NUTRACEUTICALS

1. Introduction

In our definition, nutraceuticals are products and their derivatives that occur in Nature and are constituents of plants and animal, including humans. These constituents confer a health benefit above and beyond basic nutrition or basic fortification.

Nutraceuticals are the active ingredients in functional food or nutritional supplements that deliver a health benefit. Some examples are *n*-3 long-chain fatty acids in milk to reduce cardiovascular risks, probiotics in yogurt to improve growth of beneficial intestinal flora, phytosterols in margarine to reduce cholesterol uptake, and folic acid in flour to prevent spina bifida and reduce homocysteine, a risk factor for cardiovascular disease. Combinations of essential nutrients and nutraceuticals may strenghten the health promoting potential by synergy and could be expected to contribute to risk reduction of chronic diseases, ageing, etc.

Changes of nutritional behavior, as well as in life-style, may make it difficult to have a regular intake of these nutrients. By offering these compounds as nutraceuticals, one can not only compensate for insufficiency but also contribute to a more optimal nutrition. Further, the health benefit of these ingredients can be explored beyond their functions as basic nutrients. These products have a great potential to benefit health, quality of life , healthy aging, and will contribute to improved physical and mental fitness.

For example, lutein is a carotenoid found in spinach, and zeaxanthin is the major carotenoid in corn. Both are considered to contribute to healthy vision by protecting the eye from the hazardous blue fraction of light. Lutein has been launched recently in the United States and is marketed in the multivitamin product Centrum. Zeaxanthin is in an advanced stage of development and will be introduced to the market in the near future. While lutein is derived from natural sources, zeaxanthin is being chemically synthesised by a patented process. Both products are uniquely formulated to make them stable and bioavailable. For both products, research is being conducted to demonstrate their benefical effects on human health.

Lycopene, another carotenoid, is contained in considerable amounts in tomatoes and is responsible for their red color. Lycopene has been associated in a number of epidemiological studies with a decreased risk for various cancers. Recently, mechanistic data, animal experiments, and human studies point to a role of lycopene in reducing risk for the development of prostate cancer. Lycopene is in an advanced stage of development and will be launched in the near future. It will be manufactured by a proprietary chemical process and delivered in a specially developed formulation.

From traditional medicine, it is known that green tea reduces cholesterol, that it contains antioxidants, and that it can prevent certain degenerative diseases. Epigallocatechin gallate (EGCG) has been identified as the main component responsible for these effects and EGCG has been extracted by Roche by a unique physical process that results in a highly purified and well-standardized product. Among the many health benefits of EGCG is its possible role as a cardiovascular protector. A number of *in vitro* and animal studies support this and

pave the way for human studies, including pharmacokinetic data as well as studies on the effect of EGCG on cardiovascular risk factors.

Additional new products are filling the pipeline worldwide to respond to the challenges of the dynamic nutraceutical market. A prudent vision is to bring products to the market that have an excellent potential of reducing important risk factors so to improve human health. Identification of appropriate natural constituents, development of sophisticated and unique processes to assure high quality, well defined and standardized products, and data to support efficacy and safety claims will be the hallmarks of responsible suppliers of such products.

2. Past and Current Trends In Nutrition

Food ingestion ideally produces a feeling of pleasure, satiation, and contributes to general well being (1). A fundamental role of nutrition is to ensure an adequate intake of macro- and micronutrients, permitting the smooth operation of metabolic processes. This understanding has led to the development of recommendations on nutrient intake (Table 1). As long back as in the 1940s the first recommendations for selected nutrients were issued in the United States (2) with the aim of avoiding nutrient deficiency, classically manifested, eg, as scurvy in the case of vitamin C deficiency. Later, other nutrients were included in the recommendations and published in various countries.

Eventually, it was realized that, apart from the problem of inadequate intake, certain nutrients might also be consumed in excess. For example, in the 1960s there was accumulating scientific evidence that excessive fat intake could cause a rise in blood lipids. The presence of elevated blood lipids, or hyperlipidemia, in turn represents an important risk factor in the development of atherosclerosis, as was subsequently reported (3). An excess of certain nutrients may thus also have a lasting and significant health impact, including as a cause of clinical disease.

This relatively simple concept of inadequacy *versus* excess was later refined. The 1980s saw the increasing appearance of scientific reports suggesting that essential nutrients play a part in reducing risk factors for chronic disease. One example is the role of antioxidant vitamins in arresting the development of cardiovascular disease (4).

Finally, at the end of the twentieth century, research interest in nutritional science turned increasingly to the role of nonessential nutrients in human health. Evidence on the possible role of nonessential nutrients in reducing the risk of chronic diseases continues to accumulate. For example, the U.S. Food and Drug Administration (FDA) recently authorized a further health claim to the effect that soy protein may reduce cardiovascular risk (5).

The realization that nutrition not only serves energy and nutrient intake but can also have an important impact on health has led to various efforts to develop foods with a health-promoting effect.

3. Substances and Health Benefit

What does one mean by "foods with a health-promoting effect"? Neither the scientific literature nor legislation yet provides a clear definition. Terms used as synonyms include "functional foods", "designer foods", "pharmafoods", and "agromedical foods", to name just a few examples. The term "nutraceuticals" suggests that these bioactive substances/food ingredients are understood to be part of the diet and thus distinguishes them from drugs or pharmaceuticals (6). The complexity of this new field of nutrition is underlined by the fact that not only is there a lack of a generally accepted term but also, so far, a failure to agree on what these foods are.

For example, the Food and Consumer Safety Division of the Swiss Federal Office of Public Health has proposed the following definition of functional foods, which broadly matches that of other groups and authors (7) : "... foods with a specific additional benefit that goes beyond the nutritional benefit of the nutrients they contain". In the United States, the Food and Nutrition Board (FNB) of the National Academy of Sciences has defined functional food as "a food that encompasses potentially healthful products, including any modified food or food ingredient that may provide a health benefit beyond that of the nutritional nutrients it contains" (8). This definition certainly describes very well the role of bioactive ingredients in foods with a health-promoting effect. Table 1 gives some examples of nutrients with demonstrated health benefits that go beyond their physiological effects.

In simplified terms, the development of bioactive ingredients for foods with health-promoting effects can be subdivided into the following steps, which will be discussed in greater detail below: identification, safety testing, and efficacy testing of bioactive ingredients.

3.1. Identification of Nutraceuticals. A standard way to identify bioactive substances is a selection based on available literature data. If, however, the aim is to obtain truly innovative and best-in-class bioactive substances, this approach has considerable limitations. Here, the technology of *high throughput screening* (HTS), which has been developed in recent years in pharmacological research (9), offers new opportunities for nutritional research. A modified HTS concept for identifying innovative bioactive substances suitable for developing foods with a health-promoting effect is presented below.

The first, and probably most demanding, step is to identify a molecular mechanism of action by which the sought bioactive substance is to exert its effect. Ideally, the mechanism of action should be validated in humans. In other words, a substance must be known that leads to the desired health-promoting effects by interacting with the target molecule in the human body. This effect must be measurable by suitable biomarkers and/or clinical endpoints in clinical studies. The HTS process can then be pursued in various successive steps, which will be discussed further below: establishment of an extract library, *in vitro* testing of these extracts to identify "hot fractions", and identification of the bioactive substances in these "hot fractions", including detailed structural analysis.

An extract library with a large chemical diversity of test substances is the precondition for successful HTS. Substances that are part of the food chain, such

as extracts of foods from plants, microorganisms, fungi, animals, and their combinations, can be used to establish a library of this kind. The complex mixture of substances in the extracts may lead to overlapping biological and physicochemical effects, so that active substances remain undetected or combinations of components produce false-positive results. To minimize these effects, the individual extracts can be physically separated into further fractions, eg, by high performance liquid chromatography (HPLC).

With HTS, 100,000 or more samples a day can be tested for activity in an *in vitro* test. At present, assay of activity is not the bottleneck in natural-product screening. Rather, the challenge is in the unknown composition and quantity of the substances in the complex mixtures in the extract fractions under test. To avoid the time-consuming isolation of "false-positive" components, great attention must be paid to the quality, and especially the robustness over a wide concentration range, of the test systems used in HTS.

The next step in natural-product screening is the rapid and efficient classification of biological activities and the chemical structures on which they are based. Since many substances are present in the extracts, it is vital to classify them as early in the process as possible. Identifying already known, previously isolated substances in order to exclude them allows attention to be focused on promising new candidates, and is therefore indispensable. The use of liquid chromatography and accurate determination of mass number by mass spectrometry (MS) in the form of HPLC–ESI–TOFMS (where ESI = electrospray ionization and TOF = time of flight) allows determination of the empirical formulas of a large number of components of an extract and comparison with known subatnces in chemical databases (10). Many components can be identified by combining the empirical formula with the uv spectrum and taxonomy of the plant extract in question. The remaining unknown components are classified on the basis of the exact mass number, retention time, and uv spectrum. By integrating these physical properties with the biological activities identified concurrently by HTS, it is possible to focus on the isolation and detailed structural analysis by nuclear magnetic resonance (nmr) spectroscopy of promising new substances.

The bioactive substances identified by HTS are then subjected to a series of further *in vitro* tests, such as bioavailability, stability, and solubility, before proceeding to *in vivo* testing. Interdisciplinary collaboration is essential to the success of this approach. Where molecular biological, physiological, and clinical knowledge is closely integrated with the technological know how of HTS, informatics and natural-product chemistry, new bioactive substances can be efficiently identified for foods with a health-promoting effect.

3.2. Safety of Nutraceuticals. The essential prerequisite for any bioactive substance intended for use in food is that its safety be established by generally accepted criteria. The number and scope of tests will naturally vary with different classes of substance, but in simplified terms, safety testing consists of *in vitro* tests, and use in animals and, at a later stage, also data obtained in human studies. These studies to demonstrate the safety of bioactive substances are performed in accordance with recommendations and guidelines established both by national legislation and international bodies such as the FDA and world health organization (WHO) (11). A further important element of the safety program is to obtain pharmacological and pharmacodynamic data to characterise

the activity profiles of these bioactive substances in humans. This information forms the basis for planning and performing human efficacy studies. It is worth emphasizing that all human studies—whether in the context of safety testing or the efficacy studies described below—must comply with the ICH Guideline for Good Clinical Practice (12).

3.3. Efficacy of Nutraceuticals. Most naturally occurring bioactive substances are nonessential to the human body. This means that there are no known typical signs or symptoms of deficiency established if these substances are not regularly consumed. For example, while inadequate vitamin C intake leads after a time to the classic deficiency syndrome of scurvy, such syndromes do not exist for most naturally occurring substances with a postulated health-promoting potential, such as flavonoids, polyphenols, phytoestrogens, and many more. This means that a precondition for adding these *nonessential* bioactive substances to foods is proof of their efficacy in terms of a defined health marker.

Demonstrating efficacy of a substance that occurs naturally in the food chain confronts scientists with a considerable challenge. The health-promoting effect of these naturally occurring bioactive substances must be preventive, fundamentally distinguishing them from curative drugs. This means that the effect of these natural bioactive substances on the human body may be very small over relatively short periods, ie, months or a few years, but can contribute significantly to health when they are consumed lifelong as part of the daily diet.

It is also known that many chronic human illnesses begin to develop early in life, but are becoming clinically observable only very much later. This time lag between the start of an illness and individually observable symptoms applies to cardiovascular disease, osteoporosis and many forms of cancer, and may last as long as several decades. Arterosclerotic changes can be found *in situ* in the blood vessels of adolescents and young adults, or even earlier (13). However, clinical manifestations such as myocardial infarction, stroke, or intermittent claudication will generally appear only two or three decades later. The same is true of osteoporosis, which could be described as bone weakness with an increased risk of fractures. Bone is strongest between the ages of 20 and 30. Afterward, in women earlier than in men, there is a continuous decline in bone strength, many people suffering fractures between the ages of 50 and 80. Similarly, in malignant diseases of the stomach and prostate, up to 20 years may elapse between the initiation, ie, the appearance of the first malignant cell , and clinical manifestation of the cancer. The list of examples could easily be extended.

Investigating preventive activity in these naturally occurring bioactive substances when the effect may only be moderate thus poses a serious dilemma to nutritional research. To facilitate discussion of this problem, one should first briefly examine what scientific possibilities exist for demonstrating the efficacy of these naturally occurring bioactive substances.

In simplified terms, the various categories of scientific safety tests comprise *in vitro* tests (molecular and cellular assays), animal experiments, and human studies, the latter being subdivided into epidemiological and intervention studies. Often, epidemiological studies, eg, to investigate the relationship between diet and the occurrence of a disease in a relatively large population, lead to a hypothesis that is investigated further *in vitro* and in animal experiments. Inter-

vention studies may then be performed to demonstrate efficacy in humans taking a specific amount of a bioactive substance each day under precisely defined and controlled conditions.

The measures used may be either intermediate endpoints or clinical endpoints. Clinical endpoints, such as myocardial infarction, fractures, the occurrence or course of cancers, etc, are typically used to investigate the efficacy of drugs, ie, for therapeutic purposes. However, for the reasons discussed above, clinical endpoints appear less suitable for studying the efficacy of bioactive substances for prevention. One therefore must take a closer look at the concept of biomarkers and their role in demonstrating the efficacy of bioactive substances.

The terms "biomarker", "surrogate marker", and "clinical endpoint" were proposed by Zeger at a National Institute of Health (NIH) workshop on biomarkers and surrogate endpoints (14). A surrogate endpoint, ie, a biomarker suitable for replacing a clinical endpoint, thus appears to be the appropriate parameter for demonstrating the efficacy of naturally occurring bioactive substances in human intervention studies provided it meets certain criteria (15) . It must display good specificity and sensitivity must be standardised and validated, must be noninvasive or at most minimally invasive, and it must be inexpensive if it is to be widely used.

With these provisos, the biomarker concept can be an essential element not only in the efficacy testing of bioactive substances but in nutritional science in general. With biomarkers, the moderate effects of natural bioactive ingredients on the human body can be studied without excessive strain on resources. Most biomarkers are factors that signal the risk of developing a chronic disease at an early stage. They therefore appear to be suitable parameters for investigating the preventive effect of bioactive substances, given that the goal of improved nutrition is to reduce risk factors, and hence the probability of developing chronic disease. Some examples of biomarkers follows:

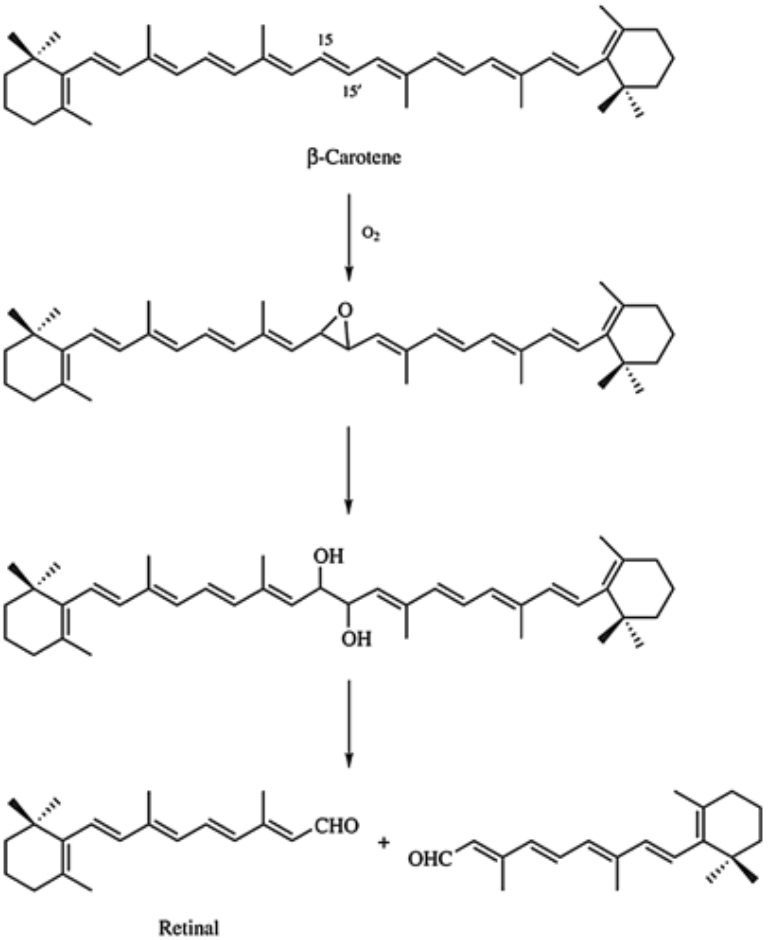
Cardiovascular
Biomarker: Serum cholesterin, blood pressure
Clinical: Cardiac infarction, apoplectic fit
Osteoporosis
Biomarker: Marker of bone, bone dence
Clinical: Fracture
Cancer, eg, prostata
Biomarker: prostata specific antigen (PSA)?
Clinical: Neoplasma of prostata or metastases

Of course, the biomarker concept also has its limitations. A single biomarker will almost certainly never be ideal. Rather a series of biomarkers will probably be required to examine the effect of a bioactive substance on human health. With this biomarker profile it will be possible to categorize the effect of a bioactive substance on risk factors for chronic diseases or other functions of the human body.

Lastly, it is important to note that in assessing the efficacy of a bioactive substance, the results of *in vitro* tests, animal experiments, and epidemiological and intervention studies in human must of course be considered as a whole.

4. Product Classes

4.1. Vitamins. Vitamins are essential organic compounds that are not synthesized in the human or animal organism. They must are consumed with the diet either as such or as a precursor, a so-called pro-vitamin, which can be converted to the vitamin, eg, β-carotene is a pro-vitamin that is converted biochemically to vitamin A (16). New work on this conversion shows that in a monooxygenase-type reaction an epoxidation of the central double bond of β-carotene followed by an unselective ring-opening reaction with water and a diol cleavage results in formation of two molecules of the aldehyde (17). The following mechanism is for the enzyme catalyzed cleavage of β-carotene.



Based on the definition above, the following 13 compounds or groups of compounds have been classified as vitamins (for humans):

Water-soluble	Fat-soluble
Vitamin B1 (thiamin)	Vitamin A (retinols)
Vitamin B2 (riboflavin)	Vitamin D (calciferols)
Vitamin B6 (pyridoxal group)	Vitamin E (tocopherols, tocotrienols)
Vitamin C (L-ascorbic acid)	Vitamin K (phylloquinone)
Pantothenic acid	
Biotin	
Folic acid	
Niacin	

Vitamins currently command a market of 4 billion US$/year. The history of vitamins is an ongoing story, and the products in a relatively mature phase of life cycle where new applications and production process alternatives and optimization are presently the main focus.

Other compounds exist that are also important for the organism and cannot be produced in sufficient quantities. These are known as "pseudo-vitamins".

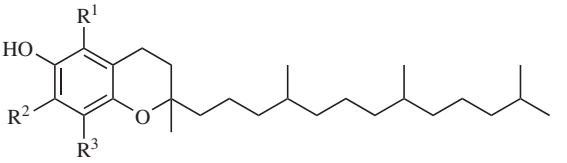
The term vitamin itself was introduced in 1911 from the words "vital amine" (18). At this time, a substance in the form of a water-soluble crystal form, which was responsible for curing beriberi, was isolated and named "beriberi vitamin". In 1920, the "beriberi vitamin" was designated vitamin B (19). The designation "vitamin" was later accepted for all essential nutritional factors, although it was recognized that not all these compounds are amines.

For the production of vitamins, chemical synthesis has been the historically preferred method. Other methods are extraction from natural sources or fermentation. The later methods can also be combined with chemical synthetic steps (20). The production of vitamins is discussed in various other sources (21,22) and is not described in detail here.

Seen as nutraceuticals, vitamins have several important functions. In the following sections, various aspects are discussed.

Vitamin E. Vitamin E is a group of compounds based on 6-chromanol. Differences between α-,β,γ,δ-tocopherol are given in the number of methyl groups on the chromane ring (23). The tocopherols have three stereocenters, at positions 2, 4′, and 8′. Synthetic α-tocopherol, a mixture that is racemic at all stereocenters, is denominated as (all-*rac*)-α-tocopherol or (2*R*S,4′*R*S,8 ′RS)-α-tocopherol. The

following structures are of tocopherols with vitamin E activity.



$R^1 = R^2 = R^3 = CH_3$: α-tocopherol
R^2 =H, $R^1 = R^3 = CH_3$: β-tocopherol
R^1 = H, $R^2 = R^3 = CH_3$: γ-tocopherol
$R^1 = R^2$ = H, $R^3 = CH_3$: δ-tocopherol

Vitamin E is the most important lipid-soluble biological antioxidant (24). Vitamin E deficiency can result from an inadequate supply of the vitamin and lipid malabsorption. Clinical vitamin E deficiency in human is rare (25). Abetalipoprotein-emia, an inborn deficiency of plasma lipoproteins, is found in rare cases (25).

Tocopherol and tocotrienol homologues, possesing an isoprenoic side chain unsaturated at position 3′, 7′, and 11′, are found in Nature, ie, plant oils such as wheat oil, soybean, sunflower, rape, corn, and peanut oil (mg/100 g). For the concentration of tocopherol and -trienol homologues in food see Refs. (26,27).

Vitamin E is thought to function primarily as a chain-breaking antioxidant that prevents the propagation of free radical reactions in this manner protecting membrane lipids and blood lipids (eg, LDL cholesterol) against oxidative damage (28–32). Because of its antioxidative properties, vitamin E is believed to help prevent diseases associated with oxidative stress, such as cardiovascular disease, cancer, chronic inflammation, and neurologic disorders.

Some nonantioxidant functions have been attributed to α-tocopherol (33). These include regulation of protein kinase C, modification of cell growth and proliferation, modification of gene transcription, protein phosphatase activation, and modifications to gene expression. In addition *in vitro* and *in vivo* studies have shown that functions of vitamin E beyond that of preventing low density lipoprotein (LDL) oxidation, include maintaining or improving endothelial function, inhibiting proliferation of smooth muscle cells and inhibiting adhesion and aggregation of blood platelets and thus supporting the protective role against coronary heart disease (34,35). In addition, some studies in animal models have shown a decrease in the atherosclerotic lesion progression with vitamin E.

Overt vitamin E deficiency almost never occurs in humans as a result of dietary limitations. However, genetic deficiencies in apolipoprotein B (apo B) or as a result of various fat malabsorption syndromes lead to severe vitamin E deficiency syndromes. The symptoms are primarily neurologic dysfunctions and include loss of deep tendon reflexes, cerebellar ataxia, dysarthria, and mental retardation, but the underlying molecular mechanisms are still unclear.

Many epidemiological studies have demonstrated that dietary vitamin E intakes are correlated with a reduced risk of cardiovascular diseases in both men and women (36). These observational studies are strongly supported by

many positive results in several animal models, including non-human primates (37) and by numerous *in vitro* evidence, which provide plausibility (38). Recently, however, intervention trials have provided mixed results indicating either a protective effect of vitamin E (19–40), whereas others have revealed neutral results (41,42) on patient with cardiovascular disease.

Although these recent clinical trials have raised some doubts on the efficacy of vitamin E in the prevention of progression of atherosclerotic lesions and cardiovascular disease, the overwhelming observational and experimental studies strongly support its positive effect on the reductions of risk of atherogenesis.

Furthermore, a large number of studies suggest that vitamin E may contribute significantly to lowering the risk of certain chronic disease, eg, in preventing prostate cancer (43) , Alzheimer′s disease (44), preeclampsia (45), and cataract (46), among others.

The Dietary Reference Intake value (DRI) includes the Recommended Dietary Allowance (RDA) and the Tolerable Upper Intake Level (UL). Compared to the previous RDA, the new recommendations for vitamin E have recently been increased by $\sim 50\%$ for men and by almost 100% for women. The current RDA for vitamin E (alpha-tocopherol) is 15 mg for adult men and women and is considered to be adequate to prevent deficiency symptoms in healthy adults as recommended by the National Academy of Sciences (47). The UL for adults is set at 1000 mg/day of any form of supplemental vitamin E (alpha-tocopherol).

In general, vitamin E has a remarkably good safety record. Extensive reviews of the medical literature have concluded that doses of > 50 times the RDA are safe for long periods of time (48–50). In clinical trials in older people with Alzheimer disease or Parkinson's disease, the very high dose of 2000 IU / day was well tolerated by most subjects for as long as 2 years (32–51).

For the production of vitamin E, two principal methods are used. In the first, isolation of mixed tocopherols from plant oil is followed by ring methylation. In the second method, a total synthesis is carried out.

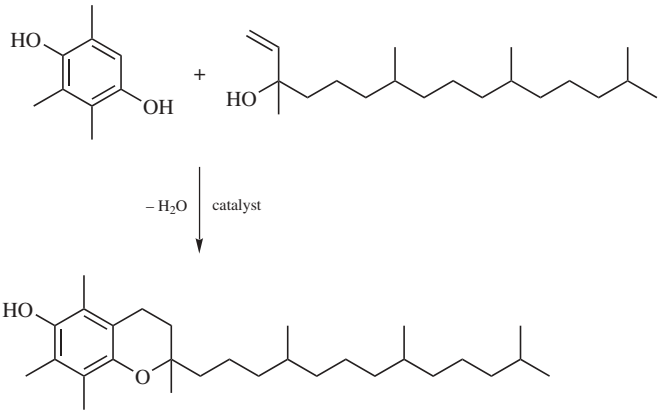
Plant oils and fats are the most important natural sources of vitamin E. Various processes are known for the purification, ie, extraction of fatty acids by esterification and distillation, crystallization of sterols, and concentration by adsorption on basic ion exchangers (52–59).

Because α-tocopherol is in a relatively low proportion in mixed tocopherols, additional methyl groups are introduced to replace unsubstituted positions. Several methods are known for this procedure, eg, hydroxymethylation with formaldehyde (60–62), chloromethylation with formaldehyde and HCl or phosphorus oxychloride (63,64), formylation with zinc cyanide and HCl (65), and Mannich aminomethylation (66,67).

The total synthesis of α-tocopherol is based on the reaction of 2,3,6-trimethyl-hydroquinone and isophytol, phytyl halides, or phytol in the presence of a catalyst, eg, Lewis and Brønsted acids (68–72). The reaction can be carried out in a wide range of solvents, organic solvents like esters or hydrocarbons or supercritical fluids (73–75).

The reaction of isophytol and 2,3,6-trimethylhydroquinone can also be catalyzed by NH-acidic or CH-acidic compounds or in the presence of sulfur containing catalysts, ie, *p*-toluenesulfonic acid, in two-phase solvent systems (76–79). Advantages of these procedures are reduced wastes, high yield and conversion,

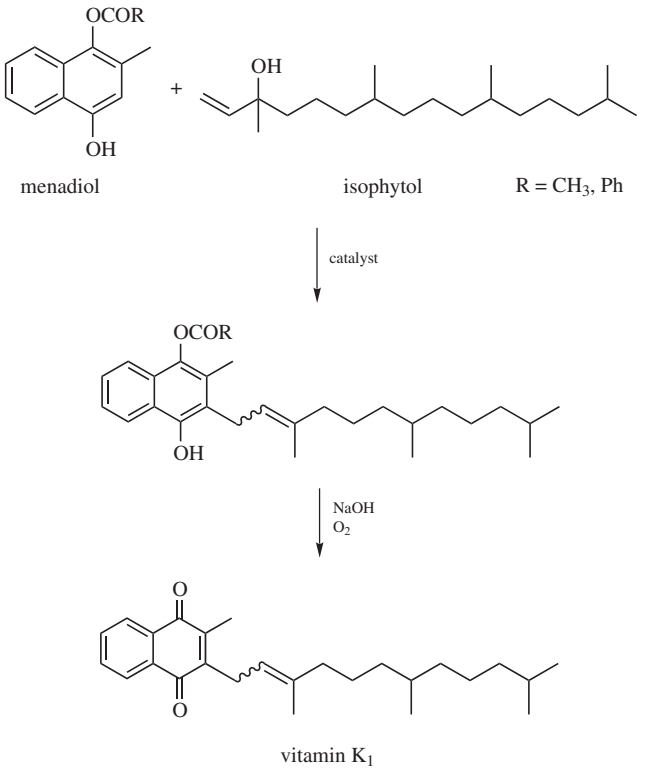
and no corrosion problems. When synthetic isophytol, or phytol, is used, an equal amount of all eight stereoisomers of α-tocopherol is formed. With natural phytol, a mixture of isomers, R,S at C-2 is produced. A practical total chemical synthesis of RRR tocopherol amenable to large scale production has yet to be achieved despite signficant effort.



Vitamin K_1. Vitamin K is the term for 2-methyl-1,4-naphthoquinone and derivatives thereof with the biological activity of phylloquinone. Phylloquinone, 2-methyl-3-phytyl-1,4-naphtoquinone, vitamin K_1, from natural sources has the configuration (2′*E*,7′*E*,11′*R* (80). Vitamin K_2, menaquinone-*n*, is 2-methyl-3-polyprenyl-1,4-naphtoquinone with *n* being the number of prenyl units, which was first isolated from rotting fish meal (and later identified as menaquinone-7) (81). Menadione, vitamin K_3, does not occur in Nature, but can be converted to menadione-4 in organisms (82). Vitamins K are lipophilic compounds, heat resistant ($\leq$373 K), stable in air, but sensitive to alkali and light (83). Vitamin K_1 is localized in chloroplasts of green leafy vegetable (84) and is also found in plant oils, eg, from soybeans, or olives (85). Table 2 shows the content of vitamin K_1 of some fruits and plant oils.Vitamin K_1 has an important role as a cofactor in the γ-carboxylation of glutamic acids (86). Deficiency of vitamin K_1 resulted in the fact that the blood clotting proteins could be produced, but they remain inactive based on the insufficient or lacking γ-carboxylation of the glutamic acid residues (86). This deficiency can result in diseases that interfere with the absorption of vitamin K_1 in the intestine, eg, pancreatic insufficiency.

The commercial synthesis of vitamin K_1 starts from menadione, which is reduced, acylated, partial saponified followed by reaction with isophytol in the presence of a catalyst, eg, boron trifluoride etherate (87). Other methods for

synthesis of vitamin K_1 are reviewed in Ref. (88).



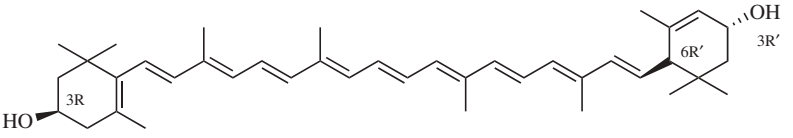
Another approach for a potentially economic synthesis of vitamin K_1 or K_2 is based on the Diels-Alder reaction of menadione, or naphtoquinone, and cyclopentadiene, treatment with strong base and alkylation with phytyl bromide, followed by a retro-Diels-Alder reaction (89,90).

In the last decade, it has become evident that vitamin K has a significant role to play in human health that is beyond its well-established function in blood clotting. There is a consistent line of evidence in human epidemiological as well as in human intervention studies, which clearly demonstrates that vitamin K can improve bone health. The human intervention studies have not only demonstrated that vitamin K can increase bone mineral density in osteoporotic people but have also shown that vitamin K can actually reduce fracture rates. Furthermore, there is evidence in human intervention studies that vitamins K and D, a classic in bone metabolism, may work synergistically on bone density. Most of these studies employed vitamin K_2 at rather high doses, a fact that has been criticized as a shortcoming of these studies. However, there is emerging evidence in human intervention studies that vitamin K_1 at a much lower dose may also benefit bone health, in particular when coadministered with vitamin D. Several mechanisms are suggested by which vitamin K can modulate bone metabolism. Besides the γ-carboxylation of osteocalcin, a protein believed to be involved in bone mineralization, there is increasing evidence that vitamin K may also posi-

tively affect Ca balance, a key mineral in bone metabolism. The Institute of Medicine has recently increased the dietary reference intakes of vitamin K to 90 μg/day for females and to 120 μg/day for males, which is an increase of ∼50% from the previous recommendations.

4.2. Folic Acid. Folic acid, *N*-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid, is *de novo* synthesized in microorganisms and plants (91–93). Folates are all members of the family of compounds based on the *N*-[(6-pteridinyl)methyl]-*p*-aminobenzoic skeleton conjugated with L-glutamic acid units. Different forms of folates occur in Nature, their stability and bioavailibity varies, and in food folates are easily oxidisable. Folic acid, the commercial form, can be reduced enzymatically to 7,8-dihydofolic acid (H2 folate), a key substance in biosynthesis, and (6*S*)-5,6,7,8-tetrahydrofolic acid (H4 folate), the most important intermediate in the formation of other folates. These compounds are essential coenzymes for the C1-unit transfer at various oxidation levels.

4.3. Carotenoids. *Lutein / Zeaxanthin.* Lutein is one of the important and widespread natural carotenoids. It is found in Nature in the (3*R*, 3′*R*, 6′*R*)-configuration (94–99). The total synthesis of lutein in larger amounts has been described but not very cost efficient compared to isolation (100). The following structure is of lutein.

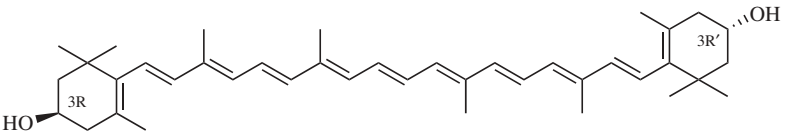


Lutein

Lutein can be extracted from natural sources, eg, from tagetes, or marigold flower petals using various organic solvents (101).

Zeaxanthin. Zeaxanthin (3*R*,3′*R*)-dihydroxy-β,β-carotene) is widespread in Nature, is found, eg, as the yellow coloring in egg yolk and corn (102,103).

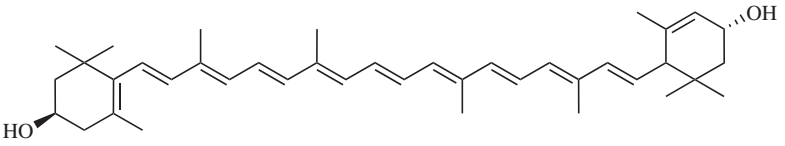
The first total synthesis of (3*R*,3′*R*)-zeaxanthin was reported by Mayer and co-workers (104,105). Various approaches to (3*R*,3′*R*)-zeaxanthin have been published. A technically feasible synthesis of (3*R*,3′*R*)-zeaxanthin based on the Wittig reaction of C_{10}-dialdehyde with a C_{15}-phosphonium salt was described by Widmer and co-workers (106). The syntheses of (*Z*) isomers, the (3*S*,3*S*′)-enantiomer, and (3*R*,3′*S*)-*meso*-zeaxanthin are also published (105). The following structure is of zeaxanthin.



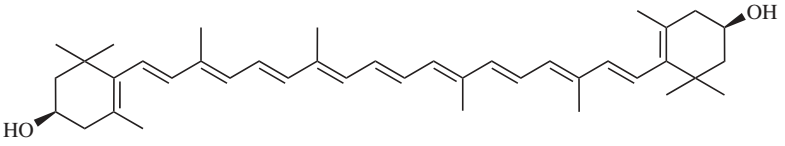
Zeaxanthin

It has been found that zeaxanthin reduces the risk of age-related macular degeneration (AMD) (107).

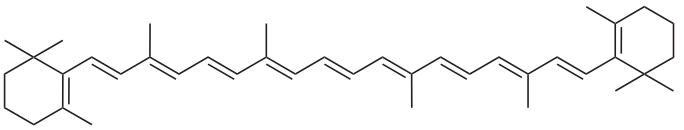
Lutein and zeaxanthin, two nutritional carotenoids that are found in a wide variety of fruits and vegetables (see Table 3), are the main chemical constituents of the macular yellow pigment that forms the yellow spot also called *macula lutea* in the center of the human retina. The macula lutea coincides with the area of highest photoreceptor density in the retina and thus is responsible for an ability to see detailed and high resolution objects. The chemical structures of lutein and zeaxanthin are shown below together with the formulas of other major plasma carotenoids.



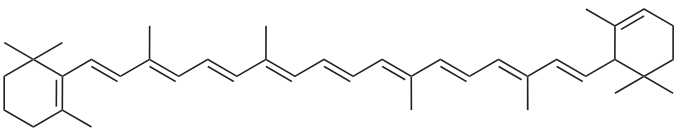
Lutein



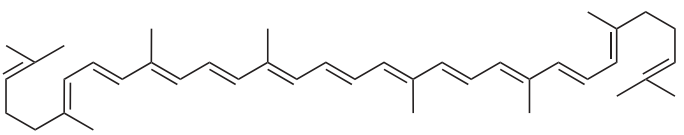
Zeaxanthin



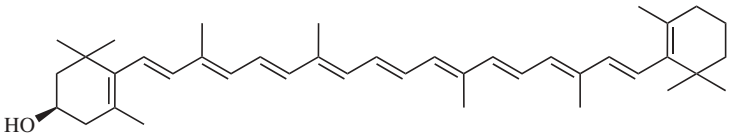
β-Carotin



α-Carotin



Lycopin



β-Cryptoxanthin

Only lutein and zeaxanthin are transported from the plasma into the retina, but not the other carotenoids and neither α-carotene, β-carotene, lycopene, or β-cryptoxanthin, carotenoids found in relatively high concentrations in plasma, can be detected in the retina. In the yellow spot these carotenoids are present at a concentration of up to ∼1 m (108), representing the highest concentration of carotenoids observed in the human body. Mammals are not able to biosynthesize carotenoids, and therefore they are exclusively dependent on their nutritional supply.

The yellow macular pigment is located in the center of the retina, in a circular or oval area also known as the *macula lutea*, which has a diameter of ∼2–3 mm. The distribution of lutein and zeaxanthin across the retina was determined by HPLC analysis of sections of postmortem retinae that were obtained from eye banks (94). Lutein and zeaxanthin occur throughout the entire retina and their highest concentrations are found in the center of the macula. The qualitative composition of the yellow pigment changes with the distance from the retinal centre of the macula. The center contains more zeaxanthin [as an ∼1:1 mixture of (3*R*,3′*R*)- and (3*R*,3′*S*)-zeaxathin] than lutein, and with increasing distance from the central retina the concentration of zeaxanthin decreases faster than that of lutein, making lutein the predominating carotenoid in the peripheral areas.

Histologically, the major part of the macular pigment is located in a retinal layer containing the axons of the photoreceptors (110). As a result, light must first pass through the yellow pigment before it reaches the light-sensitive outer segments of the photoreceptors. The macular pigment is thus an intraocular and prereceptor blue-light filter. Lutein and zeaxanthin, however, are not only present in their pre-receptor position. They are also present in the outer segments of the photoreceptors, although in substantially lower concentrations (111,112). Here, their role is actively to quench reactive oxygen species. Their antioxidant properties, their specific occurrence within the macula at high concentration has led to expectations that the intake of these carotenoids could contribute to reducing the risk for age-related macular degeneration (AMD).

Age-Related Macular Degeneration (AMD). Age-related macular degeneration (AMD) is one of the severest ophthalmologic diseases and the leading cause of irreversible visual loss in the industrialized world. Two types of macular degeneration exist, the “dry” form and the “wet” form. The dry form is characterized by the formation of drusen and progressive atrophy of the retinal pigment epithelium. In the wet form, new and undesirable blood vessels develop in the macula (neovascularisation). The latter form of AMD can impair visual performance drastically and rapidly lead to blindness.

The aetiology of AMD is not yet fully understood. Genetic factors play a part in the pathogenesis of this multifactorial disease, as do environmental risk factors such as smoking and overexposure to light (especially blue light), ie, situations that can trigger radical-induced reactions. In AMD, the integrity of the photoreceptor/retinal pigment epithelium complex is disturbed. In the course of generating light-induced electrical signals, the outer segments of the photoreceptors become increasingly damaged. One reason for this is their high polyunsaturated fatty-acid content (DHA, docosahexaenoic acid as major constituent), which makes them particularly prone to oxidative damage. Particularly in an environment such as that of the retina, characterized by the simultaneous presence of light and oxygen (113), which aids the generation of potentially harmful reactive oxygen species.

The prevalence of AMD increases rapidly from the age of ~65 years. Whereas age-related cataracts, eg, can be removed and replaced with an artificial lens, there is as yet no such remedy for an affected macula. Initial therapeutic approaches involving photodynamic therapy (114) or macula translocation (115) are still in the experimental stage. Thus any prophylactic steps to prevent or delay the onset of AMD are particularly important.

While studies *in vitro* and *in vivo* using animals [for a review see (116)] make it plausible that the ingestion of macular carotenoids could contribute to risk reduction of age-related macular degeneration, clinical trials still have to be conducted to confirm this.

A limited number of epidemiological studies have been conducted in this respect [for an overview see (116)]. Not all studies gave, the same results however. This is not surprising, since on the one hand AMD is a multifunctional disease, and on the other the disease is possibly initiated very early in life, facts that make systematic epidemiological investigation of the disease very difficult.

An interesting relationship between risk factors and the amount of macular yellow pigment was observed. Many such risk factors appear to be linked to a reduction in macular pigment density. Women are more likely to suffer from AMD and women have fewer carotenoids in the yellow spot than men (117). The same applies to people with light-colored (eg, blue) irises, who have lower concentrations of carotenoids in the macula than people with dark irises (117). Smoking is known to be another risk factor for AMD, and smokers have less macular pigment than nonsmokers (117). Two of the most important risk factors for the genesis of AMD are age and advanced AMD in one eye. In this connection, a paper published in 2001 reports a statistically significant loss of macular xanthophyll with increasing age and significantly less macular pigment in the healthy eye of patients with manifest exudative AMD in the other eye (118).

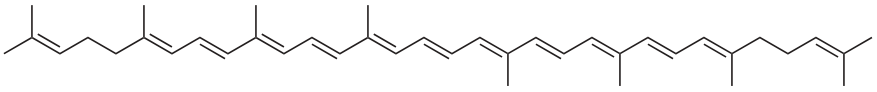
Epidemiological studies cannot be expected to furnish definitive proof of the effectiveness of lutein and zeaxanthin in AMD, however. These studies provide statistical correlations that can indicate the direction to be explored, but do not give any information on the causality of the relationships observed. Only well-structured, double-blind, randomized and placebo-controlled clinical intervention studies will provide final scientific proof of the effectiveness of lutein and zeaxanthin in reducing the risk of AMD (119). While no such proof with regard to lutein and zeaxanthin is yet available, some small-scale intervention trials have been published. In one of such study (120) 20 mg lutein/day were adminis-

tered to 58 patients with retinitis pigmentosa or Usher syndrome for a period of 6 months. Macular pigment density did not increase in some of the patients, and the authors report that in these patients the disease was observed to be more severe. Central visual acuity did not improve in this study, in contrast to two other studies. In one of these other studies, 16 patients with congenital retinopathies were given 40–20-mg lutein/day in tablet form over a period of 26 weeks (121). In the other, 14 AMD patients were treated with ~30 mg lutein/day as spinach over 26 weeks (122). After a few weeks, improvement in central visual acuity, central visual fields, and contrast intensity was observed in both investigations.

The question of whether the amount of carotenoids in the human macula, the ultimate target organ of AMD, can be raised through the diet has been investigated (123). In this study, 11 volunteers were given a diet specifically enriched with lutein (spinach) or zeaxanthin (maize) for 10 weeks. Macular pigment optical density was measured in addition to carotenoid concentrations in the plasma. On completion of the investigation, there was no change in either plasma or macula in one subject, in two others, plasma concentration was higher but not the concentration in the macula. In the other 9 subjects, an increase in the carotenoids in plasma and in the macula was detected. It would therefore appear to be possible to influence the macular pigment through the diet, although individual variation is high.

Macular pigment optical density has only been investigated in a few dietary supplement studies with lutein and/or zeaxanthin. Preliminary investigations with a small number of healthy volunteers have shown that lutein obtained from marigolds taken in a daily dose of 30 mg for a period of 140 days can lead to a 20–40 % increase in macular pigment (124). In contrast, administration of zeaxanthin isolated from flavobacteria—though in a different administrative form—gave rise to ~30% lower increases (125). A more recent study with eight volunteers (126) who were given 10-mg lutein/day for 4 months reports a monthly increase in pigment density of 4–5%.2

Lycopene. The first synthesis of lycopene was reported by Karrer and coworkers (127). A more efficient route to lycopene used the Wittig reaction of a C_{20}-building block (crocetin dialdehyde) with two C_{10}-compounds (geranyl bromide) (128). Following the $C_{15} + C_{10} + C_{15}$ route, lycopene can also be synthesized in an efficient and economic manner (129).



Lycopene (*all-E*)

Various (*E*/*Z*) isomers of lycopene are also described by using stereochemically pure phosphonium salts (130).

For the synthesis of lycopene following the sulfone coupling based on geranyl-amine, sodium chlorophenylsulfonate and ethyl chloroformate followed by treatment with butyl lithium, crocetin dialdehyde, acetic acid anhydride, and a base, see Ref. (131).

Lycopene, a carotenoid found in tomatoes (132), is a singulett oxygen catcher (133). It was demonstrated in a study that showed the consumption of 10 tomatoe meals per week decreased the risk of prostate cancer to ~50 % (134,135).

Dietary carotenoids are thought to provide health benefits by decreasing the risk of chronic diseases, particularly cancer, cardiovascular disease, and age related eye diseases. The carotenoids that have been most studied in this regard are β-carotene, lycopene, lutein, and zeaxanthin.

Lycopene (ψ,ψ-carotene) is the red, acyclic carotenoid highly abundant in the tomato. Besides lycopene, tomatoes also contain the carotenoid precursors phytoene and phytofluene, as well as β-carotene and some other minor carotenoids. In contrast to β-carotene or α-carotene, lycopene cannot be converted to vitamin A. Raw tomatoes and processed tomato products are the richest source of lycopene in western diets, containing between 8–42-μg lycopene/g wet weight depending on strain, season, and type of product (136,137). Other dietary sources are watermelon (23–72 μg/g wet weight), pink guava (~54 μg/g wet weight), pink grapefruit (~33 μg/g wet weight), and papaya (20–53 μg/g wet weight). Lycopene is highly enriched in processed tomato food like tomato paste (up to 1500 μg/g wet weight), juice (50–116 μg/g wet weight), or tomato ketchup (100–130 μg/g wet weight), (137,138). The bioavailability of lycopene from processed tomato food (139,140) and from lycopene formulated as water disperible beadlets is higher than from raw tomato (141). Food processing may improve lycopene bioavailability by breaking down cell walls and tissue matrix, thus making lycopene more accessible. The composition and structure of the food may also have an impact on the bioavailability of lycopene, and thus affect the release of lycopene from the tomato tissue matrix. In most raw foods, all-*trans*-lycopene is the predominant geometrical isomer, whereas processed tomato products also contain 5-cis, 9-cis, and 13-cis, 15- cis isomers at varying amounts (137). In human plasma, the cis isomers can contribute up to 50% to total lycopene (140,142). In tissues including prostate, the ratio of cis isomers is also relatively high (143).

The high antioxidant properties of lycopene are suggested to be linked with its effect on prevention of cancer and chronic diseases, such as cardiovascular disease. Among all carotenoids tested, lycopene, together with beta-carotene, is one of the most efficient singlet oxygen ($^1O_2$) quenchers in organic solutions (144) , and in biological environments such as liposomes (145).

Energy is transferred from $^1O_2$ to the lycopene molecule, converting it to the highly energized triplet state. Lycopene in the triplet state can be easily returned to the ground state by dissipating the energy as heat or by physical quenching. Once returned to the ground state, lycopene can react with another $^1O_2$ molecule.

Lycopene also efficiently quenches $NO_2\bullet$, a damaging radical produced in tobacco smoke (146,147), and peroxynitrite, a powerful oxidant and nitrating agent (148). Loss of protein function due to nitro-tyrosine formation has been suggested to be involved in the pathogeneses of a variety of diseases.

As a strong antioxidant, lycopene might provide protection against oxidative DNA damage suggested to be an early event in carcinogenesis. Subjects ingesting tomato juice or puree for 2 or 3 weeks increased their lycopene concentrations in plasma and blood lymphocytes (149) , and lymphocyte DNA was significantly resistant against oxidative damage by hydrogenperoxyde (149,150).

Another study demonstrated lower levels of 8-hydroxy-2′-deoxyguanosine, a marker for oxidative DNA damage, in blood lymphocytes and prostate tissue of prostate cancer patients after daily consumption of tomato sauce for 3 weeks (151). Altogether, these studies demonstrate that diets rich in lycopene can indeed reduce genetic damage in humans.

The potential beneficial effects of lycopene in human health have been reviewed extensively (154–158). Numerous observational studies have consistently shown an inverse relationship between the consumption of lycopene-rich diets, ie, tomato or tomato-based food, or lycopene plasma levels, with the risk for cancers at various sites (157,158). The strongest inverse relationship was shown for prostate cancer (157–163), one of the most prevalent cancers in western populations. Other significant inverse relations were seen for lung and stomach cancer. The "Health Professionals Follow Up" study, one of the largest cohort studies in males in the United States, followed the development of prostate cancer over 12 years and included the evaluation of three dietary questionnaires (163). Lycopene consumption was significantly associated with a decreased risk for prostate cancer by 16% (p for trend was 0.003). Interestingly, the consumption of tomato sauce, the primary source of bioavailable lycopene, was associated with a even greater reduction in prostate cancer risk. Those individuals who consumed more than two servings per week had a significant reduction of risk by 23% as compared to those having less than one serving per month. In a nested case-control study (164) embedded in the Physicians′ Health Study (a randomized placebo-controlled trial of aspirin and beta-carotene) plasma carotenoid levels of 578 men who developed prostate cancer within 13 years of follow-up were evaluated and compared to 1294 age- and smoking status-matched controls. Lycopene was the only antioxidant found at significantly lower mean levels in cancer cases than in matched controls. Individuals in the highest quintile of lycopene plasma concentrations had 25% lower prostate cancer risk than those with the lowest levels. Interestingly, for aggressive prostate cancers, the inverse association was even stronger, ie, 44% reduction of risk, comparing the highest quintile for lycopene plasma concentration with the lowest. A similar inverse association between higher lycopene plasma levels and a lower risk for aggressive prostate cancer was recently confirmed for both White and African Americans (161).

So far, no larger human intervention trials addressing the effect of lycopene in prostate cancer prevention have been published. A small trial in prostate cancer patients showed some preliminary evidence of less cancer growth and a decrease in certain biomarkers, such as PSA, after intervention with a tomato extract containing 15-mg lycopene twice daily (165). However, no conclusions can be drawn at this time since the sample size was too small. The finding that tomato intake can reduce the amount of oxidized nucleotides and thus prevent DNA mutations [see (151)] might also indicate a cancer preventive function. This finding is further supported by a number of *in vitro* studies showing the antiproliferative effects of lycopene in various cancer cell lines (166,167), including prostate cancer cell lines (168,169). As mechanisms suggested to be involved in inhibition of cancer cell growth by lycopene are the regulation of cell cycle, induction of apoptosis, interference with signal transduction pathways of cytokines, hormones and growth factors, and the increase in cell communication

(167,170–172). It has been hypothesized that lycopene may also exert its function via formation of an oxidation product, acyclo-retinoic acid. Acyclo-retinoic acid, a weak ligand for retinoic acid receptor β, inhibits cancer cell growth and induces apoptosis in prostate cancer cells (171,173,174). However, the compound has not been detected in human or animal tissues so far.

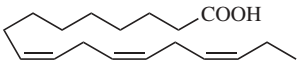
Recent evidence also indicates an association between tomato food consumption and protection against cardiovascular disease. Coronary heart disease (CHD) has a high prevalence in western countries, and is increasing in Asian countries due to changes in socioeconomic conditions, lifestyle, and diet. The link between low density lipoprotein oxidation and atherosclerosis is hypothesized to be the basis for a beneficial effect of antioxidants on the incidence of subclinical and clinical CHD.

Two prospective studies from Finland demonstrate that low levels of blood lycopene resulting from a diet low in tomatoes significantly increases the risk for heart attack and stroke, and early atherosclerosis among middle aged men (175,176). Similarly, a lower risk for aortic calification was observed in current and former smokers with higher lycopene plasma concentrations in a study in the Netherlands (177). A European multicenter case control study (EURAMIC) suggests a protective effect of lycopene for myocardial infarction (178).

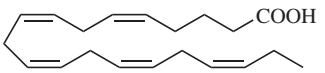
Although the observational nature of the epidemiological studies relating tomato intake with a lowered risk for cancer and CHD, offer no final proof that lycopene is the responsible compound for the preventive effects, mechanistic considerations, animal, and *in vitro* studies are strongly supportive. In conclusion, a large body of evidence suggests a protective effect of lycopene rich diets against cancer and other chronic diseases.

4.4. Polyunsaturated Fatty Acids. Polyunsaturated fatty acids (PUFA) are of interest because of their beneficial physiological activities. In fish oils, > 60 different fatty acids are found (179).

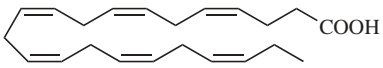
The nomenclature and structure of the main PUFAs based on a shorthand numbering from either the methyl end (*n* or ω system) or the carboxyl end (Δ system). The following structure is of polyunsaturated fatty acids.



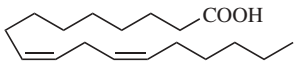
18:3ω3; 18:3n3; 18Δ9,12,15
α-linolenic acid, ALA



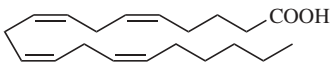
20:5ω3; 20:5n3; 20Δ5,8,11,14,17
eicosapentaenoic acid, EPA



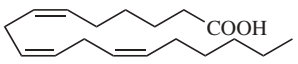
22:6ω3; 22:6n3, 22Δ4,7,10,13,16,19
decosahexanoic acid, DHA



18:2ω6; 18:2n6; 18Δ9,12
linolenic acid, LA



20:4ω6; 20:4n6; 20Δ5,8,11,14
arachidonic acid, AA

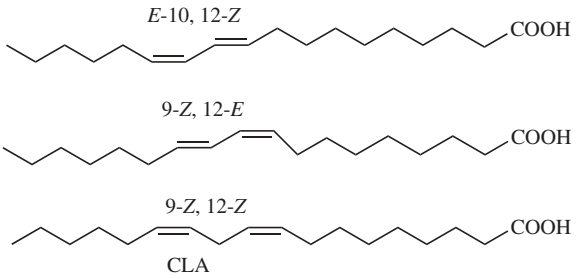


18:3ω6; 18:3n6; 18Δ6,9,12
γ-linolenic acid, GLA

ARA. Arachidonic acid (AA), (*all-Z*)-5,8,11,14-eicosatetraenoic acid, in an essential fatty acid and a precursor in the biosynthesis of prostaglandin, thromboxanes, and leukotriens. AA occurs in liver, brain, glandular organs, and was isolated from liver lipids (180), and beef (181). Several chemical total syntheses are known (182–184). For a commercial technical process AA (185).

DHA/EPA. Docosahexaenoic acid (DHA) is an omega-3 PUFA. Fish oils are rich on PUFA, especially eicosopentanoic acid (EPA, 20:5*n*-3) and docosahexanoic acid (DHA, 22:6, *n*-3). The content of EPA varies from 5 to 26%, and of DHA from 6–26% of total fatty acid (186). PUFA and their ethyl esters are soluble in sc-CO_2, and therefore it is possible to concentrate and separate them using this nonflammable and nontoxic eluent (187,188). Separation techniques based on urea precipitation or handling with silver ions are less favored because of waste and residue problems. Distillation requires high temperature with risk of thermal damage of the (*Z*)-configured double bonds. The separation of C_{18} and C_{20} compounds using separation by chain length at 373 K and 14.5 MPa is described in Ref. (189).

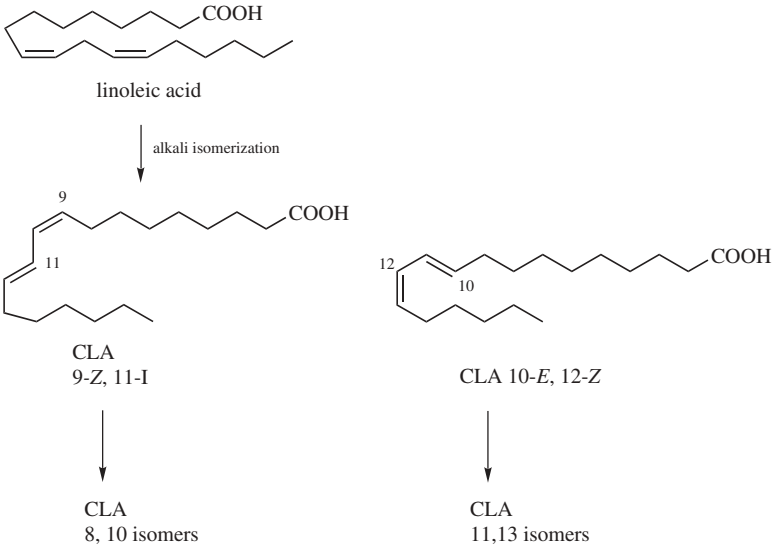
CLA. Conjugated linoleic acid (CLA) was identified in 1987 as a potential mutagen inhibitor (190). CLA is a term describing several isomers of linoleic acid (LL) and describes the position of double bonds located on carbons 9 and 12 (main isomers).



The amount of CLA varies. The 9-cis–11-trans isomer is the predominant form and responsible for the anticarcinogenic effect (191).

CLA can be prepared by isomerization of LA using tris(triphenylphosphine) chloro-rhodium, and arene chromium carbonyl complexes in homogeneous phase (192,193). A heterogeneous system for the isomerization of LA used Ni/H-MCM-41 as catalyst with the advantage of an easy separation of the catalyst (194).

The manufacturing of CLA starts from linolenic acid. The natural source must be rich in linoleic acid. The highest level of LL from botanical sources is found in oils of sunflower (64%), safflower (75%), and soybean (51%) (195). Alkaline isomerization of linoleic acid at 450 K and 4–6-h reaction time in water or glycol yields a mixture of positional isomers (196,197). Starting from the triglyceride, CLA could be obtained by isomerization using sodium hydroxide (198).



Other methods for preparation of CLA are the microbial production using cultures of *Lactobacillus sp* (199), the biotransformation using isolated linoleate isomerase enzymes (Lactobacillus reuteri, Propionobacterium acnes) (200), or the dehydration of ricinoleic acid (201).

Health Effects of PUFA. There are two main classes of PUFA, the omega-6 (*n*-6) and the omega-3 (*n*-3) series that are formed from the two essential fatty

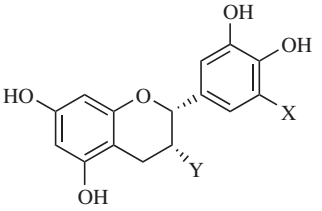
acids linoleic and α-linolenic acid, respectively. These fatty acids have a structural role in cell membranes, where they regulate fluidity and affect the function of membrane related proteins (receptors, ion channels.). In addition, PUFA are the precursors of eicosanoids, which have a variety of important biological activities and play a role in a number of diseases. The most relevant PUFA in cell membranes are arachidonic acid (AA, 20:4*n*-6) and docosahexaenoic acid (DHA, 22:6*n*-3), which are important structural and functional components of membranes especially in the central nervous system. Both DHA and AA make up a third of all lipids in the brain's gray matter. These fatty acids must be supplied in sufficient amounts during during fetal and infant brain growth. A deficit of omega-3 PUFAs during the perinatal brain growth or the retinal development can lead to disorders of the central nervous system and to impairment of vision, which may be irreversible. Many studies have reported that groups of infants who are breast-fed perform better on tests of neurodevelopment than bottle-fed infants. DHA and AA are present in human milk but not in standard infant formulas. The current evidence suggests that infants should receive a certain amount of preformed PUFA especially DHA to assure an adequate supply of this fatty acid, particularly the prematurely born infants (202).

The human diet provided appreciable amounts of linoleic acid and preformed arachidonic acid. It has been suggested that the typical "western" diet, which is high in omega-6 PUFA and low in omega-3 PUFA, may not supply the appropriate balance of PUFA for proper biological function (203). Numerous studies performed during the last two decades suggest that replacing dietary omega-6 PUFA with omega-3 PUFA to some extent is beneficial in reducing certain risks of cardiovascular disease. Epidemiological studies indicate that 2–3 portions of fatty fish per week (corresponding to 200–300-mg EPA + DHA/day) are associated with a low incidence of CVD mortality. Eicosapentaenoic acid (EPA) and DHA are considered to be the active compounds of fish oil. The cardioprotective effects of omega-3 PUFA seems to be mainly due to a combination of effects on the following risk parameters of cardiovascular health: lowering of blood triglycerides, prevention of irregular heart beat (antiarrhythmia), lowering of blood pressure, reduction of platelet aggregability (204). The triglyceride lowering effect of omega-3 PUFA is one of the most consistent finding. This effect is dose dependent and can be achieved by diet. The least amount of omega-3 PUFA to lower significantly serum triglycerides appears to be ~1 g/day as provided by a fish diet. The hypotriglyceridemic action of omega-3 PUFA in humans is believed to be primarily due to a reduction in hepatic triglyceride synthesis. Moreover, secondary prevention trials have found that omega-3 PUFA supplementation significantly reduced coronary death in postmyocardial patients. It seems probable that this reduction is due to the antiarrhythmic effects of omega-3 PUFA. Omega-3 PUFA can prevent ischemia-induced fatal ventricular arrhythmias in experimental animals. In vitro studies showed that omega-3 PUFA alter the electrophysiological properties of cardiac myocytes thereby conferring protection against arrhythmia (205). Finally, the beneficial effects of omega-3 on CVD and other chronic diseases such as antiinflammatory diseases effects may be related to the inhibition of the synthesis of pro-inflammatory prostaglandins and leukotrienes derived from arachidonic acid. Furthermore, eicosanoids derived from omega-3 possess a different potency with respect to various cellular

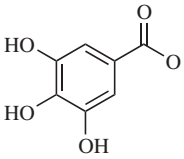
responses. Thus, modifying the relative amounts of omega-3 and omega-6 PUFA can modulate the amounts and types of eicosanoids synthesized in tissues. In humans, dietary omega-3 PUFA decreased the production of leukotriene B_4 (LTB_4), interleukin-1, tumor necrosis factor, and adhesion molecules in stimulated monocytes and neutrophils that are involved in the development of inflammation, immune reactions, and other biological processes. Moreover, evidence from laboratory animal models and clinical studies suggest that dietary omega-3 PUFA may have a beneficial effect in diseases with an inflammatory component such as rheumatoid arthritis and psoriasis (206). In addition to the well-documented effects of omega-3 PUFA on CVD, inflammatory diseases, and brain development preliminary data suggest a benefit in obesity and insulin resistance (207). Thus, increasing omega-3 PUFA in our diets, especially EPA and DHA from marine foods, may decrease the prevalence of chronic disease.

4.5. Polyphenols. In numerous studies, polyphenols have been found to have beneficial health effects. Notable are the catechins of green tea and resveratrol, a polyphenol found in grapes.

EGCG. (−)-Epigallocatechin gallate is one of the complex mixture of polyphenols found in green tea (*Camelia sinensis*) (208,209). Other catechins found in *Camelia sinensis*, eg, are epicatechin (EC), and epicatechin gallate (ECG), and epigallocatechin (EGC).



X = H, Y = OH, epicathechin
X = H, Y = $C_7H_5O_5$, epicatechin gallate
X =OH, Y = OH, epigallocatechin
X = OH, Y = $C_7H_5O_5$, epigallocatechin gallate



= $C_7H_5O_5$

Chromatographic methods are known for the isolation of green tea catechins (208,210,211). By the use of these methods, only small amounts of materials have so far been isolated in pure form. Normally, a mixture of catechins is isolated.

For large-scale production of EGCG, processes of separation and concentration are claimed (212,213). For example, EGCG can be obtained in realtively pure form by subjecting green tea extract to chromatography on a macroporous resins.

The isolation of EGCG with other polyphenols using high pressure carbon dioxide from green tea is described in Ref. (214).

EGCG is found abundantly in green tea where it quantitatively represents the most prevalent catechin. The water extractable EGCG content per gram of green tea ranges between 25 and 75 mg depending on the kind of tea, harvesting season, and extraction conditions. In Japan, the average consumption of green

tea was determined to be ~2 g/day and capita corresponding to an average daily EGCG intake of 50–150 mg/person.

In humans, EGCG is rapidly absorbed and reaches maximum concentration in plasma after ~2 h. The half-life of elimination from plasma is around 5 h. From animal studies, it is known that the absorbed compound is predominantly excreted into the bile, eventually becoming available for reabsorption. Only a smaller fraction seems to be systemically distributed. EGCG can be degraded extensively by microorganisms in the gut. Therefore, only small amounts are excreted in the feces unchanged. EGCG is widely distributed in the body, and the highest concentrations are found in the liver and the mucosa of the gastrointestinal tract.

In Asian countries, green tea is well known for its health benefits and is traditionally used for detoxification, improvement of blood flow, and overall improvement of resistance to disease for several hundred years. More recent scientific data demonstrate that the catechin fraction of green tea plays an important role in the prevention of a wide array of diseases including cardiovascular conditions, cancer, and gastrointestinal disorders as well as periodontal disease and dental cavities.

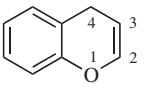
The pharmacological activity of EGCG has been further investigated in preclinical studies using both *in vitro* and animal test systems. Results demonstrate that EGCG exerts a broad range of activities that include antioxidant, antiinflammatory, antiangiogenic, antiartherogenic, antithrombotic, and antiinfectious properties. These activities are agreed upon to play an important role in fending off disease and support epidemiological data demonstrating longevity of green tea drinkers that have been found to have a lower risk for cardiovascular death and cancer.

Relying on short-term human intervention data obtained with either green tea or green tea extract high in EGCG content as well as on epidemiological data for most of the preventive activities an effective dose of 100–300-mg EGCG/person and day can be considered relevant.

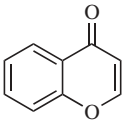
Based on presently available scientific evidence, EGCG is considered a major functional component of green tea that deserves further investigations into its disease preventive properties.

Genistein. Flavonoids and isoflavonoids are natural antioxidants and are suggested as agents responsible in the diet for the prevention of coronary diseases (215,216), breast cancer (217), and prostate cancer (218). The antioxidant behavior of flavonoids and isoflavonoids is well documented in the literature (219,223).

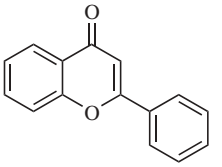
Chemically, flavonoids and isoflavonoids are derivatives of 4H-chromene. Most important are the 4-oxo-chromens, such as flavones and 3-hydroxyflavones. Another important flavone is the plant dye quercetin, 3,5,7,3′,4′-penthydroxy-flavone, one of the most important and widely disitributed of the flavonoid family. The following show structures of flavone, 4H-chromene, chromone, 3-hydroxyflavone, and quercetin.



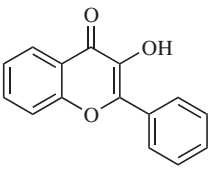
4H-Chromene



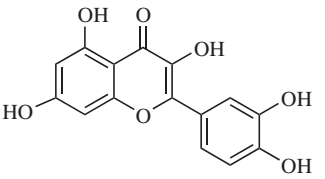
Chromone



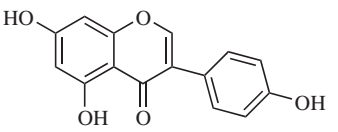
Flavone



3-hydroxyflavone



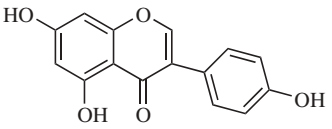
quercetin



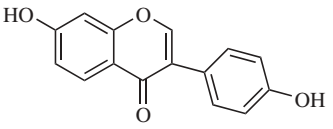
campherol

The isoflavones have the substituent in the 3-position.

Isoflavones, like genistein can be isolated from natural sources, eg, soy bean, where they occur predominately in the glycosylated form. Alternatively, they can be synthesized from phenolic precursors.



genestein

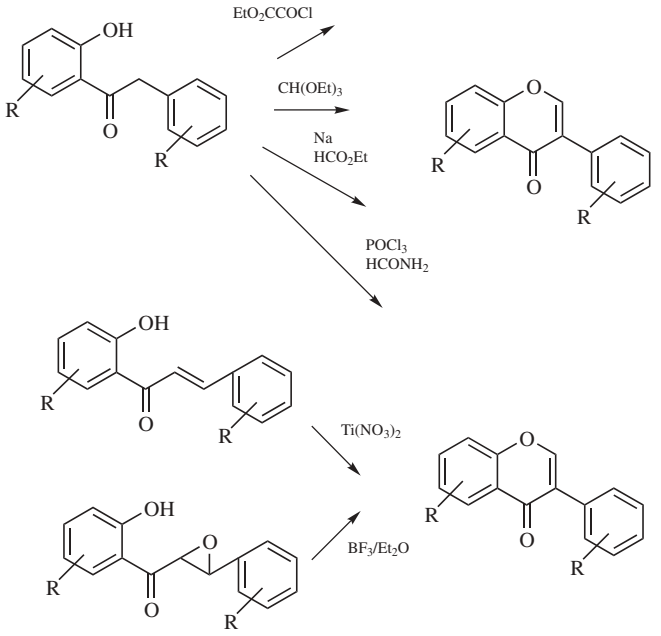


daidzein

Genistein has been isolated from soybeans (224), soybean meal (225), and *Genista tinctoria* (226).

Soy product manufacturers have developed various methods to retain isoflavones in their products (227–230). The extraction of soybeans, or soybean fractions, yields mixtures of isoflavones, which can be further concentrated and purified for use as food or nutritional products (231–239). For the isolation of genistein, direct the extractions of soybeans and further concentration have been described. Its recovery from soy molasses, which is an abundant and inexpensive vast stream of soy protein manufacture (240), is preferred. The following

synthesis shows the synthesis of isoflavones.



Genistein belongs to the group of plant derived molecules with estrogen-like activity. In the human diet, genistein is predominantly found in soy products that are regularly consumed in Asian countries like Japan, Korea, and China. The genistein content in tofu, tempeh, and soy flour was determined to be 14, 25, and 71 mg, respectively (aglycone + glycosides). The content, however, may significantly vary depending on the strain of soy beans and the food processing technique.

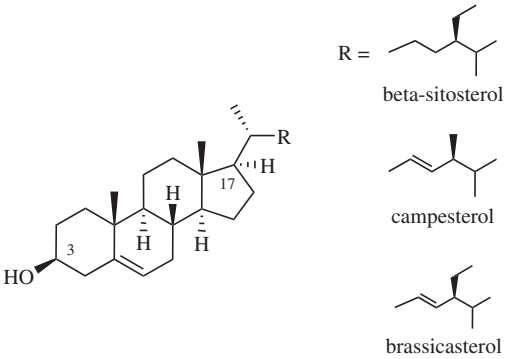
In Japan, the average dietary genistein intake has been determined to be in the range of 8–12 mg/day and capita. Genistein absorption varies considerably among individuals. This is attributed in part to differences in the microbial population in the intestine because naturally genistein predominantly occurs as glycoside that must be cleaved before absorption can take place. In Japanese people consuming a traditional diet high in soy products, an average plasma concentration of 250 nM of Genistein has been measured.

In recent years, genistein has received much attention due to its potentially preventive role against chronic disease. Based on epidemiological data suggesting an inverse correlation between soy consumption and disease risk, soy-mixed isoflavones and its main constituent genistein have been further investigated in laboratory experiments. Results indicate that genistein exerts estrogenic and antiestrogenic effects as well as effects related to cell signaling, cell growth, and death. Based on these findings, Genistein has been associated with reduced incidence of breast and prostate cancer, cardiovascular disease, and osteoporosis. Mechanistically, Genistein may exert described benefits not only by its estrogenic properties but also as regulator of gene transcription and modulator of enzyme activities.

Results of first clinical trials targeted at identifying the disease preventive properties of the soy isoflavone fraction in humans look encouring. However, further research is needed to verify the benefits of pure genistein and to identify population groups that might profit the most of it.

For further information see Ref. (241,246).

4.6. Phytosterols. Phytosterols are derived from isoprenoid biogenesis and are ubiquitous in higher plants. Usually they are C_{27-29} substances with a 3-hydroxyl-steran core with C_{8-10} alkyl and alkenyl side chains at C-17. Various additional substitution patterns exist, as exemplified by cycloartenol, gramisterol, and sitosterol. In addition to the free sterols, they also commonly occur in various derivitized forms, such as fatty 3-carboxlate esters, 3-D-glucosides, and 3-D-glucoside-6-carboxylates. Most common in the higher plants and in the human and animal food chain are phytosterols such as brassicasterol, stigmasterol, and beta-sitosterol. The following scheme shows the structure of sterols.



Phytosterols occur mostly in intracellular organelles, where they appear to play important roles in the stabilization of membranes. They have been found in nearly all plant tissues and organs, including leaves, stems, roots, blossoms, fruits, and seeds. Crude vegetable oils contain 0.5–1.5% sterols. Levels in plant-derived food oils can be significant, such as in soybean oil, which typically contains 0.2% sitosterols. Daily intake of phytosterols in a normal western human diet amounts to ~100–400 mg, higher for vegetarian diets. However < 5% of this is absorbed by the body.

Phytosterols, and especially more highly oxygenated phytosterols in general, show biochemical effects, some suggested to be beneficial biomedical effects. For example, the common dietrary phytosterols have been suggested to correlate with reduced risk of cancer and gallstones, strenthening of the immune system, and to promote cholesterol reduction (247). The common dietary phytosterols are most widely established and known for their effects on serum cholesterol. These phytosterols have been shown to competitively inhibit the normally occurring adsorption of endogenous and dietary cholesterol from the intestines. Thus they can lead to lowering of serum cholesterol levels. To obtain significant effects, the daily intake levels of phytosterols must be relatively larger than those of a normal diet, in the range of 1–10 g/day, depending on the form ingested.

Free phytosterols are relatively insoluble in oils and fats, typically only a few percent, whereas the their fatty esters can be soluble to 30% or more. It is generally believed that phytosterols must be in dissolvable or dispersable form for maximum effect and lower dosages. Accordingly, there have been patented in various forms, such as micronized or microcrystalline powders (248), physosterols with dispersants (249), emulsions, microemulsions, and other formulations (250) , fatty esters (251) , and specialized liquid blends of phytosterols, phytostanols, and their esters (252). These are variously claimed to increase solubility or dispersibility in the intestines leading to the ability to use lower amounts to achieve lowering of serum cholesterol levels, as well as increasing the possibilities for inclusion in foods forms. The esters have been shown to be enzymatically cleaved to free phytosterols in the digestive track. Some of these products have been marketed in the United States and Europe as nutraceutical products in the forms of powders, tablets or capsules, and oils, as well as included in foods such as margarines.

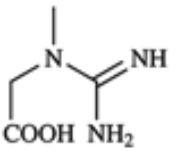
Commercial sources of phytosterols are mostly as by-products of the food oil industry, where very large quantities of edible oils are processed for use as cooking oil. Typically, the crude pressed or solvent extracted oils contain impurities, such as pigments, phosphatides, odorous substances, and certain trace metals that must be removed by further processing, eg, degumming and dewaxing, filtration over solid adsorbants, and vacuum deodorization, so as to achieve useable physical forms, acceptable organoleptic properties, and stability demanded for storage and uses. A common point of food oil processing where phytosterols are removed as process by-products is vacuum deodorization of the final product, which is normally done by steam sparging with the assistance of modest vacuum. Free phytosterols and other relatively more volatile components such as tocopherols are removed from the oil and are found in the distillate by-products. The phytosterols can be recovered by normal chemical processing technologies, such as crystallization. Esterification with fatty acids by well-known means in the industry, such as interesterificiation, can be used for preparation of the esters (253). Enzymatic methods have also been reported (254).

Another source of phytosterols is from the paper industry. In the processing of pine wood by kraft pulping, crude tall oils (mixtures of resin acids, fatty acids, and neutral oils) are also produced in very large amounts. Commercially, useful fatty acid blends and rosin are produced from them by fractional distillation and phytosterols and physterol esters can be recovered as by-products. Hydrolysis or transesterification steps are necessary to liberate the free phytosterols in order to achieve higher purities (255).

4.7. Others. *Creatine.* Creatine is a substance that occurrs in the human body. The total amount of creatine in a normal healthy person is ~120-g (70-kg) person (256–258). Phosphocreatine, the phosphorylated creatine and creatine, are important for cellular energy storage, transport, and buffering. In the human organism, creatine is formed from the amino acids argenin, glycin, and methionin.

The influence of oral creatine monohydrate administration on skeletal muscles is based on the hypothesis that increasing muscle creatine concentration may increase the availability of phosphocreatine and allow for an accelerated rate of adenosine triphosphate (ATP) synthesis (257).

Creatine pyruvate and its salts have several physiological property for treatment of obesity and overweight, and can be used to prevent free radicals and to enhance long-term performance (260–263). The creatine pyruvates can be formed to have a long shelf life by reaction of pyruvic acid and creatine (264).

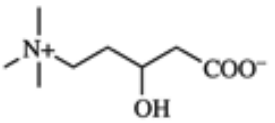


creatine
(N-(aminoiminomethyl)-N-methylglycine

Creatine monohydrate is produced from methyl isourea or cyanamide and sodium- or potassium sarkosine (265). The reaction can be carried out under addition of carbon dioxide (266).

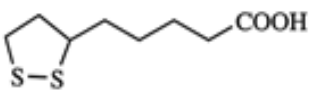
Carnitine. For therapeutical application, eg, disorder of cerebral metabolism, peripheral neuropathies, peripheral arteriopathies, *R*-(−)-carnitine, and 3-carboxy-2-hydroxypropyl trimethylammonium hydroxide, are the physiological active enatiomeric forms. Therefore, the (*R,S*) racemic mixture obtained in the production of carnitine, is resolved with D-camphoric acid (267).

Asymmeteric synthesis by stereospecific hydration of carnitine precursors using microorganisms is known (268–272). A chemical process for the synthesis of *R*-(−)-carnitine that does not start from a carnitine precursor is described in Ref. (273).



carnitine
(3-hydroxy-4-(trimethylammino)butanoate)

(R)-α-Lipoic Acid. Lipoic acid is a growth factor (274). (*R*)-(α)-Lipoic acid is the biologically active form and racemic lipoic acid is used as an antidote for liver deseases and poisoning. The synthesis of (*R*)-α-lipoic acid from chiral precursors, eg, (3*S*)-3-hydroxy octancarbonic acid esters, is claimed in the literature (275).

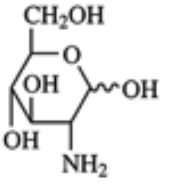


lipoic acid

L-Lipoic acid serves as a coenzyme in the oxidative decarboxylation of ketoacids and can be found in every cell of vegetable and animal organisms. The applica-

tion of a lipoic acid and its derivates in combination with vitamins is described in a patent application (275).

Glucosamin. Glucosamin from exogeneous sources, eg, food, is incorporated into the metabolic pathway of glycosaminglycan synthesis (276). Studies have shown that glucosamin increased the production of proteoglycans and sulfate uptake by articular cartilage (276,277). Glucosamin is produced by hydrolysis of seafood waste.(278).



glucoseamine
(2-amino-2-deoxyglucose)

The chemistry of glucosamine has been reviewed by Foster and Stacey (279). Glucosamine is unstable as free base but crystalline salts are easily made and handled. Most commercial glucosamine is presently sold as the hydrochloride or sulfate.

Glucosamine can be chemically synthesized by the reaction of fructose with liquid ammonia followed by treatment with hydrochloric acid and crystallization. Yields are low, < 25% (280).

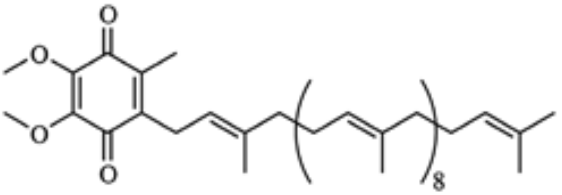
Glucosamine is the basic monomeric unit of the biopolymer chitin (from *chiton*, "coat of mail"), which forms the protective coating for invertebrate animals and is a component of the cell walls of some lower plants. Chitin is the linear polymer of beta-(1,4)-anhydro-*N*-acetyl-D-glucosamine. It comprises typically 15–30% on a dry weight basis of crustacean shells, the rest being primarily calcium carbonate and smaller amounts of protein and pigments.

The availability of hundreds of thousands of tons of crustacean processing wastes from the food industry, typically shells and heads, makes them the preferred source of glucosamine. Hydrolysis of chitin under vigorous conditions with mineral acids gives good yields of relatively pure glucosamine salts.

Processing typically involves the unit operations of treating crustacean wastes with hot aqueous base such as sodium carbonate to hydrolyze proteins, washing to remove the hydrolyzate, dissolution of the calcium carbonate with cold dilute hydrochloric acid, and washing to remove salts to yield a purified chitin. Hydrolysis of chitin with hot concentrated hydrochloric acid, charcoal treatment, and vacuum concentration gives the crystalline hydrochloride, which is further purified by recrystallization in approximate yields of from 60 to 70% (281). Ion exchange may be used to prepare other salts from the hydrochloride (282).

Recently, methods have been patented for production of glucosamine by fermentation with genetically modified organisms (283).

Coenzyme Q10. Ubiquinone is a generic term for a family of quinones (2,3-dimethoxy-5-methyl-6-polyprenyl-benzoquinone) in which the number of prenyl groups varies from 1 to 10 (or more) (284). Of special interest is ubiquinone 10, coenzyme Q_{10}, which has significant synthetic activity and is used as a cardiovascular agent.



coenzyme Q10

Industrial processes for coenzyme Q_{10} are based on biotechnical or synthetic methods (285,286). The ubiquinones can be synthesized by the condensation of 2,3-dimethoxy-5-methylhydroquinone with isopronoic alcohol and oxidation of the intermediate (284). Other approaches of ubiquinone syntheses are based on PD-catalyzed oligomerization of monoterpenes monomers (286) or Pd-catalyzed homoallyl- and homopropargyl-alkenyl coupling combined with Zr-catalyzed carboalumination (287), fermentation (285). In addition, the synthesis of ubiquinones utilizing Stille-coupling (288) or Ni(0)-catalyzed cross-coupling of an alkine and quinone is also described (289). The CoQ_{10} production by fermentation described in papers and patents reviewed stated that the highest accumulated amount is 57.8 mg/L. By mutation and monocloncal accumulation, the amount reached 50 times or more that of the original level(285). The advantages of CoQ_{10} production by using a fermentation process are that there are no raw material constraints and no by-product problems (stereoisomers of the isoprenoid side chain).

For isolation of CoQ_{10}, it is necessary to saponify the whole cells and extract the lipid or to extract it directly from dry cells followed by removing saponifiable lipid by saponification.

5. Market

The nutraceutical concept has brought a paradigm shift to the food market. Since the 1960s, essential factors like vitamins have been added to the diet. Increasingly, other beneficial subtances, such as nutraceuticals, are being sought as additives to provide additional value to food. Currently, in the food market in general, growth is between 3 and 4%. Functional foods and dietary supplements, however, are expected to grow in the immediate future with double-digit figures, reflecting the increasing health consciousness of consumers, and especially changes in the lifestyle of younger people. A better understanding of the effects of nutrients on the human body is considered to be a driving force of this emerging market. Scientists consider a significant part of chronic diseases to be associated with diet. This offers a great opportunity as well as a significant challenge

to the different groups involved in this field, such as consumers, scientists, regulatory agencies, as well as industrial suppliers.

6. Future Trends

New fields of research are being developed and may be expected to continue with increasing vigor. Molecular nutrition, molecular biology, and diagnostic tools will come together in the identification of food factors and nutrition status. It is conceivable that the status of a nutrient or constituent of a plant/organism with a recognized health benefit will be assessed in humans as appropriate and convenient diagnostic measures become available. This will provide the consumer with additional information about preventive health and enable better decisions about supplementing inividual diets. For example, phytosterols in margarine is one of the recently marketed functional foods (see the section Past and Current Trends in Nutrition). An easy to use cholesterol test, very much like the blood glucose test that is currently being used in diabetics, could allow the consumer to monitor the effect of a given product on his/her cholesterol levels. As another example, functional foods that provide folic acid will lower homocysteine serum levels, a recognized risk factor of cardiovascular disease. A test kit, which will assess homocystein levels, could help to make better choices among different products. Diagnostic tests as part of the functional food market will require that such tests are easy to perform, are precise, and are at a reasonable cost. When achieved, this would add another challenge as well as an opportunity to the functional food arena.

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Table 1. **Examples of Bioactive Nutritions with Possible Health Benefit**

Compound	Source	Beneficial effect
folic acid	vegetables	spina bifida
PUFA	fisch oil	cardiovascular disease
luteine	spinach	visual impairment
lycopene	tomatos	prostata
zeaxanthine	maize	visual impairment

Table 2. **Content of Vitamin K_1 in μg/100 g**

avocados	20	soybeans	193
apples	5	rapeseed	141
peaches	5	olive	55.5
bananas	1	Sesame	15.5
oranges	1	Walnut	15
pears	1	Safflower	9.1
pumpkins	1	Sunflower	9

Table 3. **Carotenoid Content[a] (mg/100 g) of Selected Edible Plants**

	Lutein	Zeaxanthin	β-Carotin	α-Carotin[b]	β-Cryptoxanthin[b]	Lycopene[b]
vegetables						
spinach	7.4	n.n.	4.5	n.n.	n.n.	n.n.
carrots	0.3	n.n.	9.9	3.9	n.n.	n.n.
maize	0.5	0.4	0.1	0.1	n.n.	n.n.
orange pepper	2.5	8.5	0.9	0.6	0.8	n.n.
tomato	0.1	n.n.	0.4	n.n.	n.n.	6.2
fruits and berries						
mandarines	0.1	0.1	0.3	0.01	1.8	n.n.
Satsumas	0.04	0.04	0.02	n.n.	1.2	n.n.
gou qi zi[c]		5				

[a]D. J. Hart and K.J. Scott Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the U.K. *Food Chem.* **54**, 101 (1995).

[b]Not detected = n.n.

[c]Chinese berry. See K. W. Lam and P. But. The content of zeaxanthin in Gou Qi Zi, a potential health benefit to improve visual acuity. *Food Chemistry* **67**, 173 (1999).