vitamins as Functional Ingredients Phenolic compounds derived from fruits have high antioxidative capacities and are major beneficial components in fruits and vegetables (Kong et al. 2003; Petti and Scully 2009; Kondakova et al. 2009). A wide variety of natural antioxidants and vitamins such as vitamin E,

Table 11.2 Cited examples of encapsulation of antioxidants and vitamins

Antioxidant/ vitamin	Polymer	Encapsulation method	References
Coenzyme Q10	Gum arabic/malto-dextrin/starch	Spray-drying	Bule et al. 2010
Bayberry phenols	Ethyl cellulose	Emulsion/phase separation	Zheng et al. 2011
Black currant phenols	Maltodextrin, inulin	Spray-drying	Bakowska and Kolodziejczyk 2011
Bilberry anthocyanin	Whey protein	Emulsion and heat gelation	Betz and Kulozik 2011
Garcinia cowa fruit	Whey protein isolate	Freeze-drying	Ezhilarasi et al. 2103
Vitamin C (passion fruit juice)	n-octenylsuccinate-derived starch	Spray-drying	Borrmann et al. 2013
Garlic powder	Ethyl cellulose and cellulose acetate	Modified fluid bed coater	Li et al. 2007
Lycopene	Gelatine and sucrose	Spray-drying	Shu et al. 2006
β-carotene	*Pinhao* starch	Freeze-drying	Spada et al. 2012
Eugenol/cloves	Maltodextrin/gum arabic	Spray-drying	Chatterjee and Bhattacharjee 2012
Resveratrol	Yeast cells	High pressure	Shi et al. 2008
β-carotenoids	Gum arabic	Spray-drying	Romo-Hualde, et al. 2012
α-tocopherols	Sodium alginate	Ionic gelation and size exclusion	Yoo et al. 2000
α-tocopherols	Cotton seed lipids	Spray-chilling	Gamboa, et al. 2011
Foilc acid	Alginate-pectin	Ionic gelation	Madziva et al. 2005
Folates	Alginate-pectin	Spray-drying	Shrestha et al. 2012

Co-enzyme Q10, carotenoids, polyphenols, ascorbic acid, folates, and carnosine have been shown to possess beneficial properties, and could potentially improve human health.

Several studies have reported on the protection of antioxidants and vitamins against natural degradation by encapsulation (Table 11.2). Coenzyme Q10 is an endogenous antioxidant and has been reported to be a potential candidate for the treatment of cardiovascular disease, Parkinson's disease, and Huntington's disease (Bule et al. 2010). It is vulnerable to light, oxygen, and temperature. Bule et al. (2010) microencapsulated Coenzyme Q10 using blends of gum arabic, maltodextrin, and modified starches as wall materials. In this method, different oils such as olive oil, safflower oil, coconut oil, saffola oil, flax seed oil, and rice bran oil were blended with Coenzyme Q10 using surfactants, and the resulting emulsion was homogenised and spray-dried. The results showed that the encapsulated Coenzyme Q10 was significantly stable at 30±2 °C as well as under UV light, as compared to the free Coenzyme Q 10.

Bayberry polyphenols are among the bioactive compounds found in bayberries. Due to the presence of unsaturated bonds in their molecular structure, polyphenols are vulnerable to oxidants, light, and heat, and can be easily deteriorated. Zheng et al. (2011) microencapsulated polyphenol extracts from bayberry using ethyl cellulose as a coating material and a phase separation technique to prepare the microcapsules, which were spray-dried. The results using DPPH radical scavenging method showed that the antioxidant activity of bayberry polyphenols could be effectively protected by microencapsulation.

The commonly applied methods for the microencapsulation (ME) of extracted plant phenolics, such as anthocyanins, is spray-drying. The matrix materials mainly used are polysaccharides such as maltodextrin, inulin, gum arabic, tapioca starch, citrus fibre, glucose syrup, and soy protein isolate (Betz and Kulozik 2011). By using these methods, the encapsulated plant phenolics are stabilised against degradation due to the impact of oxygen and light during storage. However, in aqueous environments, as prevalent in many foods or in the gastrointestinal tract, these water-soluble microparticles may disintegrate and lose their protective effects on the encapsulated compounds. Betz and Kulozik (2011) demonstrated that emulsion encapsulation can be used to generate water-insoluble, whey-protein-based hydrogels for the ME of anthocyanin-rich bilberry extract. In this method, a whey protein isolate (WPI) solution was mixed with bilberry extract (BE) in aqueous solution; the generated BE–WPI solution was centrifuged; the resultant supernatant was poured into sunflower oil with constant stirring; and thermal gelation was induced to obtain whey-protein-based microcapsules with encapsulated bilberry extract.

Black currants are a good source of bioactive polyphenols. Of the total polyphenols found in black currants, anthocyanins form the major component. To improve the stability of black currant polyphenols, Bakowska and Kolodziejczyk (2011) microencapsulated the polyphenols by spray-drying. The polyphenol compounds were extracted from black currant pomace and mixed with maltodextrin and inulin, and homogenised before spray-drying. The stability of the encapsulated polyphenols during 12 months of storage at 8 °C and 25 °C was evaluated. The results showed that maltodextrins DE 11 offered better protection of the phenolics during storage. Radical scavenging activity studies (antioxidant activity) demonstrated significant antioxidant activity of microencapsulated powders before and after storage, resulting in the conclusion that black currant polyphenol microcapsules represent a promising food additive for incorporation into functional foods.

Garcinia cowa fruit is considered as a functional food due to its being an excellent dietary source of (-)-hydroxycitric acid (HCA) in its fruit rinds (Jena et al. 2002). Various reports have demonstrated that HCA can regulate fatty acid synthesis (Sullivan et al. 1977), lipogenesis (Kovacs and Westerterp-Plantenga 2006), appetite (Thom 1996), and weight loss (Ramos et al. 1995). However, HCA is hygroscopic in nature and thermally sensitive. Ezhilarasi et al. (2103) microencapsulated *Garcinia cowa* fruit extract using three different wall materials such as whey protein isolate (WPI), maltodextrin (MD), and a combination of WPI and MD. In this method, the wall materials were mixed with concentrated (flash-evaporated) fruit extract in an aqueous solution, the mixture was stirred, and the resulting solution was freeze-dried to form microencapsulated powders. The microencapsulates were then incorporated into dough to prepare bread with functional food attributes. WPI effectively encapsulated and protected the HCA during bread baking and resulted in higher retention of HCA.

Passion fruit (*Passiflora)* is rich in vitamin C, vitamin B_1 and B_2, and the provitamin A, β-carotene, as well as minerals and fibres. The production and commercialisation of passion fruit juice has faced many difficulties, including reduced shelf-life during transportation. Food microencapsulation has been an efficient way of extending shelf-life during storage. Borrmann et al. (2013) microencapsulated passion fruit juice with an aqueous solution of n-octenylsuccinate (n-OSA)-derived starch using a spray-dryer. In this method, n-OSA (National Starch, United States) was added to passion fruit juice, homogenised, and the resulting mixture was spray-dried. Spray-drying of passion fruit juice encapsulated in n-OSA starch resulted in very small and stable microcapsules, in the form of a homogenous white powder that was easily reconstitutable. Microencapsulation further protected the vitamin C activity during spray-drying.

Garlic is reported to possess a vast variety of bioactive functions, such as antimicrobial, improving the immune system, and lowering serum lipid and glucose levels and blood pressure (Nagourney 1998). Garlic is processed into powder and granules, which are packaged and sold. However, it has been found that, during storage, the bioactive components in these products are often reduced or rendered inactive. The alliinase enzyme in garlic is irreversibly deactivated at the pH level in the human stomach. If garlic powder is consumed directly, there would be only an insignificant amount of allicin that can be produced inside the human body. Li et al. (2007) microencapsulated garlic powder and coated them with materials that could resist human stomach conditions to prolong the shelf-life and protect allinase activity in transit through the stomach. When in the intestine, allicin is released, resulting in enhanced absorption of this bioactive compound. Dried garlic powder was spray-dried using ethyl cellulose in acetone and isopropanol as solvents, and the coated garlic powder was further coated with cellulose acetate phthalate in acetone and isopropanol solvent. The coating of the garlic powder was done using a modified fluidised bed technique. *In-vitro* studies showed that the microencapsulated garlic powder could resist stomach pH, and that its release could be controlled in the intestine.

Lycopene is a carotenoid found in ripe tomato and is associated with decreased risk of cardiovascular diseases and preventing prostate cancer in men. Lycopene is one of the most common carotenoids found in human serum and the predominant one found in plasma (Agarwal and Rao 2000). Due to the high number of conjugated double bonds, it is considered to be one of the most potent antioxidants among the carotenoids (Dimascio, et al. 1989). However, because of the presence of unsaturated bonds in the molecular structure, lycopene is susceptible to oxidants, light, and heat, and can be easily deteriorated when exposed to such adverse environment (Lee and Chen 2002; Pesek and Warthesen 1987). Free lycopene must be protected from chemical changes before its industrial application. Shu et al. (2006) microencapsulated lycopene by spray-drying using gelatine and sucrose as encapsulant wall materials. In this method, the gelatine and sucrose were dissolved in water and lycopene dissolved in acetone was added to form an emulsion. The emulsion was then homogenised and spray-dried. The results showed that the microencapsulated capsules protected the stability of lycopene.

β-carotene, an important member of the carotenoid family, is a group of compounds widely distributed in nature, and are responsible for the yellow, orange, and red colours of fruits and vegetables (Kandlakunta, et al. 2008). These carotenoids exhibit antioxidant activity by scavenging oxygen radicals and reducing oxidative stress. However, they are susceptible to isomerisation and oxidation, which results in the loss of these properties. In a recent study, native and hydrolysed *Pinhao* (*Araucarina angustifolia* seeds) starch was used as wall materials for β-carotene microencapsulation by freeze-drying (Spada, et al. 2012). Microcapsules prepared with native starch showed the lowest stability during storage. In contrast, microcapsules encapsulated with 12-DE (dextrose-equivalent) hydrolysed starch exhibited the highest stability. Native starches exhibit poor film-forming capacity compared to hydrolysed starches, and thus hydrolysed starches form an effective barrier that protects the β-carotenes against oxidation.

Bioactive components of essential oils of species such as cinnamon, bay leaf, nutmeg, basil, and cloves contain eugenol (phenylpropene, an allyl chain-substituted guaicol) (Chatterjee and Bhattacharjee 2012). Eugenol has bioactive effects such as antioxidant, antifungal, antibacterial, and also anti-inflammatory. Essential oils are used in the formulation and preparation of functional foods. Clove oil, which contains eugenol, is used in many food preparations; however, clove oil is sensitive to light, heat, and oxygen, and has a short storage life (Shaikh, et al. 2006). At high temperatures, the antioxidant capacity of clove oil is substantially reduced. Eugenol-rich clove extract was microencapsulated using a matrix comprising maltodextrin and gum arabic and spray-drying (Chatterjee and Bhattacharjee 2012). In this study, food application in soybean oil was designed using the encapsulated clove powder as a source of natural antioxidants. The results showed that the encapsulated eugenol-rich clove extract showed antioxidant activity comparable to commercially available antioxidants such as Butylated Hydroxy Toluene (BHT).

Resveratrol, a naturally occurring non-flavonoid polyphenolic compound present in grapes, berries, and red wine, has drawn attention as a bioactive ingredient for its various beneficial biological roles. Roots of the weed *P. cuspidatum Sieb et Zucc*. are reported to be one of the richest sources of resveratrol, and have long been used in traditional Chinese medicine as a drug against inflammation, allergy, and hyperlipidaemia (Vastano et al. 2000). Resveratrol is a phytoalexin that exhibits various biological and pharmacological activities, among which antioxidative activity is predominant (Li et al. 2006). However, resveratrol is easily oxidisable and extremely photosensitive. Microencapsulation is a promising approach for improving the stability of isolated resveratrol, preserving its biological activities, and enhancing its bioavailability; hence,facilitates the incorporation of resveratrol in the development of new functional foods. Shi et al. (2008) used yeast (*S. cerevisiae)* as an encapsulation matrix to prepare microcapsules of resveratrol. In this method, yeast cells were grown in liquid culture medium, and the cells were harvested and lyophilised. Resveratrol and yeast cells in aqueous ethanol were mixed to form a homogenous solution. The homogenised mixture was then subjected to a high-pressure treatment (25 MPa) at 40 °C for 4 h, followed by centrifugation and freeze-drying. Yeast-encapsulated resveratrol showed stronger free radical scavenging activity than that of non-encapsulated resveratrol.

Among the various vitamin E categories, α-tocopherol is a representative lipid-soluble antioxidant. Nutritionally, α-tocopherol is the most important, because it prevents cell damage by inhibiting lipid peroxidation, the formation of free radicals, and cardiovascular disease; improves blood circulation; regenerates tissues; and is useful in treating fibrocystic breasts and premenstrual syndrome (Kaneko et al. 1991). The beneficial effects of α-tocopherol may be limited, however, because it is labile to heat and oxygen, and can be irreversibly converted to quinone via epoxide formation when exposed to heat and oxygen in the presence of metals such as iron (Gamboa et al. 2011). In addition, the lipophilic α-tocopherol does not dissolve in water, and thus should be assisted by surfactants or emulsifiers to increase bioavailability. Typically, materials carrying lipophilic compounds such as α-tocopherol can be homogenised, and water-in-oil emulsions can be generated using an emulsifier. However, the resulting particle size may be too large, and creaming may occur during storage. Microencapsulation technology can overcome these impediments, protecting α-tocopherol from unfavourable environments, and further allowing its solubilisation in aqueous environments for food and nutritional applications. Yoo et al. (2006) microencapsulated α-tocopherol in a sodium alginate matrix to produce microcapsules for food applications. In this method, an oil-in-water (O/W) emulsion consisting of sodium alginate as a coating material and α-tocopherol as a core material was prepared using the Tween 80 emulsifier. Microcapsules were produced by ionic gelation and size-extrusion techniques. The resulting O/W emulsion was sprayed into a calcium chloride solution by using an air-atomising system. The study found that the sodium alginate as a coating material was structurally resistant against acidic environments, and rapidly released α-tocopherol under mild alkaline conditions. In another study, lipid microcapsules containing α-tocopherol was prepared using a spray-chilling technique. Good stability of α-tocopherol was observed during storage, which indicates that the lipid matrix protected the bioactivity of the α-tocopherols (Gamboa et al. 2011).

Folic acid is essential for the healthy functioning of a variety of physiological processes in humans. Folate deficiency in the diet has been linked to malformation of the embryonic brain/spinal cord development, a condition referred to as *neural tube defects* (NTD), manifested by still-birth, mental retardation, swollen head, and poor bladder control (Czeizel and Dudas 1992). Most naturally occurring folate derivatives in foods are highly sensitive to oxygen, temperature, pH, and light, and thus their stability is affected during the processing and storage of this vitamin (Hawkes and Villota 1989). Microencapsulation technology can be used to encapsulate synthetic forms of folates, incorporate them into a food vehicle, and deliver them to consumers in more stable concentrations. Alginate and pectins have been used as wall materials to encapsulate folates (Madziva et al. 2005). In this method, aqueous polymer solutions were prepared (alginate, and alginate and low methoxyl pectin in various combinations), and homogenised. The folic acid was dispersed in the polymer solutions, and the mixture was pumped through an encapsulation nozzle with a continuous flow of nitrogen into a gently agitated aqueous solution of 0.1 M calcium chloride solution at room temperature. Discrete hydrogel beads entrapping folic acid were formed upon contact with the polymerising calcium chloride solution (ionic gelation) (Figure 11.4). Madziva et al. (2006) developed a folic-acid-fortified Cheddar cheese as a functional food by incorporating microencapsulated folates into a cheese matrix. They demonstrated that the stability of the encapsulated folic acid was enhanced in Cheddar cheese over a 3-month ripening period, compared to the free folic acid. In a recent study, 5-methyltetrahydrofolic acid (5-MTHF) was microencapsulated using a combination of pectin and sodium alginate as wall materials and spray-drying (Shrestha et al. 2012).

Encapsulation and Controlled Release of Bioactive Oils from Plant and Animal Sources A number of studies have been reported on the encapsulation of bioactive oils from plant and animal sources (Table 11.3). Drusch (2007) studied sugar beet pectin and glucose as alternate wall materials to gum arabic or milk protein to microencapsulate fish oils. In this study, fish oil rich in long-chain polyunsaturated fatty acids (PUFAs) was microencapsulated in a matrix of sugar beet pectin and glucose syrup. The results showed that, based on the physicochemical properties of the microencapsulated fish oil, sugar beet pectin could be considered as a novel wall material for microencapsulation of lipophilic food ingredients by spray-drying. In this

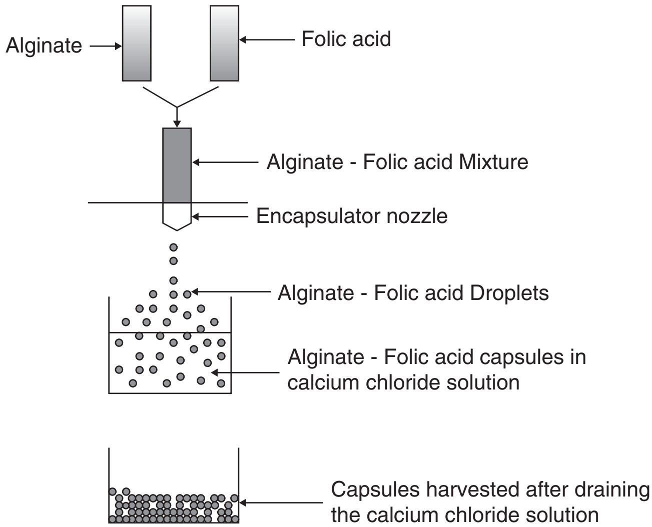

Figure 11.4 A simplified schematic illustration of microencapsulation of folic acid using alginates.

study, however, the oxidative stability of the microencapsulated fish oils was not reported.

Carbohydrates (starches, maltodextrins, and corn syrup solids) are often used as microencapsulation wall materials due to their desirable drying properties and ability to form matrices. However, carbohydrates have poor interfacial properties and must be chemically modified to improve their surface activity. Food proteins are better wall materials for microencapsulation due to their excellent emulsifying, and gel and film forming properties (Chen et al. 2006). Additionally, protein coatings are degradable by digestive enzymes, and thus can be used in developing food applications for controlled release (Chen et al. 2006). Whey proteins, caseinates, and gelatines are the most common coating materials used to encapsulate fish oil by spray-drying.

In a recent study, barley (*Hordeum vulgare L.*) protein was used as wall material to encapsulate fish oil. Barley protein powder was hydrated and, following pH adjustment, fish oil was mixed, and an emulsion formed. Microcapsules were then formed by passing the premixed emulsion through a microfluidiser system, followed by spray-drying. Barley protein conferred to the microcapsules a greater capacity to prevent oil oxidation and hence increased their usefulness in functional food applications (Wang et al. 2011).

Omega-3 fats are functional ingredients used in dietary supplements, healthy foods, and pharmaceutical products. These bioactive fatty acids have well-established health benefits and are primarily derived from fish oil. The main bioactive omega-3-fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been shown to decrease the

Table 11.3 Cited examples of encapsulation of bioactive oils from plant and animal sources

Source	Polymer	Encapsulation method	References
Fish oil	Sugar beet pectin	Emulsion and spray-drying	Drusch 2007
Fish oil	Barley protein	Emulsion and spray-drying	Wang et al. 2011
Essential oils (oregano)	Skim milk powder and whey protein concentrate	Emulsion and spray-drying	Baranauskiene, et al. 2006
Oleoresins (pepper)	Gum arabic and modified starch	Emulsion and spray-drying	Shaikh et al. 2006
Linseed oil	Gum arabic, malto-dextrin, methyl cellulose, whey protein isolate	Emulsion and spray-drying	Gallardo et al. 2013
Olive oil (extra-virgin)	Sodium caseinate, carboxymethyl cellulose, maltodextrin	Freeze-drying	Clavo et al. 2012
Flax oil	Zein	Freeze- and spray-drying	Quispe-Condori et al. 2012
Coffee oil	Gum arabic	Emulsion and spray-drying	Frascareli et al. 2012
Soya oil	Sodium-caseinate, maize starch, and corn syrup blends	Emulsion and spray-drying	Hogan et al. 2001
Sunflower oil	Dextrin and milk protein isolate	Emulsion and spray-drying	Ahn et al. 2012
Walnut oil	Sodium casienate, maltodextrin, carboxymethyl cellulose, lecithin	Emulsion and lyophilisation	Calvo et al. 2012

risk of coronary heart disease. Fish oil is the most abundant and cheapest source of EPA and DHA; however, these fatty acids are unstable and will oxidise quickly, leading to the formation of aldehydes and ketones that have unpleasant smell and taste. Various antioxidants have been used for the chemical stabilisation of EPA and DHA, but these are often not adequate to ensure desirable sensory attributes in finished food and beverage products (Shahidi and Zhong 2010). Encapsulation may therefore be a preferable approach, as demonstrated in the following text.

Essential oils from herbs such as oregano, citronella, marjoram, and pepper contribute to food flavours, as well as provide therapeutic or medicinal benefits. Herbal oils containing essential oils and oleoresins are used to develop functional foods and nutraceuticals. Flavours and therapeutic effects of these oils can be changed as a result of oxidation, chemical interaction, or volatilisation. In order to minimise these changes, microencapsulation is used in the flavour industry to entrap flavouring substances such as essential oils, oleoresins, and aroma and flavour mixtures in a protective layer of coating materials.

Baranauskiene et al. (2006) evaluated the properties of skimmed milk powder (SMP) and whey protein concentrate (WPC) as wall materials to microencapsulate essential oils from herbs (i.e., oregano, citronella, and sweet majoram) by spray-drying. In this process, coating matrices (SMP and WPC) were reconstituted, dispersed, and allowed to hydrate. Essential oils were then emulsified into the hydrated coating material and homogenised, followed by spray-drying.

Solvent-extracted oleoresins from spices exhibit better flavour profiles and have medicinal properties (Shaikh et al. 2006). However, spice oleoresins exhibit sensitivity to light, heat, and oxygen, and have shorter storage lives if not stored properly. For example, poor storability of black pepper oleoresin is a result of oxidative and polymeric changes involving the fatty oil component and monoterpenic hydrocarbons. The active constituent of pepper, piperine, is sensitive to light and oxygen, and can undergo hydrolysis to piperidine and piperinic acid, and also photolysis to iso-chavicine, which lacks the typical pepper flavour (Shaikh et al. 2006). Black pepper oleoresin was microencapsulated using gum arabic and a modified starch (HiCap100) using spray-drying to create a free-flowing powder (Shaikh et al. 2006). In this method, gum arabic and the modified starch were dispersed individually in distilled water; black pepper oleoresin was added and homogenised (Tween 80 was added to aid emulsification); and the resulting slurry was spray-dried. Gum arabic offered greater protection to the encapsulated pepper oleoresin than modified starch. Other studies on microencapsulation of spice oleoresins include: garlic oleoresin by spray-drying using edible gum as wall materials (Xiang et al. 1997); paprika oleoresin by spray-drying using gum arabic (Zilberboim et al. 1986); capsicum oleoresin by spray-drying using a mixture of carrageenan and maltodextrin (Xiang et al. 1997); and red pepper oleoresin using gum arabic and modified starch (Jung and Sung 2000).

Linseed oil contains alpha-linolenic acid (Bozan and Temelli 2008), which reportedly has beneficial effects on cardiovascular health and depression (Lucas et al. 2011). Linseed oil (omega-3-PUFA) is easily oxidised, due to its high degree of unsaturation. Gallardo et al. (2013) microencapsulated linseed oil by using different combinations of gum arabic, maltodextrin, methyl cellulose, and WPI as wall materials, and spray-drying. In this method, the dissolution of wall materials was done in deionised water separately, and they were subsequently mixed together. O/W emulsions were obtained by adding flax seed oil mixed with lecithin as an emulsifier. The wall material suspensions and the linseed O/W emulsions were homogenised and spray-dried. The microcapsules were incorporated into bread dough to prepare linseed-oil-fortified bread (functional food). However, the results showed a decrease of alpha-linoleic acid when gum arabic microcapsules containing linseed oil were incorporated into bread mix and baked into bread.

The biological activities associated with the consumption of extra-virgin olive oils (antioxidant, anti-inflammatory, chemopreventive, and anticancer) have enhanced the use of this oil in functional food and beverage development (Tortosa et al. 1999). Encapsulation will be useful to retard lipid auto-oxidation and increase the range of applications where this oil can be used. Calvo et al. (2012a) microencapsulated extra-virgin olive oil using carbohydrate-based (maltodextrin and carboxymethylcellulose) and protein-based wall materials. The emulsions formed were frozen at −80 °C, freeze-dried, and the microcapsules were ground to a powder. The protein-based wall materials were found to be more effective in preserving the quality of the microencapsulated extra-virgin olive oil.

The addition of omega-3 and omega-6 PUFAs to functional food ingredients and their consumption as dietary supplements have experienced significant increases (O'Brien 2009; Sanguansri and Augustin 2007). Flax seed oil is a good source of PUFA, especially linoleic acid (C18:3 ω3) (Bozan and Temelli 2002). However, this oil has low stability and high susceptibility to oxidation. Quispe-Condori et al. (2011) microencapsulated flax seed oil using corn protein (zein). Corn zein was dissolved in an ethanol solution, and flax oil was stirred in to form an emulsion, followed by spray-drying and freeze-drying. The freeze-dried microcapsules showed lower microencapsulation efficiency compared to spray-dried capsules.

Coffee contains polyphenols such as flavon-3-ols, hydroxycinnamic acids, favonols, and anthocyanidins (Ramirez-Coronel et al. 2004). These bioactive compounds have antioxidative effects and may potentially reduce cell damage, while roasting coffee increases the bioactivity of these components (Ramirez-Coronel et al. 2004). Coffee oil has been used recently to enhance the aromatic potential of foods and as a functional ingredient in the development of functional foods and nutraceuticals. Since exposure of the coffee oil to atmosphere can cause lipid oxidation and unpleasant odours, microencapsulation can help to reduce these adverse effects. Frascareli et al. (2012) microencapsulated coffee oil using gum arabic as the wall material. Gum arabic was first thoroughly dissolved in distilled water and homogenised with roasted coffee oil, and spray-dried. The results showed that emulsions prepared with higher coffee oil content were less stable, because the higher the oil content, the lower the gum arabic content (for the same total solids), and hence the lower the amount of wall material available to cover the oil droplets, leading to faster droplet coalescence. This study, nevertheless, showed that coffee oil could be microencapsulated into a free-flowing powder, and that microencapsulation protects coffee oil against lipid oxidation and rancidity.

Soy oil is rich in PUFA as well as other bioactive components. Preparation of a free-flowing powder containing oil can be useful in wider formulations incorporating bioactive soy oil into the development of new functional foods. Emulsions of soy oil in mixed emulsions of sodium caseinate and carbohydrates of various DE were prepared and spray-dried into free-flowing powders containing 20–75% soy oil (Hogan et al. 2001). The results showed that the ability of sodium caseinate/carbohydrate blends to encapsulate soy oil was improved by increasing the DE of the carbohydrates. Of the carbohydrates examined, corn syrup solids with a DE of 28 proved most effective when used at sodium caseinate/DE 28 ratios > 1:39 and core wall ratios < 1.5. The wall systems minimised destabilisation of oil droplets during drying and effectively encapsulated soy oil in free-flowing powder particles.

High-oleic sunflower oil is increasingly being used in the development of functional foods due to its high content of PUFA and associated health benefits. A major problem in utilising this oil is the oxidative deterioration during storage or processing. Although microencapsulation can protect seed oils from oxidation, severe lipid oxidation on the surface of the microcapsule could occur due to the high temperature during the spray-drying process and the presence of residual oils on the surface. Control of the surface free oil on the microcapsules is crucial for a prolonged shelf-life because free oil is widely spread on the surface of the microcapsules and exposed to atmosphere. Ahn et al. (2012) demonstrated that natural plant extracts (e.g., rosemary, citrus) can effectively inhibit lipid oxidation of high-oleic sunflower oil. They reported that, by employing a dextrin-coating method with wall materials such as milk protein isolate and soy lecithin in the presence of sodium triphosphate emulsifier, natural plant extracts containing high-oleic sunflower oil could be microencapsulated with greater efficiency. Homogenised emulsions of the oil and the wall materials were spray-dried. The results further showed that natural plant extracts acted as an antioxidant on the microencapsulated high-oleic sun flower oil.

It is reported that regular consumption of walnuts prevents coronary heart diseases, and this has been attributed to its high content of monounsaturated fatty acids (e.g., oleic), PUFA (e.g., linoleic acid), and omega-3 fatty acids (e.g., linolenic acid) (Pereira et al. 2008). In addition, the fatty fraction of walnut oil contains tocopherols, polyphenols, and phystosterols (Calvo et al. 2011). As with other plant oils, walnut oil is also susceptible to oxidative deterioration. A recent study reported microencapsulation of walnut oil using a combination of sodium caseinate, maltodextrin, carboxymethyl cellulose, and lecithin as wall materials (Calvo et al. 2012b). Microcapsules made from protein-based wall materials (50% sodium caseinate and 50% maltodextrin) were highly digestible (90% of the encapsulated oil was released from the microcapsules after the *in-vitro* digestion), compared to microcapsules made from carbohydrate-based wall materials (32% maltodextrin, 65.6% carboxymethyl cellulose, and 1.6% lecithin).

Encapsulation and Controlled Release of Bioactive Antimicrobials Food safety is a major issue for the food industry and consumers. Some functional foods and functional ingredients may have antimicrobial properties, and hence could improve food safety. Table 11.4 lists some of the published literature on the encapsulation of antimicrobial bioactive compounds. Studies on lemongrass (*Cymbopogom citratus*) essential oil as antimicrobial have been reported due to its applicability in the food and pharmaceutical industry (Leimann et al. 2009). The bioactive compounds that possess antimicrobial activity in lemongrass oil are α-citral (geraniol) and β-citral (neral), and they exhibit antibacterial action on Gram-negative and Gram-positive organisms. To improve the stability of lemongrass essential oil, it was microencapsulated using a simple coacervation technique (Leimann et al. 2009). Polyvinylalcohol (PVA), cross-linked with glutaraldehyde, was used as a wall polymer. The results showed that there were no significant alterations in the essential oil during the microencapsulation, and the antimicrobial activity of the encapsulated oil was not affected.

Incorporating antimicrobial bioactive substances into packaging materials has emerged as a bioactive packaging technology. The incorporation of antimicrobial agents can be done by coating and encapsulation. Lee et al. (1998) reported incorporating grapefruit seed extract, a natural antimicrobial

Table 11.4 Cited examples of encapsulation of bioactive antimicrobial compounds

Source	Coating substance	Encapsulation method	References
Lemongrass (essential oil)	Polyvinyl alcohol cross-linked with glutaraldehyde	Co-acervation	Leimann et al. 2009
Grapefruit seed extract	Low-density polyethylene and gum arabic	Emulsion and film coating	Lee et al. 1998
Carvacol and thymol	Gum arabic and polypropylene	Emulsion and film coating	Guarda et al. 2011
Curcumin	Gelation and porous starch	Emulsion and spray-drying	Wang et al. 2009
Lysozyme	Zein (prolamines)	Emulsion and supercritical antisolvent process	Zhong et al. 2009
Allylisothiocyante	Gum acasia	Emulsion and freeze-drying	Chacon et al. 2006
Allylisothoocyante	Gum arabic and Chitosan	Emulsion and spray-drying	Ko et al. 2012
Bovine lactoferrin	Whey protein Isolate and xanthan gum	Emulsion and freeze-drying	Al-Nabulsi and Holley 2007
Gallic acid	Chitosan, β-cyclodextrin, and xanthan gum	Emulsion and spray-drying	Goncalves da Rosa et al. 2013

agent, in a low-density polyethylene film (LDPE) of 30-μm thickness. The resulting encapsulated films showed inhibitory activity against *E. coli* and *S. aureus* when used for modified packaging of lettuce and soybean sprouts to extend their shelf-life. Carvacrol (isomer of thymol), a major component of oregano and thyme essential oils (Thymus and Origanum sp.), is a phenolic compound that has been used as a natural food preservative (Kulisic et al. 2004). Carvacrol and thymol were emulsified in an O/W emulsion with an aqueous solution of gum arabic adjusted to pH 6.0 with NaOH. Tween 20 was added to facilitate an aqueous continuous phase, and the emulsion was homogenised. A polypropylene film was coated with the emulsion containing the microcapsules and tested for their antimicrobial activity using a diffusion agar plate test. The results showed that the microencapsulated bioactive agents were able to inhibit growth of a broad spectrum of microorganisms. This study shows the potential of microencapsulation to incorporate antimicrobial agents into polymer films, and to use them as bioactive packaging for fresh food preservation (Guarda et al. 2011)

Curcumin is a permitted colorant used in foods, and it has a wide range of pharmacological activities including antimicrobial effects (Sowbhagya et al. 1998). Curcumin is susceptible to oxidants, light, and heat, and hence should be protected in certain forms from chemical damage before it is used in industrial applications (Hanne et al. 2002). Curcumin was stabilised by microencapsulating with edible gelatine and porous starch (Wang et al. 2009). In this method, gelatine and porous starch were dissolved in hot water to form an aqueous solution. Curcumin was pre-heated to dissolve in acetone, and the solution of curcumin was dripped into the aqueous solution of the wall materials, forming a coarse emulsion. The emulsion was homogenised and spray-dried. The results showed that curcumin retained its antibacterial and antifungal properties after microencapsulation, and hence could be a potential preservative for the food industry.

Zhong et al. (2009) microencapsulated lysozyme, an antimicrobial agent, with corn zein (prolamines) as wall material, and using a supercritical anti-solvent process (SAS). SAS is similar to spray-drying, where a feed is continuously sprayed into supercritical carbon dioxide (that acts as an antisolvent in most polymers, including zein and lysozyme). After the co-solvent in the atomised droplets is extracted out by CO_2, polymers precipitate into microparticles and nanoparticles because of the insolubility in CO_2. Encapsulated lysozyme was released gradually from the capsules; however, at lower pH (2–8), there was a rapid release.

Allyl isothiocyante (AIT), a major product of the hydrolytic action of the endogenous enzyme myrosinase on the glucosinolate singirin in aromatic herbal plants (mustard, horseradish), is a natural antimicrobial used in food matrices such as ground beef (Muthukumarasamy et al. 2003). The application of AIT has been limited due to its poor water solubility and volatility. Chacon et al. (2006) reported that gum acacia microcapsules containing AIT (prepared as freeze-dried emulsion), when incorporated into chopped refrigerated beef, eliminated a large number of *E. coli* 0157:H7 pathogens. In another study, AIT was microencapsulated using gum arabic and chitosan as wall materials, and the emulsion prepared was spray-dried. The microencapsulated powder containing AIT was tested as an additive to improve the shelf-life and quality of packaged Kimchi (a functional food) during storage at 10 °C. Encapsulated AIT, when incorporated into Kimchi, improved shelf-life without reducing quality (Ko et al. 2012).

Lactoferrin is a natural antimicrobial, the main iron-binding protein in milk, and it has antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria, fungi, and parasites (Farnaud and Evans 2003). However, divalent cations such as Ca^{2+} and Mg^{2+} reduced its antimicrobial activity. Divalent cations, after interacting with lactoferrin, induces change in the structure of the latter, yielding a less effective tetrameric form of lactoferrin, while cations are said to increase the stability of the targeted bacterial cell membranes (Ellison et al. 1988; Shimazaki 2000). To circumvent this problem, Al-Nabulsi and Holley (2007) microencapsulated bovine lactoferrin using a water-in-oil emulsion and freeze-drying. The results showed no significant difference in the antimicrobial activity between encapsulated and unencapsulated lactoferrin.

Gallic acid (3,4,5-trihydroxybenzoic acid), found abundantly in berries, citrus fruits, tea, wine, and herbs, is an antimicrobial agent. To improve the stability of gallic acid against oxygen, light, and food-processing environments, it was microencapsulated using chitosan, β-cyclodextrin, or xanthan as wall materials, and lyophilised. Gallic acid showed no loss of antioxidant capacity compared to pure gallic acid (Goncalves da Rosa et al. 2013), suggesting that encapsulated gallic acid could be used as an antimicrobial agent in the development of functional foods.

11.2.3 Encapsulation and Controlled Release of Microbial Functional Foods – Probiotics

A number of technological challenges exist in successfully incorporating probiotics into functional foods and in maintaining their viability. Processing of probiotic-incorporated foods involve heat treatment (e.g., pasteurisation), pumping, homogenising and stirring (incorporating air), and freezing (frozen dairy products), and hence it could reduce the viability of probiotic bacteria. Addition of salt (antimicrobial), drying (osmotic dehydration, e.g., powdered milk), packaging (oxygen ingress through packaging during storage), unfavourable storage conditions (e.g., post-acidification in yoghurt), heat shock (e.g., ice cream), and the possible development of antimicrobial compounds secreted by the starter cultures during fermentation could also reduce the viability of probiotic bacteria. Hence, preserving the viability of probiotic bacteria is of paramount importance in probiotic-incorporated products. In the development of functional foods, microencapsulation is used not so much for controlled release into foods but for incorporation and protecting viable cells into the products. In the recent past, probiotic bacteria have been incorporated into dairy, meat, and plant products. A number of functional nutraceuticals containing probiotics are also commercially available.

Dairy-based Functional Foods

Probiotic Yoghurts Microencapsulation in calcium-induced alginate–starch polymers (Godward and Kailasapathy 2003; Sultana et al. 2000), in potassium-induced kappa-carrageenan polymers (Adhikari et al. 2000. 2003), and in whey protein polymers (Picot and Lacroix 2004) have increased the survival and viability of probiotic bacteria in yoghurts during storage. Microencapsulation also appears to provide anoxic regions inside the capsules, thus reducing oxygen trapped inside the capsules, which prevents the viability losses of oxygen-sensitive strains (Talwalkar and Kailasapathy 2003 and 2004), in addition to protecting the cells against the detrimental effects of the acid environment in the yoghurt. McMaster et al. (2005) showed increased oxygen tolerance by bifidobacteria in gel beads. The efficiency of microencapsulation in protecting the probiotic bacteria, however, depends on the oxygen sensitivity of the bacteria and the dissolved oxygen levels in the product. The addition of starch as a filler material in the alginate capsule matrix (Sultana et al. 2000), co-encapsulation with prebiotic substances such as inulin (Iyer and Kailasapathy 2005), or coating the microbeads with chitosan (Krasaekoopt et al. 2006) appear to improve the viability of probiotic cultures.

Probiotic Cheese Among the traditional dairy foods, cheddar cheese has a markedly higher pH (4.8–5.6) than fermented milks and yoghurt (pH 3.7–4.3), which helps in providing a stable medium to support the long-term survival of acid-sensitive probiotic bacteria (Stanton et al. 1998). The metabolism of various lactic acid bacteria in cheddar cheese results in an anaerobic environment within a few weeks of ripening, favouring the survival of probiotic bacteria (Van den Tempel et al. 2002). Furthermore, the matrix of cheddar cheese and its relatively high fat content offers protection to probiotic bacteria during passage through the GI tract (Vinderola et al. 2002). Thus, it appears that microencapsulation may be only marginally beneficial in protecting probiotic bacteria in cheddar cheese. However, compared to yoghurt, cheddar cheese has longer ripening, storage, and shelf-life periods, during which the pH decreases, making the cheese acidic in nature during ripening. The combination of long maturation periods and acidic conditions could make it difficult for probiotic bacteria to survive during the 6–12-month ripening period. Additionally, compared to yoghurts, cheddar cheese contains starter and non-starter lactic acid bacteria, which may affect the survival of probiotic bacteria.

Dinakar and Mistry (1994), reported improved survival of *B. bifidum* in cheddar cheese over a 6-month ripening period. Gardiner et al. (2002) reported improved and increased survival, as well as an increased growth rate, of *L. paracasei* in cheddar cheese after 3 months of ripening. Similar results have been reported by McBreaty et al. (2001), Godward and Kailasapathy (2003), and Darukaradhya (2005). Cheese containing encapsulated *Bifidobacterium* was shown to possess similar flavour, texture, and appearance compared to the control (Dinakar and Mistry 1994; Desmond et al. 2002). Kailasapathy and Masandole (2005) has reported that the production of feta cheese incorporating encapsulated probiotic bacteria (*L. acidophilus* and *B. lactis*) is technologically feasible; however, selection of probiotic strains that are acid- and salt-tolerant, and which produces exo-polysaccharides in addition to using food polysaccharides as shell materials for encapsulation, will allow for the production of better-quality feta cheese, with greater survival rate of probiotic bacteria and an improved texture.

Probiotic Frozen Desserts Several studies have reported that probiotics entrapped in alginate or carrageenan beads have greater viability following freezing in dairy desserts (Kebary et al. 1998; Sheu et al. 1993; Godward and Kailasapathy 2003; Shah and Ravula 2000). In the manufacturing of frozen ice milk, probiotics microencapsulated with 3% calcium alginate are blended with milk, and the mix is frozen continually in a freezer. The incorporation of microencapsulated probiotics has no measurable effect on the overrun and the sensory characteristics of the products with 90% probiotic survival (Sheu et al. 1993). Addition of encapsulated cultures (*L. acidophilus* and *B. infantis*) did not have any effect on the amount of air incorporated into the ice cream (Godward and Kailasapathy 2003). The high fat content of ice cream and the neutral pH of dairy desserts may be the main factors responsible for the additional protection provided to probiotic bacteria. However, the addition of cryoprotectants such as glycerol (Sheu et al. 1993; Sultana et al. 2000) seems to improve the viability of probiotic bacteria during freezing of the dairy desserts. The milk fat in ice cream formulations may also act as an encapsulant material for probiotic bacteria during the homogenisation of the ice cream mix. The high total solids in ice cream mix, including the fat (emulsion), may provide protection for the bacteria (Kailasapathy and Sultana 2003). However, full-fat ice cream offered no extra protection for probiotic bacterial cultures (*L.* acidophilus LAFTI™ L10, *B. lactis* BLC-1, and *L. paracasei* subsp. *paracasei* LCS-1) over the low-fat product during storage, with the low-fat formulation showing improved survival of all three cultures during the freezing process (Haynes and Playne 2002).

Powdered Formulations Containing Probiotics Powders are basic types of food formulations or pharmaceutical preparations for direct use or as starting point for functional food product development. In powdered milk products, the challenge is to protect the probiotics from the excessive heat and osmotic degradation encountered during spray-drying. The addition of a thermoprotectant such as trehalose (Conrad et al. 2000) may help improve the viability during drying and storage. Some studies have examined the stability of encapsulated probiotics in dried milk. Incorporation of soluble fibre, gum acacia, into a milk-based medium prior to spray-drying the probiotic *L. paracasei* was reported to enhance its viability during storage, compared with milk powder alone (Desmond et al. 2002). However, not all the soluble fibres enhanced the probiotic viability during spray- drying or powder storage; for example, inulin and polydextrose did not influence the viability (Corcoran et al. 2005). Freeze-dried, micro-encapsulated hydrogel beads appear to be more stable during incubation at room temperature (Kailasapathy and Sureeta 2004; Capela et al. 2006). Spray-coating of a freeze-dried culture seems to be more effective for additional protection (Suita-Cruce and Goulet 2001). When a lipid coating is used, it may form a barrier to moisture and oxygen entry into the microcapsules. The nature of the packaging materials, including their oxygen-scavenging capacity, together with the addition of antioxidants, desiccants, etc., may need to be considered for effective protection of probiotic cells during storage (Hsiao et al. 2004; Miller et al. 2003). Spray-drying of starch-encapsulated bifidobacteria did not result in good survival of the organism during storage at 19–24 °C (Crittenden et al. 2006). Hence, more improved drying technology is required to enhance the viability of cells during storage of dried probiotic products.

Meat-based Functional Foods

Probiotic Meat Products Meat emulsion for the manufacture of small goods, such as dry-fermented sausages, with their low water activity, pH, curing salts, and competing starter culture organisms, presents a challenging environment for the survival of introduced probiotics during processing. When *Lactobacillus plantarum* and *Pediococcus pentosaeccus* were immobilised in alginate microcapsules, the fermentation rate was much more rapid with the encapsulated cells (Kearney et al. 1990). The rapid fermentation performance of the immobilised cells was caused by the available nutrients (i.e., skim milk) and the more protective re-hydration environment within the alginate capsules. Similar results can be obtained when microencapsulated probiotics are incorporated into a meat-fermenting product mix. Muthukumarasamy and Holley (2006) showed that microencapsulated *Lactobacillus reuteri* can be used in dry-fermented sausages to ensure that a desirable level of probiotic organisms is maintained in the final product at consumption without altering the sensory quality of these traditional small goods. In this study, alginate microcapsules, prepared by either emulsion or extrusion, were added to the salami batter (meat ingredients, starter cultures, cure mix, spice mix, and salt) at 1% (w/w). The batter was stuffed in casings, transferred to a smoke house, and allowed to ferment at 26 °C and 88% RH for 24 h, to reach a pH of less than 5.3. Fermentation was followed by drying at 13 °C and 75% RH for 25 days. *L. casei* cells, when microencapsulated in alginate beads, were more resistant to heat processing at 55–65 °C (Mandal et al. 2006). This was also demonstrated when microencapsulated alginate beads containing cultures were heat-treated at 55 °C for 15 min., and the encapsulated cells showed more stability than free cells in deMann, Rogosa and Sharpe (MRS) broth acidified to pH 5.0 (Lemay et al. 2002). These data suggests that probiotic cells microencapsulated in alginate gel beads could be used in meat processing, which requires moderate heat treatments. For meat products where a meat emulsion is initially prepared (e.g., salami, sausages), the high fat in the system may also envelop the alginate gel particles containing the bacterial cells to provide additional protection to heat during processing.

Plant-based (Vegetarian) Probiotic Products With regard to plant fermented products, probiotics are most frequently incorporated into soy products (Wang et al. 2002), although interest is increasing in the use of fermented cereals (Charalampopoulos et al. 2003; Laine et al. 2003) and vegetable pickles (Savard et al. 2003). For stabilisation of bifidobacteria during a traditional African fermented corn product, the bacterial cells were encapsulated in a mixed-polymer (gellan/xanthan) beads system (McMaster et al. 2005). Microencapsulation improved the survival of *L. rhamnosus* subjected to freezing in a cranberry juice concentrate and during storage of the frozen product (Reid et al. 2006). Microencapsulation can be of benefit to the stability of probiotic cultures; however, the way the bacteria are grown, harvested, and dried for subsequent industrial use can be as important in promoting the viability of the cultures in food systems as the microencapsulation itself. Although the probiotic bacteria show good stability in products having a low water activity, such as peanut butter ($aw = 0.24$), spray-coating of *L. rhamnosus* and incorporating into peanut butter formulations (incubated at 21 °C) showed decreased cell viability (Belvis et al. 2006). In bakery applications, stabilising viability of probiotics is a challenge, due to the high treatment during processing. Microencapsulation by spray-coating did not improve the survival and stability of added lactobacilli during bread-making (Belvis et al. 2006). However, microencapsulation in a whey protein particle was reported to be effective at enhancing the survival of probiotic lactobacilli during the heat treatment applied in biscuit manufacture (Reid et al. 2006).

Probiotics Incorporated in Nutraceuticals Probiotic-containing nutraceuticals are produced by harvesting cells from large-scale biomass industrial fermentation, microencapsulating the concentrated cell mass and drying them. They are then marketed in the form of powder, caplets, or chewable tablets. Currently, the encapsulation process is carried out by spray-coating. Examples include the STAR™ and Probiocap™ technologies (Goulet and Wozniak 2002). Fat-based polymers are used to spray-coat fine particles of probiotic cultures to enhance the survival rate against the gastric contents (Goulet and Wozniak 2002). Probiotic strains of *L. acidophilus* 50 are sold in a microencapsulated form by Institut Rosell/Lallemand The Americas, Montreal, Canada (www.lallemand.com). Probiocap ™ (microencapsulated *L. acidophilus* 50 ME in a hydrophobic matrix) claimed to have increased tolerance to gastric juices, improved survival during tableting, enhanced temperature resistance during processing, and extended shelf-life at room temperatures (Kailasapathy 2009).

Microencapsulation is beneficial in probiotic tablet manufacture. When spray-coated particles containing probiotics were exposed to compression into tablets, the survival rate was higher than with uncoated particles (Goulet and Wozniak 2002). In simulated gastric fluid (pH 1.2-2.0), free cells showed a much lower survival rate compared to the cells in the tablet (Chan and Zhang 2005). In the compression microencapsulation, the formation of a hydrogel surrounding the cell wall was thought to be the basis for cell protection (Chan and Zhang 2005).

11.3 Future Trends and Marketing Perspectives

The therapeutic effects of non-microbial and microbial functional foods and their use in preventative medicine are increasingly being reported. As clinical evidence of the beneficial effects of these functional foods accumulates, the food, nutraceutical, and pharmaceutical industries will formulate new and innovative functionally based therapeutic products. New and innovative ways of administering, developing, and controlling the release of functional foods and ingredients will be developed in the near future. Prebiotics, another increasing functional food sector, are increasingly mixed with probiotics in product formulations to enhance the stabilisation of probiotic cultures incorporated in the development of synbiotic functional foods. Recently, inulin and other prebiotics have been added to probiotics in the manufacturing of the nutrient bar called “Attune” (www.attunefoods.com), into yoghurt-covered raisins, nutrient bars, and chocolate bars (www.balchem.com).

Yoghurt and fermented milks have spearheaded the development of probiotic functional foods. The tendency is for other fermented products such as cheese, dairy-vegetable blended spreads, frozen desserts, and meat and vegetable products to follow. Designer probiotic products including food, pharmaceuticals, and nutraceutical products will be developed. These products will be innovative and consumer-convenience-based, such as pills, re-constitutable single-serve sachet products, and convenient packs with instructions on how to prepare and administer them. Commercially available food formulations, for example, to prepare probiotic yoghurts in the home kitchen using a simple yoghurt maker, are already being marketed.

A major challenge is to improve the viability of probiotic bacteria to improve their efficacy, and a large number of reports have shown that many probiotic-based food products do not have the recommended number of viable cells. Similarly, to protect the stability and to improve the bioavailability of functional ingredients is a major challenge. Thus, microencapsulation will increasingly become a valuable tool for protecting and improving the stability of functional ingredients and viability of probiotic bacteria. To date, the literature shows that microencapsulation has primarily served in the delivery of bioactives into the food matrix, and not fully explored methods for more efficient delivery in the GI tract of humans. In this respect, the development of smart polymers as wall materials, as well as new smart microencapsulation technologies, will be important in the future.

The key market drivers for functional foods include: consumer intestinal health awareness, popularity of fermented foods such as yoghurts, ageing populations, increased stress-induced lifestyles, and a busy lifestyle associated with a self-medication approach to health. The rapid growth in the probiotic sector of the functional foods market has necessitated the use of microencapsulation technology to stabilise the efficiency of probiotic bacteria in these products. Increased consumption of processed or prepared foods requires protection of the ingredients during the processing, packaging, and storage of these foods, and this is one of the key market drivers for microencapsulated food ingredients. The continuous string of public health issues surrounding the food industry, as well as increased awareness among consumers of the benefits of a healthy diet, are driving the demand for functional food products or ingredients that are natural in origin.

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12
Role and Importance of Health Claims in the Nutraceutical and Functional Food Markets

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12.1 Introduction

Several studies have attempted to establish the relationship between nutraceuticals and/or functional foods and a healthier life and/or the prevention of certain diseases. Increased scientific and consumer interest in the role of these foods have led to rapid economic and innovative expansion in this industry, as the overall economic costs of diseases, including direct health care costs and losses due to morbidity and mortality, can be very high. However, poor evidence and unfounded assumptions with limited scientific basis have sometimes been used as a basis for health claims and in the commercialization of these products. Various countries and jurisdictions have regulatory requirements for the use of health claims on labels and the public advertisement of nutraceuticals and/or functional food products. This is aimed at product safety and to protect consumers from being misled by unsubstantiated claims of the benefits of foods and food products and miraculous cures meticulous scientific evaluation is needed to ensure that health claims made on food labeling and advertising are meaningful and accurate, and will help consumers in making informed choices.

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12.2 Nutraceuticals and Functional Foods

Nutraceuticals and/or functional foods have been defined by several authors and organizations (Health Canada 1998; Zeisel 1999; AND 2013). Most of these definitions describe these foods as dietary components that may provide health benefit beyond basic nutrition, and their role in reducing or minimizing the risk of certain diseases and health conditions. The concept of food going beyond providing essential nutrients is not new. Hippocrates (460–370 BC) was one of the earlier savants to proclaim thus: "Let food be your medicine and medicine be your food." According to the World Health Organization (WHO), an unhealthy diet is one of the risk factors in cardiovascular disease (CVD), the leading cause of death globally (WHO 2013). Various studies suggest that many bioactives in foods (e.g., protein and peptides, polyunsaturated fatty acids [PUFAs], fiber, phenolics, carotenoids, probiotics, and prebiotics) may exert antioxidative, antithrombotic, hypocholesterolaemic, antimicrobial, and immunomodulatory effects (Hartmann and Meisel 2007). Plausible biological mechanisms of both the bioactive compounds and their metabolites have also been reported (Simopoulos 2002; Shimizu and Hachimura 2011 and references therein).

Various well-designed cohorts, nested case-control, randomized-controlled, placebo-controlled, double-blind trials, and other forms of assessments such as systematic reviews and meta-analyses, have been used to study the potential effects of these foods on health using various populations and study durations (Tan et al. 2012; Bao et al. 2013; Ammann et al. 2013; Roncaglioni et al. 2013). Whereas some results show clear evidence for the efficacy of these bioactive compounds, others are inconclusive. Additionally, not all observational studies have been upheld by intervention studies (McCracken 2010; Ammann et al. 2013).

The perceived healthiness of functional foods and nutraceutical products is enhanced by the ability to make a health claim, and this is used as a marketing tool worldwide. The definition and types of health claims that can be used on products vary extensively among countries. In many places, health claims are based on generally accepted scientific evidence from *in vitro* or animal experiments and/or human trials. However, in *in vitro* or animal experiments, doses much higher than what humans may be exposed to through the diet are often used. Additionally, most animals do not absorb or metabolize these active ingredients in a similar manner to humans. Human studies, on the other hand, can be complex and difficult, owing to the duration of studies, the limited number of variables that can be investigated at a time (for instance, one amino acid or a few combinations at a time), individual metabolism, intrinsic physiological state, age, appropriate and/or specific biochemical biomarkers, and measured end outcomes.

The absence of large intervention studies and/or sufficient numbers of subjects to prove statistically significant differences is also an impediment to conclusive human trials. It is often difficult to demonstrate and prove the effect of single food components in human nutrition, and attributing specific functions to health benefits is sometimes almost impossible. Diseases have multiple risk factors, and nutritional interventions that alter one of these risk factors may or may not have a beneficial effect. It is possible that the definition of populations that can benefit from these functional foods, doses, and lengths of intervention differ from uncontrolled environments, and potentially synergist ingredients may interfere with the outcome of a study (Paiva and Russell 1999). Several examples exist where these conditions are omitted from the reports. For instance; dietary intervention studies may improve cognition in adults with cognitive decline or impairment but not in individuals with established dementia (Tan et al. 2012; Ammann et al. 2013). Dietary levels of carotenoids (including β-carotene) were found to promote health, while in high-risk population (smokers and asbestos workers), high doses had adverse effects (Paiva and Russell 1999).

Similarly, mixed results on the role of some amino acids (AA) in protein and muscle synthesis have not stopped their promotion among nutrition and supplement companies. For instance, glutamine has long been promoted for numerous positive effects, such as muscle protein synthesis and increasing muscle mass. However, a recent review suggests that glutamine supplementation does not provide these benefits (Gleeson 2008; Greenfield et al. 2008). Additionally, there is no clear consensus on which combination of AA is most beneficial. Branched-chain amino acids (BCAAs; isoleucine, leucine, and valine) are among the nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) for humans; however, leucine has been shown to have the biggest effect on protein synthesis among the three BCAAs (Nogiec and Kasif 2013).

The ability of naturally occurring bioactive compounds in these bioactive foods to prevent chronic diseases largely depends on their pharmacokinetic properties, in particular absorption and distribution to the target tissue (Nogiec and Kasif 2013; Vitale et al. 2013). While native active ingredients are often tested in *in vitro* studies, they may be converted to their metabolites during processing. Conjugated isoflavones may have weaker estrogenic activity and provide no prevention to breast cancer (Kurzer and Xu 1997; Rowland et al. 2003; Christensen 2009; Patisaul and Jefferson 2010; Vitale et al. 2013). These findings suggest that *in vitro* studies may not be reflective of the bioavailability and actual benefits of the active ingredient. Moreover, the claims and efficacy of these foods are not always regulated.

12.3 Health claims

Health claims made through labeling on packaging is one of the most important ways by which manufacturers of functional foods inform consumers of the beneficial effects of a particular product. Knowledge of the type of processing method used, technical improvements, components added for enrichment or fortification, added convenience, and perceived value relative to existing products also have great influence on consumers' perception of functional foods and nutraceutical products (Childs 1999; Bech-Larsen and Grunert, 2003; Siró et al. 2008). According to a recent survey, a large percentage of shoppers who bought oatmeal/hot cereal and yoghurt/yoghurt drink did so because of specific health concerns and for the products being marketed to address specific health concerns (Table 12.1). Of the 64% of shoppers who regularly bought oatmeal or hot cereal, 57% did so in part because of specific personal or household health concerns, whereas 51% of these consumers bought hot cereal brands and products because they were marketed or labeled targeting specific health concerns. Although some food and beverages products such as soy beverages, meal replacement drinks, and low-glycemic grain-based foods had lower purchasing rates (10–17%), a larger percentage (10–43%) of shoppers who purchased these products did so due to specific health concern as well as the marketing or labeling used to target the specific health concerns (35–43%). The survey also reported that 84, 76, 72, 72, and 72% of the shoppers bought food and beverages targeting sports/fitness performance, anti-aging, cancer, joint/bone health, and male health, respectively.

The growing trend in functional foods consumption to address health concerns has called for better legislation and the development of policies and regulations to govern health claims for both manufacturers and consumers. As stated earlier, various countries and jurisdictions define health claims differently, which can make it even more confusing. The different definitions and regulatory requirements in different countries have led to different claims being permitted in different countries. Table 12.2 provides a list of some of the regulatory bodies in various countries and jurisdictions. Most of these regulatory bodies have laws (e.g., article 10(1) of Regulation (EC) No. 1924/2006) prohibiting making health claims on foods unless authorized and included in a list of permitted claims (EU 2006). Other jurisdictions have adopted regulations that stipulate harmonized rules for the use of health claims on foods based on their nutrient profiles and strong scientific evidence (Shimizu 2003; Health Canada 2009; 2014; Shimizu and Hachimura 2011). Due to these differences, different levels of scientific support for similar claims are required in these different countries. For instance, there are only a few permitted health claims in Canada (Health Canada 2014). These include ground whole flaxseed, barley, and oat products, whose claim of cholesterol-lowering effect has been substantiated (Table 12.3). However, dietary fat/fiber/grain product and whole grains are not permitted to claim cancer and coronary heart disease reduction, respectively, in Canada, owing to the position that there is insufficient evidence from clinical trials and other studies. Some of the permitted health claims in the European Union (EU), Untied States, and Japan are presented in Tables 12.4–12.6.

Table 12.1 Purchasing of selected types of grocery products with targeted health and wellness positioning, 2011 (percentage of U. grocery shoppers)

Grocery Product	Buy regularly (%)	Buy for specific health concern (%)	Buy products marketed for specific health concern (%)
Oatmeal/hot cereal	64	57	51
Yoghurt/yoghurt drinks	66	54	46
Soy-based meals or entrees	8	45	37
Fish products	45	43	27
Soy beverages	17	43	35
Meal-replacement drinks	10	42	43
Low-glycemic-index, grain-based foods such as cereals, bread, or pasta	12	40	37
Fruit/vegetable food products (other than fresh)	64	34	18
Margarine-type spreads (e.g., Benecol, Smart Balance)	52	33	32
Ready-to-eat cold cereal	77	32	32
Juice/juice drinks	72	31	23
Snack/cereal/nutrition bars	53	31	28
Fresh eggs	84	30	18

Source: Sprinkle 2012. Reproduced from Nutraceuticals World (www.NutraceuticalsWorld.com) with permission from Rodman Media.

The food industry is also subjected to an uneven playing field, and consumers are often left confused. While the rules vary among countries and jurisdictions, it is clear that, in all countries and jurisdictions, there is a need for clearer and simplified claim(s) that are easy to understand by the consumer, and which are substantiated by scientific evidence. All countries and jurisdictions disallow health claims on functional foods that include medical claims such as claims to "prevent," "cure," "treat," or "diagnose" diseases. Ultimately, in order to successfully obtain and maintain an approved health claim, it is essential to understand and comply with the regulatory requirements in the jurisdiction where the application is submitted (Health Canada 2009; EFSA 2013a).

Japan was one of the first countries to regulate functional foods. As early as 1991, the Japanese Ministry of Health, Labor and Welfare (MHLW) became the first regulatory agency to recognize functional foods as a unique food category (Shimizu 2003). Functional foods that claim health benefits with sufficient evidence to support the claim may apply for approval through the "food for specified health uses" (FOSHU) regulatory system (Shimizu 2003) and are allowed to use the seal of approval if successful (Figure 12.1). Shimizu (2003) provided a detailed description of the FOSHU application and approval process. FOSHU-approved products have been classified into eight groups according to their specific health claims (Table 12.6). Between 1993, when

Table 12.2 Regulatory bodies governing nutrition health claims

Country	Regulatory body
Australia and New Zealand	Food Standards Australia New Zealand
Brazil	National Health Surveillance Agency
Canada	Health Canada
China	State Food and Drug Administration
European Union	European Food Safety Authority
France	French Food Safety Agency
Japan	Ministry of Health, Labour and Welfare
The Netherlands	Netherlands Nutrition Centre
Sweden	Swedish Nutrition Foundation
United Kingdom	Joint Health Claims Initiative
United States of America	Food and Drug Administration

Source: AAFC 2009. Consumer Trends: Functional Foods; http://www.gov.mb.ca/agriculture/market-prices-and-statistics/food-and-value-added-agriculture-statistics/pubs/consumer_trends_functional_foods_en.pdf ISSN 1920–6593 Market Analysis Report AAFC No. 11061E (Table 3: Regulatory Bodies Governing Nutrition Health Claims in Various Countries Around the World).

the first FOSHU product was approved, and August 2010, there were a total of 950 FOSHU-approved foods commercially available. More than 70% of the FOSHU-approved products are used to promote gut health (Table 12.7). Since 2001, the regulatory range of FOSHU products has been broadened to accept products in the form of capsules and tablets in addition to conventional foods. In April 2001, the MHLW enacted a new regulatory system called the "Foods with Health Claims," which includes the existing FOSHU system and the newly established "Foods with Nutrient Function Claims" (FNFC) (Shimizu 2003). The FNFC are equivalent to the nutrient function claims standardized by Codex Alimentarius (Codex 1997). FNFC products include 12 vitamins (vitamins A, B1, B2, B6, B12, C, E, D, biotin, pantothenic acid, folic acid, and niacin) and two minerals (Ca and Fe). FNFC products are also required to contain information on the upper and lower limits.

The EU has also distinctively defined the two main claims that can be made on foods. A nutrition claim states or suggests that a food has beneficial nutritional properties, such as "low fat," "no added sugar," and "high in fibre," whereas a health claim is "any statement on labels, advertising or other marketing products that health benefits can result from consuming a given food or from one of its components such as vitamins and minerals, fibre, and 'probiotic' bacteria." The three different types of health claims permitted in the EU are listed in Table 12.8 (EFSA 2013b). Health claims of foods are allowed on labels and marketed after an authorization has been procured from the panel on Dietetic Products, Nutrition and Allergies (NDA) of the European Food Safety Authority (EFSA), as required under regulation EC 1924/2006 (EU 2006). The panel verifies the scientific basis of

Table 12.3 Some permitted health claims in Canada

Food or food category	Claim	Conditions of use of the claim
Ground whole flaxseed	[serving size from Nutrition Facts table in metric and common household measures] of (brand name) [name of food] supplies/provides X% of the daily amount [of ground (whole) flaxseed]* shown to help reduce/lower cholesterol. The "daily amount" referred to in the primary statement is 40 g of ground whole flaxseed. For example, 16 g (two tablespoons) of ground flaxseed supplies 40% of the daily amount shown to help lower cholesterol.	The food: a. contains at least 13 g of ground whole flaxseed i. per reference amount and per serving of stated size, or ii. per serving of stated size, if the food is ground whole flaxseed, whole flaxseed, or a prepackaged meal; b. contains at least 10% of the weighted recommended nutrient intake (WRNI) of a vitamin or mineral nutrient i. per reference amount and per serving of stated size, or ii. per serving of stated size, if the food is a prepackaged meal; c. contains 100 mg or less of cholesterol per 100 g of food; d. contains 0.5% or less alcohol; e. contains i. 480 mg or less of sodium per reference amount and per serving of stated size, and per 50 g if the reference amount is 30 g or 30 ml or less, or ii. 960 mg or less of sodium per serving of stated size, if the food is a prepackaged meal; f. meets the conditions for "free of saturated fatty acids" or "low in saturated fatty acids" (items 18 and 19, respectively, in the table following section B.01.513 of the Food and Drug Regulations)

(*continued overleaf*)

Table 12.3 (*continued*)

Food or food category	Claim	Conditions of use of the claim
Barley products	"[serving size from Nutrition Facts table in metric and common household measures] of (brand name) [name of food] [with name of eligible fiber source] supplies/provides X% of the daily amount of the fiber shown to help reduce/lower cholesterol." The "daily amount" referred to in the primary statement is 3 grams of barley beta-glucan. For example, 125 ml (1/2 cup) of cooked pearled barley supplies 60% of the daily amount of the fiber shown to help lower cholesterol.	a. the food contains at least 1 g of beta-glucan from barley grain products per reference amount and per serving of stated size; b. the food contains at least 10% weighted recommended nutrient intake (WRNI) of a vitamin or mineral nutrient i. per reference amount and per serving of stated size, or ii. per serving of stated size, if the food is a prepackaged meal; c. the food contains 100 mg or less of cholesterol per 100 g of food; d. the food contains 0.5% or less alcohol; e. the food contains i. 480 mg or less of sodium per reference amount and per serving of stated size, and per 50 g if the reference amount is 30 g or 30 ml or less, or ii. 960 mg or less of sodium per serving of stated size, if the food is a prepackaged meal; f. the food meets the conditions for "low in saturated fatty acids" or "free of saturated fatty acids"

Oat products	"[serving size from Nutrition Facts table in metric and common household measures] of (brand name) [name of food] [with name of eligible fiber source] supplies/provides [X% of the daily amount] of the fibers shown to help reduce/lower cholesterol." If the oat fiber source is specified in the brand name (e.g., Quaker Oatmeal), it does not need to be repeated subsequently in the claim. Names of eligible sources of oat fiber are oat bran, rolled oats/oatmeal, and whole oat flour. The term "oats" may also be used as a synonym or generic name for these sources. For example, if the eligible fiber source is a food itself: "1 cup (X g) of Quaker Oatmeal supplies X% of the daily amount of the fibers shown to help reduce cholesterol." If the eligible fiber source is an ingredient: "1 muffin (X g) with oat bran provides X% of the daily amount of the fibers shown to help lower cholesterol."	a. contain at least 0.75 g beta-glucan oat fiber per reference amount and per serving of stated size from the eligible sources; b. contain at least 10% of the weighted recommended nutrient intake of a vitamin or a mineral nutrient per reference amount and per serving of stated size; c. contain 100 mg or less of cholesterol per 100 g of food; d. contain 0.5% or less of alcohol; e. contain 480 mg or less of sodium per reference amount and per serving of stated size, and per 50 g if the reference is 30 g or less; and f. meet the definition of "free of saturated fatty acids" or "low in saturated fatty acids"

(*continued overleaf*)

Table 12.3 (*continued*)

Food or food category	Claim	Conditions of use of the claim
Plant sterols	"[serving size from Nutrition Facts table in metric and common household measures] of [naming the product] provides X% of the daily amount* of plant sterols shown to help reduce/lower cholesterol in adults." The "daily amount" referred to in the primary statement is 2 g. "Plant sterols help reduce [or help lower] cholesterol." This statement, when used, shall be shown in letters up to twice the size and prominence as those of the primary statement. "High cholesterol is a risk factor for heart disease." This statement, when used, shall be shown in letters up to the same size and prominence as those of the primary statement.	The food: a. contains a minimum level equivalent to 0.65 g of free plant sterols or stanols per reference amount and per serving of stated size; b. contains at least 10% of the weighted recommended nutrient intake of a vitamin or mineral per reference amount and per serving of stated size; c. contains 100 mg or less of cholesterol per 100 g of food; d. contains 0.5% or less alcohol; e. contains 480 mg or less of sodium per reference amount and per serving of stated size, and per 50 g if the reference amount is 30 g or 30 ml or less; f. meets the criterion "low in saturated fatty acids"

Table 12.4 Some permitted health claims in the EU

Food or food category	Claim	Conditions of use of the claim
α-linolenic acid (ALA)	ALA contributes to the maintenance of normal blood cholesterol levels	The claim may be used only for food that is at least a source of ALA as referred to in the claim "SOURCE OF OMEGA-3 FATTY ACIDS" as listed in the Annex to Regulation (EC) No. 1924/2006. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 2 g of ALA.
Arabinoxylan produced from wheat endosperm	Consumption of arabinoxylan as part of a meal contributes to a reduction of the blood glucose rise after that meal.	The claim may be used only for food that contains at least 8 g of arabinoxylan (AX)-rich fiber produced from wheat endosperm (at least 60% AX by weight) per 100 g of available carbohydrates in a quantified portion as part of the meal. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained by consuming the arabinoxylan (AX)-rich fiber produced from wheat endosperm as part of the meal.
β-glucans from oats and barley	Consumption of β-glucans from oats or barley as part of a meal contributes to the reduction of the blood glucose rise after that meal.	The claim may be used only for food that contains at least 4 g of β-glucans from oats or barley for each 30 g of available carbohydrates in a quantified portion as part of the meal. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained by consuming the β-glucans from oats or barley as part of the meal.
Docosahexaenoic acid (DHA)	DHA contributes to maintenance of normal brain functions.	The claim may be used only for food that contains at least 40 mg of DHA per 100 g and per 100 kcal. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 250 mg of DHA.
Docosahexaenoic acid (DHA)	DHA contributes to the maintenance of normal vision.	The claim may be used only for food that contains at least 40 mg of DHA per 100 g and per 100 kcal. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 250 mg of DHA.

(*continued overleaf*)

Table 12.4 (*continued*)

Food or food category	Claim	Conditions of use of the claim
Eicosapentaenoic acid and docosahexaenoic acid (EPA/DHA)	EPA and DHA contribute to the normal function of the heart.	The claim may be used only for food that is at least a source of EPA and DHA as referred to in the claim "SOURCE OF OMEGA-3 FATTY ACIDS" as listed in the Annex to Regulation (EC) No. 1924/2006. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 250 mg of EPA and DHA.
Lactulose	Lactulose contributes to an acceleration of intestinal transit.	The claim may be used only for food that contains 10 g of lactulose in a single quantified portion. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a single serving of 10 g of lactulose per day.
Live yoghurt cultures	Live cultures in yoghurt or fermented milk improve lactose digestion of the product in individuals who have difficulty digesting lactose.	In order to bear the claim, yoghurt or fermented milk should contain at least 108 colony-forming units live starter microorganisms (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) per g.
Olive oil polyphenols	Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress	The claim may be used only for olive oil that contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.
Wheat bran fiber	Wheat bran fiber contributes to an increase in fecal bulk.	The claim may be used only for food that is high in fiber as referred to in the claim "HIGH FIBER" as listed in the Annex to Regulation (EC) No. 1924/2006.

Source: EU (2012) Commission Regulation (EU) No 432/2012. http://eur-lex.europa.eu, © European Union, 1998–2014.

Table 12.5 Some approved health claims in the United States

Food or food category	Model health claim	Conditions of use of the claim
Dietary lipids (fat) (1993) *Nature of the food*: The food shall meet all of the nutrient content requirements of §101.62 for a "low-fat" food; except that fish and game meats (i.e., deer, bison, rabbit, quail, wild turkey, geese, ostrich) may meet the requirements for "extra-lean" food in §101.62.	a. Diets low in fat may reduce risk of some cancer. b. Eating a healthful diet low in fat may help reduce the risk of some types of cancers. Development of cancer is associated with many factors, including a family history of the disease, cigarette smoking, and what you eat.	a. The claim states that diets low in fat "may" or "might" reduce the risk of some cancers; b. In specifying the disease, the claim uses the following terms: "some types of cancer" or "some cancers"; c. In specifying the nutrient, the claim uses the term "total fat" or "fat"; d. The claim does not specify types of fats or fatty acids that may be related to the risk of cancer; e. The claim does not attribute any degree of cancer risk reduction to diets low in fat; and f. The claim indicates that the development of cancer depends on many factors

(*continued overleaf*)

Table 12.5 (*continued*)

Food or food category	Model health claim	Conditions of use of the claim
Dietary saturated fat (1993) *Nature of the food*: The food shall meet all of the nutrient content requirements of §101.62 for a "low-saturated-fat," "low-cholesterol," and "low-fat" food; except that fish and game meats (i.e., deer, bison, rabbit, quail, wild turkey, geese, and ostrich) may meet the requirements for "extra-lean" food in §101.62.	a. While many factors affect heart disease, diets low in saturated fat and cholesterol may reduce the risk of this disease. b. Development of heart disease depends upon many factors, but its risk may be reduced by diets low in saturated fat and cholesterol and healthy lifestyles. c. Development of heart disease depends upon many factors, including a family history of the disease, high blood LDL cholesterol, diabetes, high blood pressure, being overweight, cigarette smoking, lack of exercise, and the type of dietary pattern. A healthful diet low in saturated fat, total fat, and cholesterol, as part of a healthy lifestyle, may lower blood cholesterol levels and may reduce the risk of heart disease. d. Many factors, such as a family history of the disease, increased blood- and LDL cholesterol levels, high blood pressure, cigarette smoking, diabetes, and being overweight, contribute to developing heart disease. A diet low in saturated fat, cholesterol, and total fat may help reduce the risk of heart disease. e. Diets low in saturated fat, cholesterol, and total fat may reduce the risk of heart disease. Heart disease is dependent upon many factors, including diet, a family history of the disease, elevated blood LDL cholesterol levels, and physical inactivity.	a. The claim states that diets low in saturated fat and cholesterol "may" or "might" reduce the risk of heart disease; b. In specifying the disease, the claim uses the terms "heart disease" or "coronary heart disease;" c. In specifying the nutrient, the claim uses the terms "saturated fat" and "cholesterol," and lists both; d. The claim does not attribute any degree of risk reduction for coronary heart disease to diets low in dietary saturated fat and cholesterol; and e. The claim states that coronary heart disease risk depends on many factors.

Fiber-containing grain products, fruits, and vegetables (1993) *Nature of the food*: a. The food shall be or shall contain a grain product, fruit, or vegetable. b. The food shall meet the nutrient content requirements of §101.62 for a "low-fat" food. c. The food shall meet, without fortification, the nutrient content requirements of §101.54 for a "good source" of dietary fiber.	a. Low-fat diets rich in fiber-containing grain products, fruits, and vegetables may reduce the risk of some types of cancer, a disease associated with many factors. b. Development of cancer depends on many factors. Eating a diet low in fat and high in grain products, fruits, and vegetables that contain dietary fiber may reduce your risk of some cancers.	a. The claim states that diets low in fat and high in fiber-containing grain products, fruits, and vegetables "may" or "might" reduce the risk of some cancers; b. In specifying the disease, the claim uses the following terms: "some types of cancer," or "some cancers"; c. The claim is limited to grain products, fruits, and vegetables that contain dietary fiber; d. The claim indicates that development of cancer depends on many factors; e. The claim does not attribute any degree of cancer risk reduction to diets low in fat and high in fiber-containing grain products, fruits, and vegetables; f. In specifying the dietary fiber component of the labeled food, the claim uses the terms "fiber," "dietary fiber," or "total dietary fiber"; and g. The claim does not specify types of dietary fiber that may be related to the risk of cancer. Nature of the food. a. The food shall be or shall contain a grain product, fruit, or vegetable. b. The food shall meet the nutrient content requirements of §101.62 for a "low fat" food. c. The food shall meet, without fortification, the nutrient content requirements of §101.54 for a "good source" of dietary fiber.

(*continued overleaf*)

Table 12.5 (*continued*)

Food or food category	Model health claim	Conditions of use of the claim
Soy protein (1999) *Nature of the substance:* a. Soy protein from the legume seed *Glycine max*	a. 25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of food] supplies __ g of soy protein. b. Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease. One serving of [name of food] provides __ g of soy protein.	a. The claim states that diets that are low in saturated fat and cholesterol and that include soy protein “may” or “might” reduce the risk of heart disease; b. In specifying the disease, the claim uses the following terms: “heart disease” or “coronary heart disease”; c. In specifying the substance, the claim uses the term “soy protein”; d. In specifying the fat component, the claim uses the terms “saturated fat” and “cholesterol”; e. The claim does not attribute any degree of risk reduction for CHD to diets that are low in saturated fat and cholesterol and that include soy protein; f. The claim does not imply that consumption of diets that are low in saturated fat and cholesterol and that include soy protein is the only recognized means of achieving a reduced risk of CHD; and g. The claim specifies the daily dietary intake of soy protein that is necessary to reduce the risk of CHD, and the contribution that one serving of the product makes to the specified daily dietary intake level. The daily dietary intake level of soy protein that has been associated with reduced risk of coronary heart disease is 25 g or more per day of soy protein.

Plant sterol/stanol esters (2000, 2001, 2003, 2005) *Nature of the substance*: a. *Plant sterol esters* Prepared by esterifying a mixture of plant sterols from edible oils with food-grade fatty acids. The plant sterol mixture shall contain at least 80% beta-sitosterol, campesterol, and stigmasterol (combined weight). b. *Plant stanol esters* Prepared by esterifying a mixture of plant stanols derived from edible oils or by-products of the kraft paper pulping process with food-grade fatty acids. The plant stanol mixture shall contain at least 80% sitostanol and campestanol (combined weight).	For plant sterol esters: a. foods containing at least 0.65 g per serving of plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the food] supplies ___g of vegetable oil sterol esters. b. Diets low in saturated fat and cholesterol that include two servings of foods that provide a daily total of at least 1.3 g of vegetable oil sterol esters in two meals may reduce the risk of heart disease. A serving of [name of the food] supplies ___g of vegetable oil sterol esters. For plant stanol esters: a. foods containing at least 1.7 g per serving of plant stanol esters, eaten twice a day with meals for a total daily intake of at least 3.4 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of the food] supplies ___g of plant stanol esters. b. Diets low in saturated fat and cholesterol that include two servings of foods that provide a daily total of at least 3.4 g of vegetable oil stanol esters in two meals may reduce the risk of heart disease. A serving of [name of the food] supplies ___g of vegetable oil stanol esters.	a. The claim states that plant sterol/stanol esters should be consumed as part of a diet low in saturated fat and cholesterol; b. The claim states that diets that include plant sterol/stanol esters "may" or "might" reduce the risk of heart disease; c. In specifying the disease, the claim uses the following terms: "heart disease" or "coronary heart disease"; d. In specifying the substance, the claim uses the term "plant sterol esters" or "plant stanol esters," except that if the sole source of the plant sterols or stanols is vegetable oil, the claim may use the term "vegetable oil sterol esters" or "vegetable oil stanol esters"; e. The claim does not attribute any degree of risk reduction for CHD to diets that include plant sterol/stanol esters; f. The claim does not imply that consumption of diets that include plant sterol/stanol esters is the only recognized means of achieving a reduced risk of CHD; and g. The claim specifies the daily dietary intake of plant sterol or stanol esters that is necessary to reduce the risk of CHD, and the contribution that one serving of the product makes to the specified daily dietary intake level. Daily dietary intake levels of plant sterol and stanol esters that have been associated with reduced risk of are: i. 1.3 g or more per day of plant sterol esters ii. 3.4 g or more per day of plant stanol esters h. The claim specifies that the daily dietary intake of plant sterol or stanol esters should be consumed in two servings eaten at different times of the day with other foods.

Source: FDA (2013) Health Claims Meeting Significant Scientific Agreement (SSA).

Table 12.6 Categories of health claims approved by FOSHU

Categories	Food component	Biomarkers	Allowed claim on product
Gastrointestinal conditions	Carbohydrates, oligosaccharides, dietary fibres, and chitosan	• Frequency and volume of evacuation • Number and ratio of *bifidobacteria*	"Products containing carbohydrate helps increase intestinal *bifidobacteria*, thus aiding in the maintenance of good gastrointestinal condition."
Blood pressure	Lacto-tripeptide from fermented milk, dodeca-peptide from casein, a group of peptides from sardine and soya protein	• Systolic blood pressure • Diastolic blood pressure	"Products containing these ingredients are suitable for individuals with slightly elevated blood pressure."
Serum cholesterol	Soya protein/peptide, chitosan, low-molecular sodium alginate and phytosterol, plant sterol, Stanol	• Serum total cholesterol, LDL cholesterol, and HDL cholesterol levels	"Products containing these ingredients helps individuals decrease their serum cholesterol levels"
Blood glucose	Indigestive dextrin, wheat albumin, L-arabinose	• Blood glucose • Glycohaemoglobin • Fructosamine • Blood insulin levels	"Products containing these ingredients are helpful for those who are concerned about their blood glucose levels."

Absorption of minerals	Fructo-oligosaccharides, caseinphosphopeptide	• Improve Ca absorption in the gut intestine • Supply iron (Fe) of higher bioavailability	"Products provide high bioavailability for human consumers and are suitable for Ca supplementation." "Products containing haem-Fe from haemoglobin are suitable for those with mild anaemia, who need supplemental Fe."
Blood lipids	Diacylglycerol, globin digest, coffee	• Blood lipid levels • BMI	"Helps reduce postprandial serum triacylglycerol levels." "Products containing diacylglycerol inhibits an increase in levels of body fat."
Dental health	Xylitol, maltitol, erythritol, and palatinose (low in cariogenicity), green tea polyphenol casein (non-cariogenic), and phosphopeptide amorphous calcium phosphate (anti-cariogenic)	• Oral pH • Demineralization of enamel slabs in the human mouth	"Products containing these ingredients are low- or non-cariogenic." "Products containing these ingredients make teeth strong and healthy."
Bone health	Vitamin K2, milk basic protein, and soya isoflavones	• Bone mineral density • Gla-osteocalcin in blood	"Products containing these ingredients help promote bone calcification." "Product helps increase bone density to make healthy bones."

Source: Shimizu 2003. Reproduced with permission of Cambridge University Press.

Figure 12.1 Seal of approval used by FOSHU regulatory system in Japan, symbolizing "jumping for health". *Source*: Adapted from Shimizu 2003. Reproduced with permission of Cambridge University Press.

the health claims of the food. As of January 31, 2008, the EC had received more than 44,000 health claim requests from member states, albeit with many duplications. The national lists were subsequently compiled into a consolidated list (4,637), with some member states withdrawing claims before their evaluation by the EFSA. Health claims corresponding to submissions covering different claimed effects, or brought together under the same claimed effect and which comply with the requirements of Regulation (EC) No. 1924/2006, are authorized under Article 13(3) of Regulation (EC) No. 1924/2006, and included in a list of permitted claims (EU 2012). In pursuance of Article 20(1) of Regulation (EC) No. 1924/2006, a Union Register (the Register) containing all the authorized nutrition and health claims made on foods and, inter alia, the conditions of use applying to them, as well as a list of rejected health claims and the reasons for their rejection, is established and maintained. Between July 2008 and March 2010, the EFSA published 125 opinions providing scientific advice for more than 900 health claims, out of the consolidated list (4,637) of health claims submitted. As of May 16, 2012, the commission regulation (EC) No. 432/2012 has an established list of 222 permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health (EU 2012). Some of the permitted health claims are listed in Table 12.4. In some of the permitted claims, the conditions and/or restrictions of use of the food and/or additional statement or warning may be required. For instance, foods containing guar gum or pectins should warn of choking in people with swallowing difficulties, as well as provide advice on the need to

Table 12.7 Functional foods and intestinal nutrient absorption

Functions	Functional ingredient	Mechanisms of action
Enhance mineral absorption	Casein phosphopeptide (CPP)	Increase calcium solubility in the lower small intestine
	Fructooligosaccharide	Increase calcium solubility by lowering intestinal pH
Suppress blood glucose increase	Wheat albumin	Inhibit α-amylase activity
	L-Arabinose	Inhibit α-glucosidase activity
	Guava tea polyphenol	Inhibit α-amylase activity
	Touch extract	Inhibit α-glucosidase activity
Suppress blood cholesterol increase	Soybean protein/peptide	Capture and accelerate excretion of cholesterol and bile acids
	Plant sterol, stanol	Inhibit incorporation of cholesterol into mixed micelles in the intestinal tract
Suppress blood triglyceride increase and body fat accumulation	Globin digests	Inhibit intestinal absorption of triglyceride
	Coffee mannooligosaccharide	Inhibit intestinal absorption of triglyceride
	Polymerized tea polyphenol	Inhibit lipase action

Source: Shimizu and Hachimura 2011. Reproduced with permission of Elsevier.

be taken with plenty of water to ensure these substances reach the stomach. Information on the variability of lactose tolerance and statements on the need to seek advice on the effects of lactose in the diet are required in health claims made on foods containing lactose. The Commission Regulation (EU) No. 536/2013 of June 11, 2013, amended (EC) No. 432/2012 and authorized six new health claims on food products containing alpha-cyclodextrin, DHA, DHA/EPA (two different health claims), dried plums of “prune” cultivars (*Prunus domestica* L.), and fructose, and this became effective on January 2, 2014 (EU 2013).

An estimated 95% of foods that do not contain vitamins and minerals reportedly receive negative opinions from the EFSA, while most vitamin and mineral food supplement health claims are approved (Brookes 2010). For instance, zinc has 18 different permitted health claims, including its role in maintaining normal nails, bones, hair, and skin, to contributions toward normal cognitive function, protein synthesis, and carbohydrate synthesis. The prohibitions are often perceived as unjustified by the EU food sector because the products/substances have been legally sold with health claims for many years without being challenged under their national legislation.

Table 12.8 Types of health claims in the European Union

Claim	Definition	Example
General function (Article 13.1)	This refers to the role of a nutrient or substance in the growth, development, and body functions; psychological and behavioural functions; slimming and weight control; and satiety or reduction of available energy from the diet. These claims do not include those related to child development or health or disease risk reduction.	"Food can help reinforce the body's natural defences or enhance learning ability."
New function (Article 13.5)	Claims based on newly developed scientific evidence and/or for which protection of proprietary data is requested.	"A combination of red spinach, green spinach, red chicory, green chicory, green leaf chard, red leaf chard, red Swiss chard, golden Swiss chard and white Swiss chard and maintenance of normal blood cholesterol concentration."
Disease risk reduction and child development or health (Article 14)	This refers to the reduction of disease risk or to children's development or health.	Eicosapentaenoic acid (EPA) and "reduces the AA/EPA ratio in blood. A high AA/EPA level is a risk factor in the development of attention difficulties in children with attention deficit hyperactivity disorder (ADHD)-like symptoms." "Plant sterols have been shown to reduce cholesterol levels, a risk factor in the development of coronary heart disease."

Source: EFSA 2013b. © EFSA.

Health claim prohibitions by regulatory bodies can have grave economic consequences on the sector and can result in increased uncertainty, cause loss of employment and profitability, threaten industrial viability, and raise barriers against introducing and sustaining new products (Brookes 2010). Some perceive negative opinions and such prohibitions as harsh, with the likely impact of substantially reducing research and development of new products, limiting product competitiveness, and increasing the market share of products available through the Internet and mail orders, which are often not subject to regulatory requirements. Additional cost is incurred for amending labels and promotional literature; making changes to packaging; marketing and promotional activities; and, in extreme scenarios, for recall and disposal.

In the EU, a health claim application that receives a negative decision is prohibited from use after a 6 months grace period. In the quest to protect consumers from being misled, decisions made by regulatory bodies, when overly severe, may sometimes have negative consequences on consumers. Rejected health claims may mean that consumers lose out from reduced choice and possibly higher prices, owing to fewer products and less competition in the market. Consumers may also have difficulties in understanding the basis of the rejection. At the request of the European Commission, the EFSA organized an information session on November 20, 2013, to streamline the preparation and presentation of applications reporting human studies for scientific substantiation and authorization of health claims submitted for evaluation (EFSA 2013a).

In the EU, for instance, the cost of submitting health claims can be high (€980–€1,663 for Article 13.1, and €6,400–€8,000 for Article 13.5/Article 14); the average time taken to deliver opinions on health claim applications is >2 months, and this varies with the type of health claim (Brookes, 2010). Where human clinical trials are required to produce proprietary data to support health claim applications (Article 13.5/Article 14), the costs can be in the range of €0.25 million–€1 million plus.

In the United States, a health claim is one of the three claims (along with nutrient content claims and structure/function claims) that can be used on food and dietary supplement labels. The Food and Drug Administration (FDA) describes health claim as "a relationship between a food, food component, or dietary supplement ingredient, and reducing risk of a disease or health-related condition." The food or part of a food must surpass the rigorous stand of "significant scientific agreement" of a health claim to be approved. Several approved health claims about reduced risks of coronary and cardiovascular disease (CVD) and cancer in relation to consumption of dietary lipids, soluble fiber, fiber-containing grain products, fruits and vegetables, soy protein, and stanols/sterols have been approved by the FDA (FDA 2013a). A few examples of these approved health claims in the United States are provided. Qualified health claims – such as atopic dermatitis risk and partially hydrolyzed whey protein infant formula; reduced risk of cancer and tomatoes and/or tomato sauce and green tea; and reduced risk of CVD and nuts and omega-3 fatty acids – are subjected to enforcement discretion due to minimal-to-non-conclusive scientific evidence (FDA 2013b).

In addition to the regulatory bodies in different countries and jurisdictions, Codex Alimentarius provides global/universal recommendations to assist member countries in the use, evaluation, and substantiation of health claims, which may also serve as a basis for national legislation (Codex Alimentarius 1997). Under Codex Alimentarius, there are three types of health claims, namely: nutrient function claims, enhanced function claims, and reduction of disease risk claims (Table 12.9). Codex Alimentarius recommends the

Table 12.9 Definition from Codex

Nutrition and health claims	Definition
Nutrition claim	Any representation that states, suggests, or implies that a food has particular nutritional properties, including but not limited to the energy value and to the content of protein, fat, and carbohydrates, as well as the content of vitamins and minerals. The following are not considered to be nutrition claims: (a) the mention of substances in the list of ingredients; (b) the mention of nutrients as a mandatory part of nutrition labeling; (c) quantitative or qualitative declaration of certain nutrients or ingredients on the label if required by national legislation.
Nutrient content claim	Nutrition claim that describes the level of a nutrient contained in a food (e.g., "source of calcium"; "high in fiber and low in fat")
Health claim	Any representation that states, suggests, or implies that a relationship exists between a food or a constituent of that food and health. Health claims include: *Nutrient function claims* – A nutrition claim that describes the physiological role of the nutrient in growth, development, and normal functions of the body. (For example, "Nutrient A (naming a physiological role of nutrient A in the body in the maintenance of health and promotion of normal growth and development). Food X is a source of/high in nutrient A.") *Enhanced function claims* – Claims concern specific beneficial effects of the consumption of foods or their constituents, in the context of the total diet on normal functions or biological activities of the body. Such claims relate to a positive contribution to health or to the improvement of a function or to modifying or preserving health. (For example, "Substance A (naming the effect of substance A on improving or modifying a physiological function or biological activity associated with health). Food Y contains X g of substance A.") *Reduction of disease risk claims* – Claims relating the consumption of a food or food constituent, in the context of the total diet, to the reduced risk of developing a disease or health-related condition. Risk reduction means significantly altering a major risk factor(s) for a disease or health-related condition. Diseases have multiple risk factors, and altering one of these risk factors may or may not have a beneficial effect. The presentation of risk reduction claims must ensure, for example, by use of appropriate language and reference to other risk factors, that consumers do not interpret them as prevention claims. (For example, "A healthful diet low in nutrient or substance A may reduce the risk of disease D"; "Food X is low in nutrient or substance A"; or "A healthful diet rich in nutrient or substance A may reduce the risk of disease D. Food X is high in nutrient or substance A.")

Source: Codex Alimentarius 1997; http://www.codexalimentarius.org/standards/list-of-standards/.

inclusion of the following details on the label: quantity of the constituent that is the subject of the claim; the target group; vulnerable groups (how to use it, or individuals who need to avoid it); how to use the food to obtain the claimed benefit, other lifestyle factors, or other dietary sources; maximum safe intake; how the food or food constituent fits within the context of the total diet; and a statement on the importance of maintaining a healthy diet. Codex Alimentarius does not permit health claims in foods for infants and young children, except where specifically provided for in the relevant Codex standards or national legislation. In 2009, Codex Alimentarius adopted the Codex Committee on Food Labelling's recommendations on the scientific substantiation of health claims.

Harmonizing permitted health claims and labeling to international standards may be of help to both consumers and food manufacturers. Both the Codex Alimentarius and an Economic Union project in 1999 proposed the enhanced function claim and the disease risk-reduction claims. Most of the regulations described by these countries and jurisdictions share some similarities. The structure/function claims in the United States (enacted by the Dietary Supplement Health and Education Act in 1994) is similar to the FOSHU system and enhanced function claims of the Codex Alimentarius. The nutrient function claims was standardized by the Codex Alimentarius in 1997, and it is similar to the FNFC from Japan.

12.4 Conclusion

Unambiguous and conclusive reports, detailing the benefits of functional foods and nutraceuticals, the populations that can benefit from consuming these, the recommended doses, lengths of intervention, and whether potentially synergist ingredients may interfere with the outcome, are required. The rigorous scrutiny that health claims are subjected to by regulatory agencies prior to approvals helps to boost consumer confidence. At the same time, extensive regulatory hurdles may be counterproductive, as they may prevent consumers from having access to products that may help to improve health and wellness.

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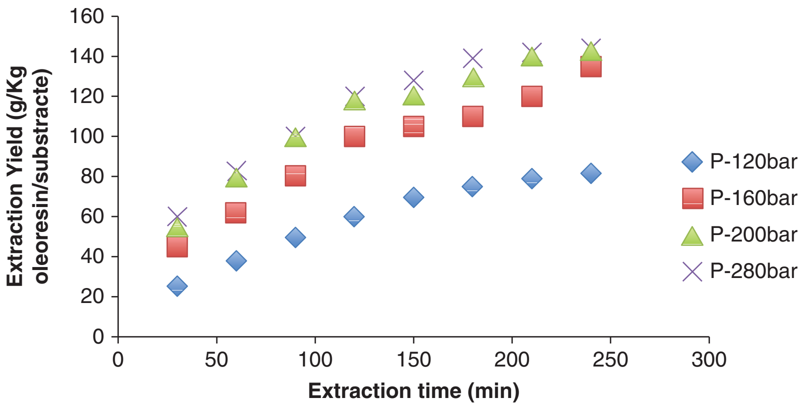

Plate 1 The effect of pressure and time on cumulative yield of hop oleoresin from cone pellets using supercritical CO_2 at 40 °C. (Modified from Del Valle, J. M. et al. (2003b). Supercritical CO_2 extraction of Chilean hop (Humulus lupulus) ecotypes. *Journal of the Science of Food and Agriculture,* **83**: 1349–1356.)

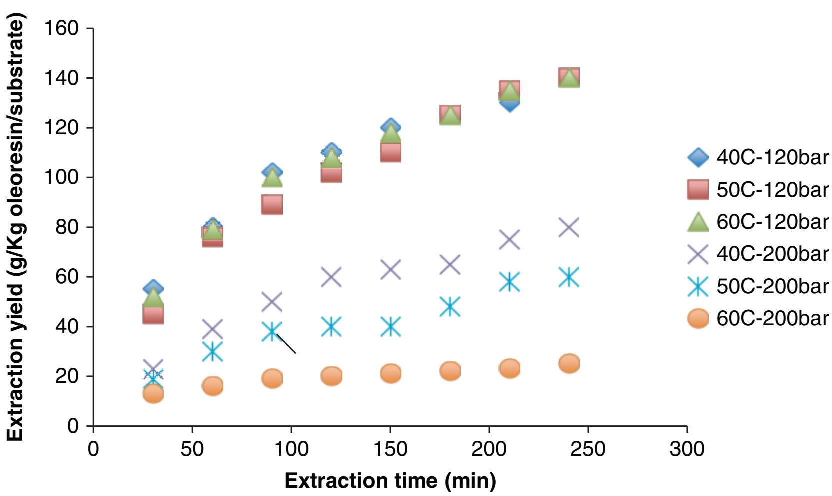

Plate 2 The effect of temperature (40, 50, and 60 °C), pressure (120, 200 bar), and time (30–240 min) on cumulative yield of hop oleoresin from cone pellets using supercritical CO_2. (Modified from Del Valle, J. M. et al. (2003b). Supercritical CO_2 extraction of Chilean hop (Humulus lupulus) ecotypes. *Journal of the Science of Food and Agriculture,* **83**: 1349–1356.)

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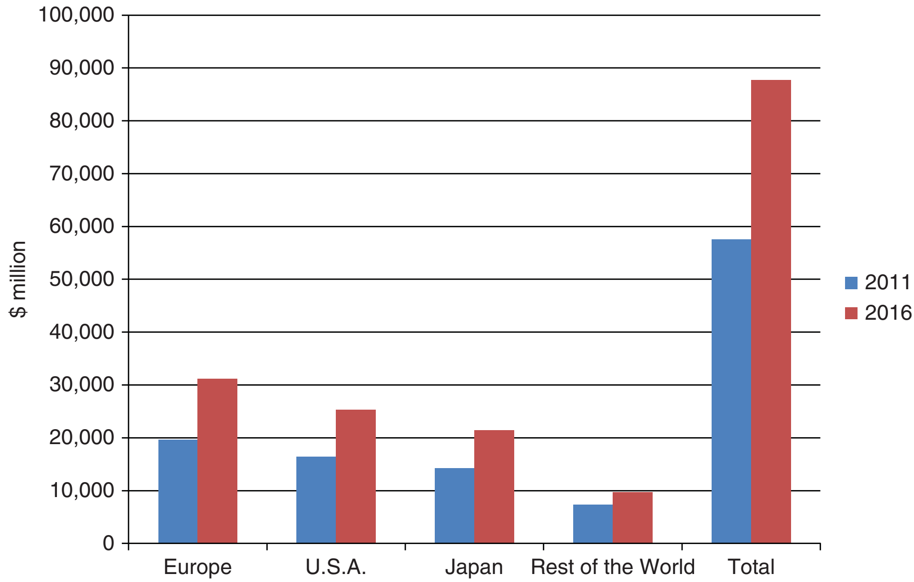

Plate 3 Top global functional beverage markets, 2011 and 2016. *Source*: BCC Research (2011). Market research report. *Nutraceuticals: Global Markets and Processing Technologies*

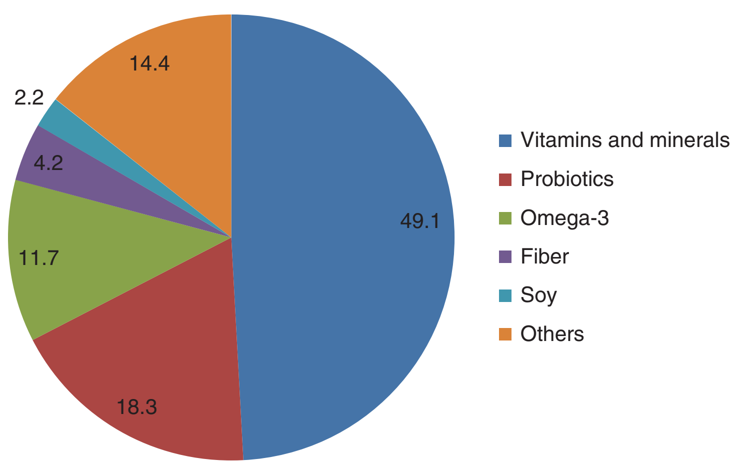

Plate 4 Global nutraceutical beverage market, by ingredient market share, 2011. *Source*: BCC Research (2011). Market research report. *Nutraceuticals: Global Markets and Processing Technologies*

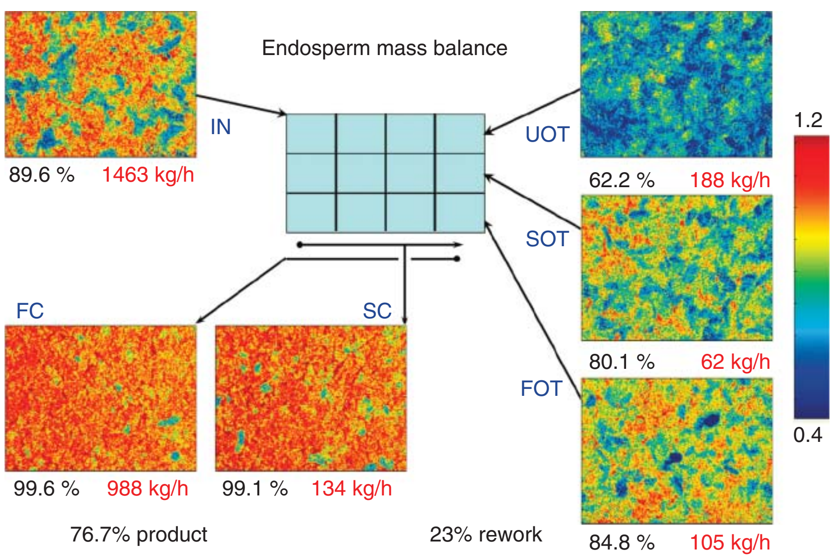

Plate 5 Endosperm mass balance of commercial flour mill purifier. The rectangular diagram in the center represents a purifier (left to right) with the incoming stock (IN, top left) entering, and the first cut (FC) and second cut (SC) product streams. From IN to FC, the endosperm content has been enriched from 89.6% to 99.6%, while the quantity has been reduced from 1,463 kg/h to 988 kg/h. The by-product material is divided into three streams, designated as upper overtails (UOT), second overtails (SOT), and first overtails (FOT) of varying percentage endosperm and endosperm flow. The summation of FC and SC products was 76.7%, and the combined by-product streams subject to rework was 23.3%. (From Wetzel, D. L. et al. (2010). InSb focal plane array chemical imaging enables assessment of unit process efficiency for milling operation. *Applied Spectroscopy*, **64**(12): 1320–1324.)

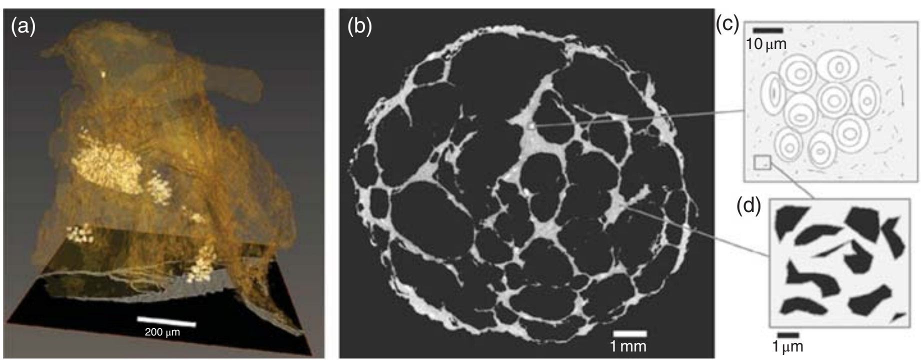

Plate 6 (a) 3D reconstruction of extruded whole-grain barley flour with whey protein isolate. The starch granules are rendered in white, showing their clustered distribution within the sample (voxel size 682 nm); (b) X-ray microtomography image of cross-section of extrudate; (c and d) Sketch of small cluster of granules and air-filled micropores inside the solid matrix. (From Penttilä, P. A. et al. (2011). X-ray characterization of starch-based solid foams. *Journal of Materials Science*, **46**: 3470–3479.)