Chapter 9

Oilseed Processing and Fat Modification

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Chapter Outline

I. INTRODUCTION

Edible oils originate from seeds (oilseeds), animal tissues (lard, tallow, or fish), fruits (e.g. palm), processing by-products of cereals (e.g. rice bran oil), and fisheries industries (fish oil, cod liver oil, etc.), among others. In general, cleaning, followed by oil recovery are essential first steps. Thus, seed crushing and refining are the major operations in the procurement of edible oils.

If the oil comes from seeds, it must be cleaned before extraction to remove unwanted solid particles. The seeds are then subjected to drying followed by heating to temper them, thus helping to deactivate enzymes that may lead to degradation of oil or other matters in the seed and better release of the oil from oil compartments (Meshehdani *et al.*, 1990).

In the intact seeds, the enzymes are separated from the oil compartments within the cells. However, if seeds are damaged the enzymes present may come into contact with the oil or other sensitive material, such as glucosinolates in canola. This would lead to adverse reactions, to different degrees, depending on the temperature, moisture, and the extent of damage. In some cases, oilseeds may subsequently undergo dehulling, such as in soybean. In this way, not only may a better oil result following dehulling, but the resultant deoiled soybean meal would contain a higher content of proteins, e.g. 48% versus 44%. Procurement of a better oil may also be achieved in cases where the hulls contain less desirable components such as waxes in sunflower or corn which would otherwise need to be removed at a later stage. However, dehulling must be carried out carefully to avoid rupture of oil cells and their adsorption by the hulls.

Depending on the seeds, they may be heated before crushing and pressing by a screw, which generally releases about one-third of the oil. The screw press provides a continuous operation that allows ejection and separation of the oil by draining. The solid material that still contains two-thirds of the oil is often a sticky solid mass, known as the 'cake'. The large pieces of cake may then be subjected to flaking for size reduction before extraction of the remaining oil by appropriate solvents (Kemper, 2005).

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Oil extraction is often achieved in crown extractors using a counter-current flow which reduces the amount of solvent required. In this process, the solvent flows over the surface of the particles and diffuses through the miscella during the percolation process (Kemper, 2005).

The solvents used for oil extraction are hexanes, in a mixture of about 60% *n*-hexane and other hexane isomers. Since hexane is flammable, the use of other solvents has been considered, but none of these is yet in commercial use because of the lower yield of oil and other considerations. Following solvent extraction, the oil is separated and the resultant solids are passed to the desolventizer-toaster to remove the remaining hexanes. The residual oil in the resultant meal is usually less than 1%. The toasting process is needed when the meal is to be used for food or feed. The nearly desolventized flakes are removed from the system using a vapor-tight cyclone or by vapor desolventizing with steam, leading to the production of a desolventized meal. This meal may then be ground to the desired size depending on its end use. Crude oil is also obtained following the removal of hexanes by distillation and mixing of the pressed oil with the solvent-extracted oil.

In the case of fruit oils, such as those of the palm, oil may be procured from both the fruit and the kernel. The processing of olive fruit and palm differs somewhat, but details will not be provided here. Bunches of palm fruits are harvested at the desired stage of maturity and transported to the mill, where they are sterilized by heating with pressurized steam for about an hour to inactivate the enzymes present so that the oil is not hydrolyzed.

Following sterilization, the fruits are stripped from stalks, and then transferred to a digester where the material is reheated to about 100°C for a few minutes to loosen the pericarp from the nuts and to break and release the oil from oil-bearing cells. This material is then subjected to continuous screw-pressing. The press liquor contains nearly two-thirds oil along with water and some solids, while the press cake contains the nuts and the flesh fiber. Addition of water to the press liquor facilitates the settling of the solids upon filtration and the resultant crude oil may be separated by decanting or centrifugation. The oil is then cooled and stored while the solids (press cake) are transferred to an aspirator, which allows separation of the nuts (kernels) from the fiber. The resultant fiber is often burned to provide heat for the steam boiler. The seed may then be conditioned by drying to loosen the kernels from the shells. The nuts may then be cooled and stored or cracked and the kernels separated from the shells based on density differences. Kernels are subsequently screw-pressed or solvent-extracted to produce palm kernel oil and palm kernel meal (Fairhurst and Mutert, 1999).

For the recovery of oil of animal origin, both wet and dry rendering may be practiced. This subject is beyond the scope of this chapter, but in wet rendering the material is cooked by steam and this eventually leads to three phases, with precipitated denatured proteins as solids, the water phase, and the fat layer on the top which can be separated by decanting using centrifuges (Henry, 2009). The crude oil thus obtained may be subjected to further processing, as required.

Cold-pressing is another method used for producing oils that may otherwise be sensitive to oxidation or to procure the prime quality of oil that is often used in the crude form. In this case the oil is extracted by traditional methods or commonly by screw-pressing, but usually in a batch processor. The pressed oil is released through small holes or slots. The cold-pressed oil has the advantage of retaining minor components of the oil without many changes. Following oil extraction and refining, other possible steps may be carried out to prepare products. As an example, the oils may be cooled slowly to about 4°C in a process known as winterization, which removes saturated fats that could precipitate out during refrigerated storage. The oils may also be subjected to the introduction of water to prepare margarines. In all these cases, oils could also be partially hydrogenated, a process that has raised concern due to the production of *trans* fats. Nonetheless, this process is often necessary to produce more solid-like fats that provide mouth-feel and texture to the end products. However, more recent efforts have concentrated on producing formulations that make use of palm and other more saturated oils to provide texture and mouth-feel without introducing *trans* fats into products.

Finally, the stability of edible oils is of much interest to the industry and consumers alike because adequate shelf-life of products is necessary, especially when products are used in frying or high-temperature operations. In such cases, the use of antioxidants, especially for more unsaturated oils, is practiced. Nonetheless, the oilseed industry has moved gradually towards producing high-oleic products, such as high-oleic sunflower oil, to obtain more stable oils with extended shelf-life characteristics.

II. PROCESSING OF EDIBLE OILS

The crude oil obtained following pressing and extraction may further be subjected to a series of processing steps known as refining. Specialty oils and lard, as well as tallow, are usually consumed without further refining.

The process of refining is carried out to produce a bland oil, mainly triacylglycerols (TAGs), commonly known as triglycerides. The non-TAG components present in the crude oil may include small amounts of a number of products and other solids as well as phospholipids, free fatty acids (FFAs), monoacylglycerols, diacylglycerols, pigments, tocopherols and/or tocotrienols, phytosterols, waxes, and possibly sulfur-containing compounds, along with hydroperoxides, secondary oxidation products, and possibly squalene and other hydrocarbons, including carotenoids. Phenolic compounds other than tocols may also be present in certain oils, such as sesame seed and olive oils (Pokorny, 1991).

During the refining process, oils are subjected to degumming, refining, bleaching, deodorization and, in certain cases, winterization. Antioxidants are sometimes also added to the oil following deodorization to enhance the oxidative stability of the oil. Some unit operations lead to the removal of certain beneficial components, such as tocophenols and sterols, from the oil.

A. Removal of Phospholipids: The Degumming Process

Phospholipids present in the oils are generally more unsaturated in nature and darken in color upon storage as a result of oxidation. These phospholipids exist in hydratable (HPL) and non-hydratable (NHPL) forms. The HPLs (phosphatides) may be removed by water washing as they are precipitated out from the oil. The sludge of phospholipids is referred to as 'gums' and hence the process is known as degumming. The NHPLs therefore require acidification to remove them from the oil. Thus, industrially, the oil may be subjected to acidification with 0.05−0.2% of phosphoric acid at a temperature of 70−80°C over a period of 5−30 minutes along with stirring (Zufarov *et al.*, 2008). This process may also help to remove some of the chlorophyll in the oil that may otherwise act as a photosensitizer, thus speeding up oil deterioration via photooxidation. More recently, the use of citric or malic acid has been considered for the degumming process. After acidification, the oil is subjected to water washing and removal of phospholipids. The phospholipids removed may be used in different applications. For example, phospholipids from soybean oil processing, known as soy lecithin, may be purified and used as a dietary supplement or in other applications (Ceci *et al.*, 2008).

The first refining step involves the removal of phospholipids using the degumming process. Degumming purifies the seed oils, which normally contain impurities in the colloidal state or in solution (Bernardini, 1985). Crude vegetable oil is degummed to produce an oil substantially free of materials that settle down during transportation or storage. Solvent-extracted vegetable oils contain considerable amounts of phosphatides and other mucilaginous materials which form deposits in the storage tanks. It is vital to remove the phosphatides from the crude oil because their presence would impart undesirable flavor and color to the oil, and shorten its shelf-life. They also lead to increased refining losses by emulsifying considerable amounts of neutral oil, which is lost in the soap stock. The recovered phosphatides can be further processed to produce lecithin for use as an emulsifier in products such as margarine, chocolate, and emulsion paints, or as a dietary supplement.

The common oilseeds such as soybean, cottonseed, sunflower, and rapeseed are rich sources of phospholipids (Indira *et al.*, 2000; Willem and Mabel, 2008), which are either hydratable (HPLs) or non-hydratable (NHPLs). Most of the phospholipids in crude sunflower and rapeseed oils are hydratable and can be removed by water degumming (Zufarov *et al.*, 2008). The NHPLs cannot swell and form gels or precipitate from the oil (Szydlowska-Czerniak, 2007), hence their removal requires a more complex process at increased temperatures with the use of phosphoric acid, citric acid, malic acid, or other degumming agents.

The resulting insoluble, hydrated gum, following acid treatment, is separated as a sludge by centrifugal action and, when dried, affords crude lecithin. The degummed oil can be dried and pumped to storage containers or can proceed to the refining step. Excessive amounts of phosphoric acid should be avoided, as it may increase the content of phosphorus in the medium, which is difficult to remove and may contribute to further refining problems. Different degumming processes, namely dry degumming, water degumming, acid degumming (Andersen, 1962), and total degumming (Dijkstra and Opstal, 1989) have been described in detail. More recently, enzymatic degumming, simultaneous degumming/dewaxing, and membrane degumming have received attention for their role in reducing the refining loss and color intensity of the finished oil.

The dry degumming process involves the removal of gums through precipitation by acid conditioning and via filtration during the bleaching process, not via centrifugal separation. This process is used for low-phosphatide oils such as palm oil, lauric oils, and edible tallow, and is suitable for preparing oils for subsequent physical refining. Water degumming consists of treating the natural oil with a small amount of water, followed by centrifugal separation. The process is applied to many oils that contain phospholipids in significant amounts. Phosphatidylcholine

and phosphatidylinositol are completely hydratable. Phosphatidylethanolamine and phosphatidic acid are only partially hydratable or non-hydratable, when they form salts with divalent cations (mainly calcium and magnesium) or when they are not in the dissociated form. The phosphatide−metal complexes can be decomposed by the addition of acid or a complexing agent, followed by hydration with water. Partial neutralization of acid is used to avoid the migration of phosphatides back to the oil phase (Kovari, 2004).

For physical refining of vegetable oils, water degumming is not sufficient. In the acid degumming process, gums are precipitated by an acid-conditioning process (using e.g. phosphoric, citric, malic, or tartaric acid) and subsequently removed by centrifugal separation. The total degumming process, known by its Dutch acronym TOP, is designed to further treat the oil that has already been water degummed. The process has two variations to cater for different needs (Dijkstra and Opstal, 1987). In the first approach a dilute acid is finely dispersed into the oil. After a sufficient contact time a base is added and mixed into the acid-in-oil dispersion. The base can be sodium hydroxide, sodium carbonate, or sodium silicate. During the process the acid initially decomposes metal−phosphatidic acid complexes into insoluble metal salts and phosphatidic acid (in acid form). Phosphatidic acid is then hydrated by partial neutralization with the base added, and removed from the oil by centrifugation. The second approach uses a combination of two centrifuges to remove the hydrated phospholipids with high efficiency and minimal loss. The first one removes the bulk of the gum phase. Clearly, the quality of the water-degummed oil is critical to TOP degumming. When the water-degummed oil has a higher content of calcium and magnesium, the TOP process becomes less effective (Cleenewerck and Dijkstra, 1992). The removal of phospholipids from vegetable oils by membrane technologies is a relatively new development (Ochoa *et al.*, 2001).

The traditional degumming processes, including water degumming, TOP degumming, acid treatment, and others, cannot guarantee the low phosphorus contents required for physical refining, and they are not always optimally suited for all oil qualities because of the high content of NHPLs (Copeland and Belcher, 2005).

The latest degumming processes are soft degumming and enzymatic degumming. The soft degumming process involves complete elimination of phospholipids by a chelating agent, such as ethylenediaminetetraacetic acid (EDTA), in the presence of an emulsifying agent. Different kinds of crude oil are degummed by the soft degumming method; the content of phospholipids in the treated oil samples is lowered to approximately 5 ppm. However, the high cost of EDTA does not make this process industrially attractive (Choukri *et al.*, 2001).

The first enzymatic degumming process that was used industrially was the EnzyMax1 process, which launched in 1992 and used porcine phospholipase A_2. Subsequently, various microbial phospholipases with different specificities have been developed. They have the advantage of being kosher and halal, and of having an unlimited availability and a low cost. Enzymatic degumming is probably the best process available for reducing the phosphorus content of crude oils below the 5 ppm level (Chakrabarti and Rao, 2004). The enzyme, phospholipase A_2, catalyzes the non-hydratable phosphatides into hydratable lysophospholipids, which are then removed by centrifugation, yielding oil that is low in phosphorus. Owing to the low temperature used, this process produces no color deterioration in the degummed oil compared to conventional phosphoric acid degumming. Moreover, the oil content of the gums from enzymatic degumming is only 25−30% compared to 50−60% in the conventional method for rice bran oil. The oryzanol present in crude rice bran oil remains almost intact during the enzymatic process. Processes for simultaneous dewaxing and degumming use water and an aqueous solution of calcium chloride, followed by centrifugation or low-temperature (20°C) crystallization, which facilitates the precipitation of hydratable and non-hydratable phosphatides along with wax, and the phosphorus content decreases to below 5 ppm (Kaimal *et al.*, 2000; Rajam *et al.*, 2005).

The economic feasibility of these processes is quite good owing to the elimination of one step from the entire process. The membrane process is usually used in extraction plants. According to Lin *et al.* (1997), membrane separation is primarily a size-exclusion-based, pressure-driven process. It separates different components according to their molecular weight or particle size and shape and is dependent on their interactions with membrane surfaces and other components of the mixture. Phospholipids can be separated from TAGs in the miscella stage using appropriate membranes. The membrane-based crude oil degumming produces a permeate and a retentate containing TAGs and phospholipids, respectively. The majority of the coloring bodies and some of the FFAs and other impurities are included in phospholipid micelles and also removed (Lin *et al.*, 1997).

B. Refining

The degumming step, prior to chemical refining, is not always necessary. The best known and the most widely used chemical refining procedure is the caustic soda process. Refining of oil is practiced as a purifying

treatment designed to remove FFAs. Usually, the refined oils are neutral (i.e. neither acidic nor alkaline), free of materials that separate on heating (break material), lighter in color, less viscous, and more susceptible to oxidation.

The refining process may be carried out in either a batch or a continuous system. In batch refining, the aqueous emulsion of soaps formed from FFAs, along with other impurities (soapstock), settles to the bottom and is drawn off. In the continuous system the emulsion is separated by centrifugation. Essentially all soybean oils in the USA are refined by the continuous process. Based on the FFA content of the oil, caustic with an excess of 0.10−0.13% is proportioned into the crude oil and mixed in a high-shear in-line mixer. The soap−oil mixture is heated to 75−80°C and fed to a pressure or hermetic type centrifuge for separation into light- and heavy-density phases. Light-phase discharge consists of the refined oil containing traces of moisture and soap; the heavy phase is primarily soap, insoluble material, free caustic, phosphatides, and a small quantity of neutral oil. Refined oil is washed with 10−20% by weight of soft water at 90°C. The water washing process removes about 90% of the soap content in the refined oil; the remainder of the soap is removed in the bleaching process (Mounts and Khym, 1980). Soapstock and wash water are combined and treated with sulfuric acid to convert the soap into crude fatty acids. Most of the acidified soapstock is used as a high-energy ingredient in animal feed. Depending on market demand, acidified soapstock may be sold to fatty acid producers who recover the crude fatty acids by distillation as a valuable by-product of oil refining (Mounts, 1981). The residue from this distillation is a good source of sterols.

Conventional chemical refining is time consuming and has several disadvantages. It has substantial energy requirements and the by-products formed (soapstock and deodorizer distillate) are neither environmentally friendly nor commercially valuable. Furthermore, the chemical process leads to considerable oil loss; soapstock can hold as much as 50% of its weight of neutral oil. Despite having several disadvantages, it is still used in many industries because of the successful reduction of FFA content to an acceptable level. The refining of high FFA oil has been accomplished by miscella refining as it provides three immediate benefits: a lower refining loss; a lighter colored refined oil without bleaching; and elimination of the need for water washing of the refined oil or miscella (Canavag, 1976). The miscella refining process has been commercialized in Japan for many years. It is a simple process that is carried out in an explosion-proof system. However, the cost of the equipment is somewhat higher than that of an ordinary refining plant and control of the process is more difficult. Miscella deacidification involves slight modification of the chemical refining process, in which the oil is mixed with hexanes to create miscella. The mixed solvent process using hexane as the main solvent and ethanol or isopropanol as the second solvent has been carried out for refining of high FFA rice bran oil (Ghosh, 2007). The miscella are mixed with sodium hydroxide in a neutralization step and then reacted with phosphatides. This process also induces decolorization. Soapstock is removed by centrifugation, which results in minimal loss of neutral oil; however, it is very expensive and solvent removal requires several steps. Miscella deacidification is only used for the refining of cottonseed oil because a lighter colored final product is obtained compared to using the classical methods (Bhosle and Subramanian, 2005).

Physical refining processes use steam stripping under vacuum to avoid chemical neutralization. This is a simplified operation that removes FFAs, unsaponifiable matter, and pungent compounds, and also reduces the amount of oil lost. Physical refining is also known as deacidification (deodorization) by steam distillation in which FFAs and other volatile components are distilled off from the oil using an effective stripping agent, which is usually steamed under suitable processing conditions (Ceriani and Meirelles, 2006). It consumes less steam, water, and power, and requires less capital investment than the chemical refining process (Cvengros, 1995). Physical refining of crude vegetable oil has several advantages over the traditional alkali refining process. For example, there are improvements with regard to simplicity of the procedure, product yield, energy conservation, and reduced generation of environmental pollutants. There are also many drawbacks as not all types of oils are suitable for this process. The use of high temperature and high vacuum often results in the formation of side products such as polymers and *trans* isomers (Sengupta and Bhattacharyya, 1992). Steam refining of certain high-FFA oils has been carried out in Europe for many years. The economics of deacidification by steam refining versus caustic refining normally favor steam refining only when high-FFA oils are processed (Sullivan, 1976).

A new supercritical fluid-based process has been developed which permits the counter-current refining (treatment) of extracted soybean oil to produce a refined feedstock suitable for direct deodorization. The process makes use of a packed vessel that facilitates interfacial contact between the high-pressure carbon dioxide and a liquid soybean oil in a counter-current mode (List *et al.*, 1993).

C. Bleaching

Bleaching of alkali-refined oils removes entrained soaps and reduces color bodies in the oil as well as decomposing hydroperoxides to secondary oxidation products; it is more appropriately referred to as adsorption treatment. Carotenoids, chlorophylls, residual soap, phospholipids, metals, and oxidized products are removed by bleaching. Bleaching often reduces the resistance of oils to rancidity, because some natural antioxidants are also removed with impurities. Three types of bleaching method can be used in the edible oil industry: adsorption bleaching; heat bleaching; and chemical oxidation.

The fat may be treated with various bleaching agents. Heated oils are treated with fuller's earth (a natural earthy material that will decolorize oils), activated carbon, or activated clays. Effective adsorption requires a large surface and highly specific surface area and the use of a very porous adsorbent. The channels by which molecules reach this surface depend on the type of molecule involved. The nature of the process must allow acceptably firm bonds, chemical or physical, between the bleaching clay and the adsorbate (Patterson, 1992). Therefore, to attain maximum bleaching performance, an efficient bleaching earth is required which has surfaces of the correct chemical composition and a pore distribution that is selectively attractive to the detrimental components present in crude TAG oils. A few types of bleaching agent are used in the vegetable oil industry, such as acid-activated bleaching earth, natural bleaching earth, activated carbon, synthetic silicates, and synthetic resins. Many impurities, including chlorophyll and carotenoid pigments, are adsorbed onto such agents and removed by filtration. Trace metal complexes, such as those of iron and copper, phosphatides, and oxidation products are also removed by the adsorptive effect of the bleaching earth and any residues of phosphoric acid are also removed during this stage. Usually, bleaching earth does not remove all the color-producing materials; many of these are removed by thermal destruction during the subsequent deodorization process.

Although batch atmospheric bleaching is still used to some extent in the USA, batch or continuous vacuum bleaching is generally practiced. Bleaching generally improves oil quality with respect to color, initial and aged flavor, and oxidative stability, but the process also has other less obvious effects, some of which are desirable and some undesirable. Several factors affect the degree of bleaching of an oil. Adsorption of pigments by the adsorbent and a reduction in color through oxidation of certain pigments are two favorable factors. Color increases brought about by oxidation of other pigments and stabilization of oxidized pigments against adsorption are unfavorable factors. Bleaching earth has been shown to catalyze such oxidation reactions. Vacuum bleaching minimizes these unfavorable events. Such reactions and color changes are complex, for example, oxidation and heat can bleach carotenoids; and these conditions may also encourage the formation of new pigments (Mounts, 1981). Natural and activated earths that have little or no acidity will produce little or no change in the FFA content of the neutralized oil. Some of the more acidic activated earths may increase FFA content by 0.05−0.10%, especially if the contact time is long or if moisture or soap is present.

Some pigments, such as the carotenes, become colorless if heated sufficiently. When many oils are heated to above 175°C, a phenomenon known as heat bleaching takes place. Apparently, heat decomposes some pigments, such as the carotenoids, and converts them to colorless compounds. However, this will leave the pigment molecules in the oil and may have adverse effects on oil quality. According to Gunstone and Norris (1983), if this oil comes into contact with air, colored degradation products such as chroman-5,6-quinones from γ-tocopherol may be formed and these are very difficult to remove. In addition, oxidation of carotenoids invariably affects the acylglycerols and may destroy natural antioxidants present in the oil. Consequently, oxidation-mediated bleaching is never used for edible oil, but is restricted to oils for technical purposes, such as soap making.

De-Smet and Alfa-Laval now offer counter-current bleaching and steam-agitated bleaching plants for crude rice bran oil processing. Industrial grade rice bran oil is often bleached by conventional chlorate bleaching and used in soap production.

Conjugation of oxidized polyunsaturated fatty acids during bleaching is known to occur. Oxidation of the oil before or during bleaching will promote conjugation; therefore, procedures such as deaerating both the initial oil and the adsorbent and vacuum bleaching would help to suppress the conjugation reaction; *trans*-isomerization has been shown to occur with acid-activated earths, but only at temperatures of 150°C and above, which far exceed those normally employed in the bleaching process (Mounts, 1981).

D. Deodorization

In the edible oil refinery, deodorization is the last process step used to improve the taste, odor, color, and stability of the oil by the removal of undesirable substances. All commercial deodorization, whether in continuous,

semicontinuous, or batch units, is essentially a steam stripping of the oil for the removal of FFA and other volatile compounds.

During the process, peroxide decomposition products, color bodies, and their decomposition products are eliminated, and the contents of sterols, sterol esters, and tocopherols are reduced. The goal of the deodorization is to produce a finished oil that has a bland flavor, an FFA content of less than 0.05%, and a zero peroxide value. The modern commercial deodorizers are equipped with a pollution control system that consists of three steps: the deodorizer distillate recovery system; the closed circuit condensing water system; and the vapor scrubbing system. The distillate recovery system removes 80−90% of the distillate from the steam train before it reaches the condenser. The short-chain fatty acid (SCFA) fractions pass through the recovery unit and are recovered from the vapor scrubbing system. The deodorizer distillate is a concentrate of tocopherols and sterols and is a valuable source of these materials that could be used as value-added components in different applications.

Deodorization is primarily a high-temperature, high-vacuum, steam-distillation process. To produce a high-quality finished product, each deodorizer installation must deaerate the oil, heat the oil, steam strip the oil, and cool the oil, all with zero exposure to air. Steam deodorization is feasible because the flavor and odor compounds that are to be removed have appreciably greater volatility than TAGs present. Operation at high temperatures increases the volatility of these odoriferous compounds, and the introduction of stripping steam into the deodorizer greatly increases the rate at which these compounds are volatilized. Reduced pressure operation further aids in the removal of the odoriferous compounds and protects the oil from atmospheric oxidation. The deodorization process consists of blowing steam through heated oil held under a high vacuum. Small quantities of volatile components, responsible for taste and odor, distill, leaving a neutral, virtually odorless oil that is suitable for the manufacture of bland shortening or delicately flavored margarine. Originally, deodorization was a batch process, but increasingly, continuous systems are being used in which hot oil flows through an evacuated column counter-current to the upward passage of steam. In Europe, a deodorization temperature of 175−205°C is common, but in the USA higher temperatures of 235−250°C are usually employed. About 0.01% of citric acid is commonly added to deodorized oils to inactivate trace-metal contaminants such as soluble iron or copper compounds that would otherwise promote oxidation and the development of rancidity. The oil has to be given an adequate residence time for proper deodorization and the destruction of heat-labile pigments. The deodorized oil is counter-currently cooled first by the incoming oil and then by water to around 50°C. The cooled, deodorized oil is then passed through a polishing press to give it a transparent look.

Olive oil is invariably marketed in the undeodorized form. The natural flavor is an important asset, and olive oil commands a premium price in the market because of its distinctive flavor. The common cooking oils of Asia − soybean, rapeseed, peanut, sesame, and coconut − are consumed in their crude form as expressed from oilseeds. In contrast, deodorized oils are in particular demand in the USA and Europe. For many years the only important vegetable oil consumed in the USA was cottonseed oil, which in its crude form has such a strong and unpleasant flavor that further processing is necessary to render it suitable for consumption. Because of the widespread sale of neutrally flavored cottonseed oil products over many years, a general preference was developed for odorless and tasteless oils.

Another reason for the practice of deodorizing edible oils in Europe and the USA relates to differences in oil quality by Western and Eastern extraction techniques. In China and Southeast Asia, edible oils have been produced principally by small, relatively crude equipment. The yield of oil is relatively low, and a minimum amount of non-acylglycerol substances is expressed from the seed, with the result that the flavor of the oil is fairly mild. In Europe and the USA, oil extraction is carried out in large factories that operate on an extremely competitive basis. Very-high-pressure expression or solvent extraction is used, and to improve yields the seeds are heat-treated before extraction. Oils obtained in high yield under such conditions are stronger in flavor than oils prepared by low-pressure expression, and the refining and deodorizing steps are required to improve palatability. The improvement in yields more than compensates for the added costs of refining and deodorizing.

E. Winterization and Hydrogenation

Cloud formation during storage under low temperature is a concern for many edible oils. A winterization process is usually carried out to separate the crystallized part (waxes or triacylglycerols) from the oil and thus prevent turbidity of the oil in the winter or during cold storage. Waxes in general are esters of long-chain aliphatic fatty alcohols and long-chain fatty acids (LCFAs) that have 36−60 carbon atoms. They can be divided into soluble waxes, with chain lengths lower than C40; partially soluble waxes, with lengths between C40 and C42; and crystallizable waxes, with lengths of more than C44, which are responsible for the turbidity or sediment formation during oil storage and need to be removed by winterization. Winterization operations in the processing of edible oils are basically the separation of

oils into two or more fractions with different melting points. In the winterization process, the oils are cooled in a simple way and kept at a low temperature for some time. The liquid and the solid fractions are generally separated by filtration. To separate the waxes from the oil by filtration, it is necessary to mix the winterized oil with a filter aid. This is to form a layer of the filtering material on the filter mats, giving a suitable drainage system. The wax-containing oil can then be pumped through the filter to remove waxes and the filter aid, as performed in winterization of sunflower oil (Raβ *et al.*, 2008). Winterization has a broad application in edible oil technology, including the production of cocoa butter equivalents from palm oil, palm-kernel oil, and shea fat, and from hydrogenated soybean and cottonseed oils (Kreulen, 1976).

Winterization can be difficult for certain oils such as palm olein because of the high content of the higher melting point crystallized part and the high viscosity of the oil that may hinder filtration (Leiboritz and Ruckenstein, 1984). In such cases, solvent winterization, with hexane or acetone, is used to reduce viscosity and facilitate crystallization of the waxes. Additives may also be used to help separate waxes from the oil. Calcium chloride and sodium lauryl sulfate solutions have been used in the winterization of rice bran oil to facilitate crystallization. The crystals that become dispersed in the water phase are separated by centrifugation (Ghosh, 2007).

When different degrees of hardness are required, the liquid oils may be subjected to a hydrogenation process to produce stable semi-solid plastic fats such as margarine and shortening. Processing conditions for hydrogenation can be modified to create a range of fats and oils with different melting and other characteristics. One such example is that of soybean oil, where relatively unstable linolenic acid (C18:3) is converted to more stable fatty acids. The hydrogenation process converts unsaturated fatty acids into their saturated or less unsaturated counterparts by the addition of hydrogen to the double bonds. Two types of process are involved in hydrogenation, namely saturation and isomerization. Saturation is where a molecule of hydrogen is added directly across a double bond in the presence of catalysts to give a saturated single bond. Hydrogenation is usually accompanied by the generation of *trans* fatty acids through the *cis* bond isomerization. *Trans* fatty acids formed during hydrogenation have been implicated in an increased risk of coronary heart disease, and have compelled consumers, health authorities, and manufacturers to reconsider the process. The process may also be accompanied by a shift in the double-bond location, leading to the formation of conjugated dienes and trienes.

The hydrogenation of edible oils is complex. Conventional hydrogenation uses a nickel catalyst and hydrogen gas at high temperatures of 140−230°C. It is a three-phase process with hydrogen in the gas phase, the liquid oil, and the solid catalyst, usually nickel. To achieve high reaction rates, an active catalyst as well as good mass transfer conditions between the gas and liquid and between the liquid and the catalyst are required. The high temperature produces high levels of *trans* fatty acids in the final oils. Low-temperature electrocatalytic hydrogenation may serve as an alternative method for the production of hydrogenated edible oils low in *trans* fatty acids. Low-temperature electrocatalytic hydrogenation uses an electrically conducting catalyst such as Raney nickel or platinum black as a cathode. Electrocatalytic hydrogenation has been used to produce a variety of organic compounds such as aromatic compounds, phenols, ketones, nitrocompounds, dinitriles, and glucose (Jang *et al.*, 2005). However, different catalysts are required for low-temperature hydrogenation since nickel catalysts are not very active below 120°C. Nickel can be replaced by precious metal catalysts, which are active at the low temperature of 70°C. Palladium, platinum, and ruthenium were the most potential precious metal catalysts for the hydrogenation of vegetable oils. Each metal catalyst has different characteristics in selectivity, reactivity, and *cis*−*trans* isomerization during hydrogenation. It has generally been accepted that platinum catalysts produce the least amount of *trans* fatty acids during hydrogenation (Jang *et al.*, 2005). Another hydrogenation method is supercritical fluid state hydrogenation, which improves the mass transfer of multiphase systems and reduces the formation of *trans* fat. A supercritical fluid state improves hydrogen transfer to the catalyst surface during hydrogenation by providing a good homogeneous phase. The efficacy of hydrogenation of oleochemicals and vegetable oils using supercritical carbon dioxide or propane as the solvent has been investigated (Macher *et al.*, 1999; Macher and Holmqvist, 2001; King *et al.*, 2001).

III. FAT MODIFICATION

Lipids have long been recognized for the richness they impart to foods as well as their satiety value in the diet. Lipid is an important component of the diet, because it provides both energy and essential fatty acids (EFAs). It is the most concentrated energy source in the diet, with an average energy value of 9 kcal/g compared to 4 kcal/g for carbohydrates and proteins. Lipid is an essential constituent of the membranes of every cell in the body. In addition to its role as a structural component of cells, lipid plays other roles in the body that include serving as an energy reserve, a regulator of body functions, and an insulator against heat loss. The role of dietary lipids in health and disease

(notably, coronary heart disease, obesity, hyperlipidemia, and cancer) is one of the most active areas of research in modern food science, nutrition, and biochemistry. In this respect, the role of structured lipids should also be considered.

Structured lipids are TAGs or phospholipids containing SCFAs and/or medium-chain fatty acids (MCFAs) along with LCFAs located in the same glycerol molecule, and are produced by a chemical or an enzymatic process (Senanayake and Shahidi, 2000). These specialty lipids may be synthesized via direct esterification, acidolysis, alcoholysis, or interesterification reactions. However, the common methods reported in the literature for the synthesis of structured lipids are based on the reactions between two TAG molecules (interesterification) or between a TAG and an acid (acidolysis). These specialty lipids have been developed to fully optimize the benefit of various fatty acid moieties. Structured lipids have been reported to have beneficial effects by affecting a range of metabolic parameters including immune function, nitrogen balance, and improved lipid clearance from the bloodstream (Quinlan and Moore, 1993). Structured lipids are also produced to improve or change the physical and/or chemical properties of TAG. Research on structured lipids remains an interesting area that holds great promise for the future.

Nutraceutical is a term used to describe commodities procured from foods, but used in the medicinal form; they provide demonstrated physiological and health benefits beyond those ascribed to their nutritional value (Scott and Lee, 1996). These products may be incorporated into products that have the usual appearance of food to provide specific health benefits (Scott and Lee, 1996). Structured lipids can be designed for use as medical, nutraceutical, or functional food ingredients, depending on the form of use.

Lipids, mainly TAGs, can be modified to incorporate specific fatty acids of interest to achieve desired functionalities. Structured lipids may be synthesized via the hydrolysis of fatty acyl groups from a mixture of TAG followed by random re-esterification onto the glycerol backbone (Babayan, 1987). Various fatty acids, including different classes of saturated, monounsaturated, and *n*-3 and *n*-6 polyunsaturated fatty acids (PUFAs) or their mixtures may be used in this process, depending on the desired metabolic effect. Structured lipids containing MCFAs and LCFAs exhibit changes in their absorption rates because MCFAs are rapidly oxidized for energy while LCFAs are oxidized very slowly. These specialty lipids are structurally and metabolically different from the simple physical mixtures of medium-chain triacylglycerols (MCTs) and long-chain triacylglycerols (LCTs) (Akoh and Moussata, 1998).

A. Structured Lipids and their Fatty Acid Constituents

The unsaturated fatty acids from both the *n*-3 and *n*-6 families, as well as those from the *n*-9 family, may be included in structured lipids to promote health. The clinical advantages of these specialty lipids are derived from the combined effect of the short-, medium-, and long-chain fatty acids and the uniqueness of the structured lipid molecule itself. Many of these effects are due to the existing differences in the metabolic fate of the various fatty acids involved. Here, a brief description of SCFAs, MCFAs, and EFAs or PUFAs belonging to the *n*-3 and *n*-6 families is provided.

SCFAs are saturated fatty acids with two to four carbon atoms and include acetic acid (2:0), propionic acid (3:0), and butyric acid (4:0). These fatty acids are volatile and produced in the human gastrointestinal tract via bacterial fermentation of dietary carbohydrates (Stein, 1999). SCFAs are present in the diet in small amounts, for example, acetic acid in vinegar and butyric acid in bovine milk and butter. They may also be present in fermented foods. In humans, SCFAs contribute to 3% of total energy expenditure (Hashim and Babayan, 1978) and these are more easily absorbed in the stomach and provide fewer calories than MCFAs and LCFAs. Thus, acetic, propionic, and butyric acids have caloric values of 3.5, 5.0, and 6.0 kcal/g, respectively.

In certain nutritional applications, the use of SCFAs as alternatives to their MCFA and LCFA counterparts has been of interest. SCFAs are easily hydrolyzed from TAG and rapidly absorbed by the intestinal mucosa (Ruppin *et al.*, 1980). These fatty acids go directly into the portal vein for transportation to the liver, where they are broken down to acetate via β-oxidation. The acetate can then be metabolized for energy or use in new fatty acid synthesis. SCFAs affect gastrointestinal function by stimulating pancreatic enzyme secretion (Harada and Kato, 1983) and increasing sodium and water absorption in the gut (Roediger and Rae, 1982).

MCFAs are saturated fatty acids with six to 12 carbon atoms (Senanayake and Shahidi, 2000) and are commonly found in tropical fruit oils such as those of coconut and palm kernel (Bell *et al.*, 1991). For example, coconut oil naturally contains some 65% MCFA (Young, 1983). One of the first medical foods developed, as an alternative to conventional lipids, was based on MCTs. MCTs serve as an excellent source of MCFAs for the production of structured and specialty lipids. Pure MCTs have an energy value of 8.3 kcal/g. However, they do not provide EFAs (Heird *et al.*, 1986; Lee and Hastilow, 1999). MCFAs are more hydrophilic than their LCFA counterparts, and hence solubilization as micelles is not a prerequisite for their absorption (Ikeda *et al.*, 1991).

MCTs can also be directly incorporated into mucosal cells without hydrolysis and may be readily oxidized in the cell. MCTs pass directly into the portal vein and are readily oxidized in the liver to serve as an energy source. Thus, they are less likely to be deposited in the adipose tissues (Megremis, 1991) and are more susceptible to oxidation in tissues (Mascioli *et al.*, 1987).

MCTs are liquid or solid products at room temperature. They have a smaller molecular size, lower melting point, and greater solubility than their LCFA counterparts. These characteristics account for their easy absorption, transport, and metabolism compared to LCT (Babayan, 1987). MCTs are hydrolyzed by pancreatic lipase more rapidly and completely than are LCTs (Bell *et al.*, 1991). They may be directly absorbed by the intestinal mucosa with minimum pancreatic or biliary function. They are transported predominantly by the portal vein to the liver for oxidation (Heydinger and Nakhasi, 1996) rather than through the intestinal lymphatics. In addition, MCFAs are more rapidly oxidized to produce acetyl-coenzyme A and ketone bodies, and are independent of carnitine for entry into mitochondria.

MCTs need to be used with LCTs to provide a balanced nutrition in enteral and parenteral products (Ulrich *et al.*, 1996; Haumann, 1997a). In many medical foods, a mixture of MCTs and LCTs is used to provide both rapidly metabolized and slowly metabolized fuel as well as EFAs. Clinical nutritionists have taken advantage of the simpler digestion of MCTs to nourish individuals who cannot utilize LCTs owing to fat malabsorption. Thus, patients with certain diseases (Crohn's disease, cystic fibrosis, colitis, enteritis, etc.) have shown improvement when MCTs are included in their diet (Kennedy, 1991). MCTs are also increasingly used to feed critically ill or septic patients who presumably gain benefits in the setting of associated intestinal dysfunction. MCTs may be used in confectionery and in other functional foods as carriers for flavors, colors, and vitamins (Megremis, 1991). MCTs have clinical applications in the treatment of lipid malabsorption, maldigestion, obesity, and deficiency of the carnitine system (Bach and Babayan, 1982).

The EFAs are PUFAs belonging to the *n*-3 or the *n*-6 fatty acid families. The biological activity of PUFAs depends on the position of the double bond in the molecule nearest to the methyl end of the chain, being *n*-3 when the double bond is located between the third and fourth carbon atoms and *n*-6 when it is between the sixth and seventh carbon atoms. The parent compounds of the *n*-3 and *n*-6 groups of fatty acids are linoleic acid and α-linolenic acid (ALA), respectively. Within the body, these parent compounds are metabolized by a series of alternating desaturation (in which an extra double bond is inserted by removing two hydrogen atoms) and elongation (in which two carbon atoms are added) (Figure 9.1), but only to a limited extent of up to 4−5% for producing long-chain PUFAs (Plourde and Cunnane, 2007). The enzymes metabolizing linoleic acid and ALA are thought to be identical (Horrobin, 1990).

The role of EFAs as precursors of a wide variety of short-lived hormone-like substances called eicosanoids has received much attention. These 20-carbon endogenous biomedical mediators are derived from EFAs, notably arachidonic acid and dihomo-γ-linolenic acid (DGLA) from the *n*-6 family, and eicosapentaenoic acid (EPA) from the *n*-3 family (Branden and Carroll, 1986). Eicosanoids include prostaglandins, prostacyclins, thromboxanes, leukotrienes, and hydroxy fatty acids, which play a role in regulating the cell-to-cell communication involved in cardiovascular, reproductive, respiratory, renal, endocrine, skin, nervous, and immune system actions. Arachidonic acid is derived from linoleic acid, which gives rise to series-2 prostaglandins, series-2 prostacyclins, series-2 thromboxanes, and series-4 leukotrienes. These end products of *n*-6 fatty acid metabolism induce inflammation and immunosuppression. Prostanoids (the collective name for prostaglandins, prostacyclins, and thromboxanes) of series-1 and leukotrienes of series-3 are produced from DGLA. When *n*-3 fatty acids are processed in the eicosanoid cascade, series-3 prostaglandins, series-3 prostacyclins, series-3 thromboxanes, and series-5 leukotrienes are formed.

The biological activities of the eicosanoids derived from *n*-3 fatty acids differ from those produced from *n*-6 fatty acids. For example, series-2 prostaglandins formed from arachidonic acid may impair the immune function while series-3 prostaglandins produced from EPA ameliorate immunodysfunction. Thromboxane A_2 produced from arachidonic acid is a potent vasoconstrictor and platelet aggregator, whereas thromboxane A_3 synthesized from EPA is a mild vasoconstrictor and has shown antiaggregatory properties (von Schacky, 2003). Furthermore, *n*-3 fatty acids competitively inhibit the formation of eicosanoids derived from the *n*-6 family of fatty acids.

The *n*-3 fatty acids, such as ALA, EPA, and docosahexaenoic acid (DHA), have a myriad of health benefits related to cardiovascular disease, inflammation, allergies, cancer, and immune and renal disorders. Bang and Dyerberg (1972, 1986) suggested that the relatively high *n*-3 fatty acid content (especially EPA and DHA) of the diet of Inuits was related to their lower incidence of cardiovascular disease. Research has shown that DHA is essential for the proper function of the central nervous system and visual acuity of infants. The *n*-3 fatty acids are essential for normal growth and development throughout the life cycle of humans and therefore should be included in the diet. Fish and

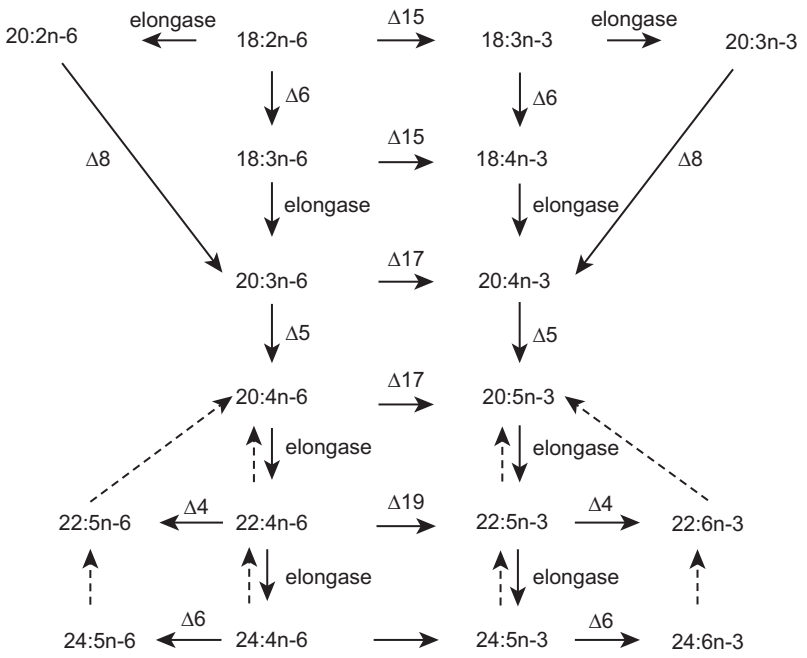

FIGURE 9.1 Biosynthesis of *n*-3 fatty acids.

marine oils are rich sources of *n*-3 fatty acids, especially EPA and DHA. Most fish oils, such as cod liver, menhaden, and sardine oils, contain approximately 30% EPA and DHA.

The *n*-6 fatty acids exhibit various physiological functions in the human body. The main functions of these fatty acids are related to their roles in the membrane structure and in the biosynthesis of short-lived derivatives (eicosanoids) which regulate many aspects of cellular activity. The *n*-6 fatty acids are involved in maintaining the integrity of the water-impermeable barrier of the skin. They are also involved in the regulation of cholesterol transport in the body.

γ-Linolenic acid (GLA) has shown therapeutic benefits in a number of diseases and syndromes, notably atopic eczema, cyclic mastalgia, premenstrual syndrome, cardiovascular disease, inflammation, diabetes, and cancer (Horrobin, 1990). While arachidonic acid is found in meats, egg yolk, and human milk, GLA is present in oats, barley, and human milk. GLA is also found in higher amounts in plant seed oils such as those from borage, evening primrose, and blackcurrant (Gunstone, 1992). Algae such as *Spirulina* and various species of fungi also seem to be desirable sources of GLA (Carter, 1988).

B. Production of Structured and Specialty Lipids

Production of specialty lipids used for confectionary fat formulations and nutritional applications may be carried out using enzyme-catalyzed reactions. In the area of confectionary fats, interesterification of high oleic sunflower oil and stearic acid using immobilized *Rhizomucor miehei* lipase produces mainly 1,3-distearoyl-2-monoolein (StOSt) (Macrae, 1983). Other reactants may also be used for production of specialty confectionary fats. In particular, there are many reports on the enzymatic interesterification of mixtures of palm oil fractions and stearic acid or its esters to produce fats containing high concentrations of StOSt and 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POSt) (Macrae, 1983). These products are the main components of cocoa butter, and hence enzymatic interesterification may produce fats with similar compositions and physical properties to cocoa butter (Macrae, 1985).

Enzyme-catalyzed reactions have been used to synthesize a human milk fat substitute for use in infant formula (Quinlan and Moore, 1993). An acidolysis reaction of a mixture of tripalmitin and unsaturated fatty acids, using an *sn*-1,3-specific lipase as a biocatalyst, afforded TAG derived entirely from vegetable oils rich in palmitate in the 2-position with unsaturated fatty acyl groups in the *sn*-1 and *sn*-3 positions. These TAG closely mimic the fatty acid distribution found in human milk fat, and when they are used in infant formula instead of conventional fats, the presence of palmitate in the *sn*-2 position of the TAG has been shown to improve digestibility of the fat and absorption of other important nutrients such as calcium. Hamam and Shahidi (2008) investigated the effect of chain

length, number of double bonds, location and geometry of double bonds, reaction conditions, and reactivity of different lipases on the incorporation of selected LCFAs into TAGs, such as trilinolein (tri C18:2) and trilinolenin (tri C18:3). The conditions for the synthesis of structured lipids were also optimized using response surface methodology (RSM) (Hamam and Shahidi, 2006a). Structured lipids having LCFAs at the *sn*-2 and medium-chain caprylic acid (8:0) at their *sn*-1,3-positions from corn oil were also prepared and the effects of substrate mole ratio, amount of enzyme, and reaction time on the incorporation of caprylic acid into corn oil were optimized (Ozturk *et al.*, 2010).

Enzyme-assisted reactions may also be used for the production of common lipid commodities such as margarine hardstocks and cooking oils. When non-specific lipases such as *Candida cylindraceae* and *Candida antarctica* are used as biocatalysts for the interesterification of oil blends, the TAG products are very similar to those obtained by chemical interesterification (Macrae, 1983), but the process is not economically attractive. However, enzymatic reactions may best be employed for the production of fats and oils containing nutritionally important PUFAs, such as EPA and DHA. For example, various vegetable and fish oils have been enriched with EPA and DHA by enzyme-catalyzed reactions (Senanayake and Shahidi, 1999a, b, 2001). The use of this technique to produce structured lipids with MCFAs and PUFAs located specifically in either the *sn*-2 or *sn*-1,3 position of the TAG has been described. Enzymatic processes are particularly suitable for the production and modification of lipids containing PUFAs, owing to the instability of the fatty acids involved and their susceptibility to oxidation under harsh chemical processing conditions. Wang and Shahidi (2010) examined the effect of chemical randomization on the stability of menhaden and seal blubber oils.

Interesterification of blends of palm and fully hydrogenated canola oils, and cottonseed and hydrogenated soybean oils using *sn*-1,3-specific lipases as catalysts, gave fats with a low *trans* fatty acid content that were effective as margarine hardstock (Mohamed and Larsson, 1994). Reaction of mixtures of palm stearine and lauric fats, using immobilized *R. miehei* as a catalyst, also produced fats that were functional as margarine hardstocks (Posorske *et al.*, 1988). With these enzymatically interesterified fats, margarine could be formulated without using hydrogenated fats.

In addition to the food applications, the process of interesterification or acidolysis could provide medical or nutritional benefits. For example, structured lipids may be used to improve the nutrition profile of certain TAGs (Osborn and Akoh, 2002). Saturated fatty acids at the *sn*-2 position are beneficial in terms of providing increased caloric intake through infant formula and enteral supplements (Decker, 1996). Structured lipids also have beneficial effects on a range of metabolic parameters including immune function, nitrogen balance, and improved lipid clearance from the bloodstream (Senanayake and Shahidi, 2005).

C. Structured Lipids Containing *n*-3 Polyunsaturated Fatty Acids

Plant seed oils such as those from borage, evening primrose, and blackcurrant are predominant sources of GLA (18:3*n*-6). GLA has been used in the treatment of certain skin disorders as well as a variety of other pathological conditions. The *n*-3 PUFAs have potential in the prevention of cardiovascular disease, arthritis, hypertension, immune and renal disorders, diabetes, and cancer (Senanayake and Shahidi, 2000). Structured lipids containing both GLA and *n*-3 PUFAs may be of interest because of their desired health benefits. Enzymatic synthesis of structured lipids containing GLA, EPA, and DHA in the same glycerol molecule, using selected oils such as those of borage and evening primrose as the main substrates, has been reported (Senanayake and Shahidi, 1999a, 2001). In these studies, three microbial enzymes, namely lipases from *C. antarctica* (Novozym-435), *Mucor miehei* (Lipozyme-IM), and *Pseudomonas* sp. (Lipase PS-30) were used as biocatalysts with free EPA and DHA as acyl donors. A higher incorporation of EPA + DHA (34.1%) in borage oil was obtained with *Pseudomonas* sp. lipase, compared to 20.7% and 22.8% EPA + DHA, respectively, with *C. antarctica* and *M. miehei* lipases (Table 9.1). Similarly, in evening primrose oil *Pseudomonas* sp. lipase afforded the highest degree of EPA + DHA incorporation (31.4%), followed by lipases from *M. miehei* (22.8%) and *C. antarctica* (17.0%) (Table 9.2). The modified borage and evening primrose oils thus obtained may be useful in the treatment of certain clinical disorders.

The incorporation of *n*-3 fatty acids into the acylglycerols of borage oil was also reported by Ju *et al.* (1998). In this work, borage oil was selectively hydrolyzed using immobilized *Candida rugosa* lipase and the resultant product was then used with *n*-3 fatty acids in the acidolysis reaction. The total content of *n*-3 and *n*-6 fatty acids in acylglycerols was 72.8% following acidolysis. The contents of GLA, EPA, and DHA in the structured lipid so prepared were 26.5%, 19.8%, and 18.1%, respectively. The *n*-3/*n*-6 ratio was increased from 0 to 1.09 following the acidolysis reaction. In another study, the fatty acid composition of borage oil was modified using EPA ethyl ester with an immobilized lipase from *C. antarctica* (Akoh and Sista, 1995). The highest incorporation (31%) was obtained with 20% *C. antarctica* lipase. At a substrate mole ratio of 1:3, the ratio of *n*-3 to *n*-6 fatty acids was 0.64. Under similar

TABLE 9.1 Fatty Acid Composition of Borage Oil Triacylglycerols Before and After Modification with EPA and DHA

		After Modification		
Fatty Acid (wt%)	Before Modification	Lipozyme-IM	Novozym-435	Lipase PS-30
16:0	9.8	6.9	7.3	5.5
18:0	3.1	2.6	2.8	2.0
18:1*n*-9	15.2	11.6	13.3	10.4
18:2*n*-6	38.4	28.8	31.2	26.1
18:3*n*-6	24.4	22.3	19.3	17.6
20:1*n*-9	4.1	3.1	3.3	2.6
20:5*n*-3	–	11.8	8.4	24.1
22:1*n*-11	2.5	1.8	2.1	1.7
24:1	1.5	–	–	–
22:6*n*-3	–	11.0	12.3	10.0

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

conditions, the *n*-3 fatty acid content (up to 43%) of evening primrose oil was increased, with a corresponding increase in the *n*-3/*n*-6 ratio from 0.01 to 0.6. Sridhar and Lakshminarayana (1992) modified the fatty acid composition of groundnut oil by incorporating EPA and DHA using an *sn*-1,3-specific lipase from *M. miehei* as the biocatalyst. The modified groundnut oil had 9.5% EPA and 8.0% DHA.

Incorporation of EPA and capric acid (10:0) into borage oil using two immobilized lipases, SP435 from *C. antarctica* and IM60 from *R. miehei*, as biocatalysts has also been reported (Akoh and Moussata, 1998). Higher incorporation of EPA (10.2%) and 10:0 (26.3%) was obtained with IM60 lipase, compared to 8.8% and 15.5%, respectively, with SP435 lipase. In another study, Huang *et al.* (1994) incorporated EPA into crude melon seed oil by two immobilized lipases, IM60 from *M. miehei* and SP435 from *C. antarctica*, as biocatalysts. Higher EPA incorporation was obtained using EPA ethyl ester than using EPA itself for both enzyme-catalyzed reactions. Furthermore, immobilized lipases, IM60 from *M. miehei* and SP435 from *C. antarctica*, were used to modify the fatty acid composition of soybean oil by incorporation of *n*-3 fatty acids (Huang and Akoh, 1994). The transesterification reaction was carried out with FFA and ethyl esters of EPA and DHA as acyl donors. With free EPA as the acyl donor,

TABLE 9.2 Fatty Acid Composition of Evening Primrose Oil Triacylglycerols Before and After Modification with EPA and DHA

		After Modification		
Fatty Acid (wt%)	Before Modification	Lipozyme-IM	Novozym-435	Lipase PS-30
16:0	6.2	4.5	4.9	3.6
18:0	1.7	1.3	1.4	1.0
18:1*n*-9	8.7	6.4	7.1	6.2
18:2*n*-6	73.6	55.1	60.6	49.7
18:3*n*-6	9.9	9.7	9.0	7.5
20:5*n*-3	–	12.3	8.4	22.0
22:6*n*-3	–	10.5	8.6	9.4

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

M. miehei lipase gave a higher incorporation of EPA than *C. antarctica* lipase. However, when ethyl esters of EPA and DHA were the acyl donors, *C. antarctica* lipase gave a higher incorporation of EPA and DHA than *M. miehei* lipase.

Marine oils can also be modified to incorporate MCFAs to improve their nutritional properties. Specific structured lipids with PUFA residues at the *sn*-2 position and MCFA residues at the *sn*-1,3 positions have high potential in biomedical and nutraceutical applications. In this form, the PUFA residues are protected against oxidation by the two saturated MCFA residues. In addition, PUFAs are better absorbed in the intestinal tract as 2-monoacylglycerol (2-MAG) upon hydrolysis by pancreatic lipase. MCFAs are absorbed efficiently and are a quick source of energy without being deposited in the adipose tissues. An immobilized *sn*-1,3-specific lipase from *M. miehei* was used to incorporate capric acid (10:0; an MCFA) into seal blubber oil. Upon enzymatic reaction, the fatty acid composition of seal blubber oil was modified and under optimum reaction conditions, a structured lipid containing 27.1% capric acid, 2.3% EPA, and 7.6% DHA was obtained (Table 9.3). Positional distribution of fatty acids in the structured lipid revealed that *M. miehei* lipase incorporated capric acid predominantly at the *sn*-1,3-positions of TAG molecules (Senanayake and Shahidi, 2002). In another study, structured lipids were successfully produced using seal blubber oil or menhaden oil with GLA and using lipase PS-30 from *Pseudomonas* sp. For the acidolysis reaction, optimum reaction conditions were 1:3 mole ratio of oil to GLA, reaction temperature of 40°C, reaction time of 24 hours, and enzyme concentration of 500 enzyme activity units/g oil. Under these conditions, incorporation of GLA into seal blubber oil was 37.1%, and 39.6% incorporation was achieved when menhaden oil was used (Spurvey *et al.*, 2001). Incorporation of capric acid (10:0) into fish oil TAG using immobilized lipase from *R. miehei* (IM 60) has also been reported (Jennings and Akoh, 1999). The fish oil concentrate used contained 40.9% EPA and 33.0% DHA. After a 24-hour incubation in hexane, there was an average of 43% incorporation of capric acid into fish oil, while the content of EPA and DHA decreased to 27.8% and 23.5%, respectively. Furthermore, capric acid (10:0) and EPA were incorporated into borage oil using lipase from *C. antarctica* and *R. miehei* as biocatalysts. Higher incorporation of EPA (10.2%) and 10:0 (26.3%) was obtained with *R. miehei* lipase, compared to 8.8% and 15.5%, respectively, with *C. antarctica* lipase.

A single-cell oil (produced by a marine microorganism, *Schizochytrium* sp.) containing docosapentaenoic acid (DPA; 22:5*n*-6) and DHA and caprylic acid (8:0) were used to produce a structured lipid using lipases from *R. miehei* and *Pseudomonas* sp. (Iwasaki *et al.*, 1999). The products contained caprylic acid at the *sn*-1 and *sn*-3 positions and DHA or DPA at the *sn*-2 position of glycerol. When *Pseudomonas* sp. was used, more than 60% of total fatty acids (palmitic, myristic, pentadecanoic, stearic, DHA, and DPA) in single-cell oil was exchanged with caprylic acid. The amount of TAG containing two caprylic acid and one DHA or DPA residue was 36%. With *R. miehei* lipase, the

TABLE 9.3 Enzymatic Modification of Seal Blubber Oil with Capric Acid to Produce Structured Lipids

Fatty Acid (wt%)	Before Modification	After Modification
10:0	–	27.1
14:0	3.4	2.7
14:1	1.0	0.8
16:0	5.0	3.7
16:1*n*-7	15.1	11.9
18:1*n*-9 and *n*-11	26.4	19.3
18:2*n*-6	1.3	1.7
20:1*n*-9	15.0	9.1
20:5*n*-3	5.4	2.3
22:1*n*-11	3.6	1.9
22:5*n*-3	4.9	3.0
22:6*n*-3	7.9	7.6

incorporation of caprylic acid was only 23%. A large amount of DHA and DPA remained unexchanged with this enzyme, so that the resulting oil was rich in TAG species containing two or three DHA or DPA residues (46%). The difference in the degree of acidolysis by the two enzymes was suggested to be due to their different selectivity towards DPA and DHA, as well as the difference in their positional specificities.

Hamam and Shahidi (2005, 2006b) prepared a number of structured lipids containing long-chain *n*-3 PUFAs and studied their oxidative stability. They found that a loss of endogenous tocopherols in the oil during the enzymatic esterification was responsible for the compromised oxidative stability of the structured lipids. It was further demonstrated that tocopherols were esterified with the FFAs in the medium and hence provided support for the mechanism by which tocopherols were lost during the process (Hamam and Shahidi, 2006b).

D. Production of *n*-3 Polyunsaturated Fatty Acid Concentrates from Marine and Algal Oils

Concentrates of *n*-3 fatty acids from marine and algal oils may be obtained using a number of techniques. The resultant concentrates may be in the form of FFAs, simple alkyl esters, or acylglycerols. Techniques that may be used include urea complexation, low-temperature crystallization, chromatography, distillation, supercritical fluid extraction, and enzyme-assisted reactions (Shahidi and Wanasundara, 1998).

For the preparation of PUFA concentrates on a large scale, each of the above physical and chemical methods has some disadvantages in terms of low yield, a requirement for large volumes of solvent or sophisticated equipment, a risk of structural changes in the fatty acid products, or high operation costs. Lipases work under mild temperature and pH conditions (Gandhi, 1997), and hence their potential use for the enrichment of PUFAs in oils is of much interest. Lipases (EC 3.1.1.3) are enzymes that catalyze the hydrolysis, esterification, interesterification, acidolysis, and alcoholysis reactions. The common feature among lipases is that they are activated by an interface. Lipases have been used for many years to modify the structure and composition of food lipids. Lipases act on neutral lipids and generally hydrolyze the esters of PUFAs at a slower rate than those of more saturated fatty acids (Villeneuve and Foglia, 1997). This relative substrate specificity has been used to increase the concentration of *n*-3 PUFAs in seal blubber and menhaden oils by subjecting them to hydrolysis by a number of microbial lipases (Wanasundara and Shahidi, 1998). The concentration of *n*-3 fatty acids by enzyme-assisted reactions involves mild reaction conditions and provides an alternative to the traditional concentration methods such as distillation and chromatographic separation. Furthermore, concentration via enzymatic means may also produce *n*-3 fatty acids in the acylglycerol form, which is nutritionally preferred.

In general, PUFAs in TAG molecules are resistant to *in vitro* hydrolysis by pancreatic enzymes. Microbial lipases have also been found to discriminate against PUFAs in enzyme-catalyzed reactions. Therefore, it is possible to concentrate *n*-3 fatty acids of marine oils. Preparation of *n*-3 PUFA-enriched acylglycerols from seal blubber and menhaden oils has been attempted via enzymatic hydrolysis (Wanasundara and Shahidi, 1998). Several microbial enzymes, namely lipases from *C. cylindraceae*, *Rhizopus oryzae*, *Pseudomonas* sp., *Chromobacterium viscosum*, *Geotrichum candidum*, *Rhizopus niveus*, *M. miehei*, and *Aspergillus niger*, were screened to enrich *n*-3 PUFAs in both oils. All microbial lipases tested were able to hydrolyze fatty acids in both oils, but at different rates (Table 9.4). Among the enzymes examined, *C. cylindraceae* was found to be the most effective biocatalyst for the production of *n*-3 fatty acid concentrates from seal blubber oil. However, in menhaden oil, lipase from *R. oryzae* gave the highest degree of hydrolysis. Other lipases studied gave lower degrees of hydrolysis than *C. cylindraceae* and *Rhizopus oryzae* lipases in both oils. At a given hydrolysis time, all lipases had considerably higher degrees of hydrolysis in seal blubber oil than in menhaden oil (data not shown). This difference may be due to the presence of higher amounts of PUFAs, especially EPA and DHA, in menhaden oil than in seal blubber oil, which exhibit resistance to enzymatic hydrolysis.

Among the enzymes tested, lipase from *C. cylindraceae* appeared to be the most active biocatalyst in increasing the contents of total *n*-3 fatty acids in the non-hydrolyzed fraction of both seal blubber and menhaden oils (Table 9.4). In menhaden oil, the total *n*-3 fatty acid content increased from 30% (original oil) to 46.4% and 46.1% after 75 hours of hydrolysis by lipases from *R. oryzae* and *C. cylindraceae*, respectively. In seal blubber oil the maximum increase in total *n*-3 fatty acids, from 20.2% to 45.0%, was reached when lipase from *C. cylindraceae* was used under similar experimental conditions (Table 9.4). The use of enzymes to produce *n*-3 fatty acid concentrates has an advantage over traditional methods of concentration (chromatographic separation, molecular distillation, etc.) since such methods involve extremes of pH and high temperatures which may partially destroy the natural all-*cis* *n*-3 PUFAs by oxidation and by *cis*–*trans* isomerization or double-bond

TABLE 9.4 Enzymatic Hydrolysis of Seal Blubber and Menhaden Oils

	Seal Blubber Oil		Menhaden Oil	
Enzyme Source	Hydrolysis (%)[a]	Total *n*-3 Fatty Acid Content (%)	Hydrolysis (%)[a]	Total *n*-3 Fatty Acid Content (%)
Candida cylindraceae	84	45	60	46
Rhizopus oryzae	77	34	70	46
Pseudomonas sp.	70	26	53	39
Mucor miehei	59	29	51	40
Chromobacterium viscosum	52	25	50	38
Geotrichum candidum	40	30	33	43
Rhizopus niveus	38	25	25	37
Aspergillus niger	26	23	5	35

[a]*After 75 hours of hydrolysis.*

migration. Therefore, mild conditions used in enzymatic hydrolysis provide a promising alternative that could also save energy and increase product selectivity.

The mechanism of resistivity of lipases towards the long-chain *n*-3 PUFAs in marine oils has been demonstrated by Bottino *et al.* (1967). The presence of carbon−carbon *cis*-double bonds in the fatty acids results in bending of the chains. Therefore, the terminal methyl group of the fatty acid lies close to the ester bond, which may cause a steric hindrance effect on lipases. The high bending effect of EPA and DHA is due to the presence of five and six double bonds, respectively, which enhance the steric hindrance effect; therefore, enzymes cannot reach the ester linkage of these fatty acids and glycerol. However, saturated and monounsaturated fatty acids do not present any barriers to lipase approach and could be easily hydrolyzed. Therefore, the fatty acid selectivity of a lipase for EPA and DHA allows separation of these fatty acids from the remaining fatty acids in marine oils, which is very important in the production of *n*-3 fatty acid concentrates. In addition, lipases have frequently been used to discriminate against EPA and DHA in concentrates containing both of these fatty acids; this enables the preparation of both EPA- and DHA-enriched concentrates.

Much attention has also been paid to using microbial lipases to produce *n*-3 fatty acid concentrates either by hydrolysis or by transesterification reactions of marine oils. Tanaka *et al.* (1992) used a number of microbial lipases (from *C. cylindraceae*, *R. oryzae*, *Pseudomonas* sp., *Ch. viscosum*, and *A. niger*) to hydrolyze tuna oil and found that *C. cylindraceae* lipase was the most effective biocatalyst in increasing the DHA content in the concentrates. This enzyme was able to increase the DHA content in the non-hydrolyzed fraction to three times that present in the original tuna oil; however, other lipases did not increase the DHA content in the oil. Shimada *et al.* (1994) reported that hydrolysis of tuna oil by lipase from *G. candidum* increased the content of both EPA and DHA from 32.1% to 57.5%. In another study, Hoshino *et al.* (1990) used several lipases for selective hydrolysis of cod liver and sardine oils. The best hydrolysis results were obtained for the non-specific *C. cylindraceae* lipase and *sn*-1,3 specific *A. niger* lipase, but none of the enzymes were able to raise the EPA content of the acylglycerols to any great extent. However, over 50% of total fatty acids were produced when these two enzymes were used.

The *n*-3 PUFA concentrates obtained using urea complexation and/or other concentration methods (Shahidi and Wanasundara, 1998) may be reacted with glycerol by enzymatic means to produce concentrates in the acylglycerol form. This form of *n*-3 fatty acid is preferred over FFAs and their alkyl esters. While TAG could be prepared, the presence of partial acylglycerols cannot be easily avoided. Various enzymes are known to catalyze the formation of acylglycerols. Osada *et al.* (1992) used lipases from *Ch. viscosum* and *C. cylindraceae* for direct esterification of glycerol with individual FFAs, including EPA and DHA. The lipase from *Ch. viscosum* was superior to *C. cylindraceae* lipase and resulted in 89−95% incorporation. With the latter lipase, 71−75% incorporation was obtained for all fatty acids, except for DHA, which reached 63% incorporation.

E. Low-Calorie Structured and Specialty Lipids

The high incidence of obesity due to overconsumption of lipids or high-calorie foods has encouraged researchers to develop products that are only partially digestible and provide fewer calories than regular fats and oils while being similar to conventional lipids in other aspects. Thus, the production of low-calorie lipids, which are characterized by a combination of SCFAs and/or MCFAs and LCFAs in the same glycerol backbone, is an active area of research and development in the field of structured and specialty lipids. Interest in these types of products emerged from the fact that they contain 5−7 kcal/g compared to the 9 kcal/g of conventional fats and oils, because of the lower caloric content of SCFAs or MCFAs compared to their long-chain counterparts. Reduced-calorie specialty lipids are designed for use in baking chips, coatings, dips, bakery and dairy products, or as cocoa butter substitutes. Currently, such products are synthesized by random chemical interesterification between a short-chain triacylglycerol (SCT) and an LCT, typically a hydrogenated vegetable oil such as soybean or canola oil (Smith *et al.*, 1994). Examples of commercially available low-calorie lipids include Caprenin®, Salatrim®, and Neobee®, as briefly reviewed here.

Caprenin is composed of one molecule of a very long-chain saturated fatty acid, behenic acid (C22:0), and two molecules of medium-chain saturated fatty acids, caprylic acid (C8:0) and capric acid (C10:0), and is a commercially available reduced calorie structured lipid. It provides 5 kcal/g compared to 9 kcal/g of conventional fats and oils. This product was first produced by Procter & Gamble Company. The constituent fatty acids for Caprenin synthesis are obtained from natural food sources. For example, caprylic and capric acids are obtained by fractionation of palm kernel and coconut oils while behenic acid is produced from rapeseed oil. Behenic acid, being a very long-chain saturated fatty acid, is poorly absorbed regardless of its position on the glycerol moiety. The MCFAs provide fewer calories than absorbable LCFAs. Caprenin displays functional characteristics similar to cocoa butter and can be used as a cocoa butter substitute in selected confectionary products. It is digested, absorbed, and metabolized by the same pathway as other TAGs (Artz and Hansen, 1996). Caprenin is a liquid or semi-solid product at room temperature, has a bland taste and is fairly heat stable. A petition by Proctor & Gamble to the United States Food and Drug Administration (FDA) for Caprenin for use as generally recognized as safe (GRAS) in soft candy bars and in confectionary coatings for nuts, fruits, and cookies was made, but subsequently withdrawn.

Salatrim, another reduced calorie structured lipid, is composed of a mixture of very short-chain fatty acids (C2:0−C4:0) and LCFAs (predominantly C18:0) (Smith *et al.*, 1994). The SCFAs are chemically transesterified with vegetable oils such as highly hydrogenated canola or soybean oil. The very short-chain fatty acids reduce the caloric value to approximately 5 kcal/g and LCFAs provide lipid functionality. Salatrim was developed by Nabisco Foods Group and is now marketed under the brand name Benefat™ by Cultor Food Science, Inc. It has the taste, texture, and functional characteristics of conventional fats. It may display different melting profiles depending on the amounts of SCFA and LCFA used in its chemical synthesis. Reduced fat baking chips are one of the products in the market that contain Salatrim and were introduced to the market in 1995 by Hershey Food Corporation. Salatrim received FDA GRAS status in 1994 and can also be used as a cocoa butter substitute. It was intended for use in chocolate-flavored coatings, chips, caramel, fillings for confectionery and baked goods, peanut spreads, savory dressings, dips and sauces, and dairy products (Kosmark, 1996).

Neobee, another caloric reduced fat, is composed of capric and caprylic acids and produced by Stepan Company. This class of specialty lipids includes different products. For example, Neobee 1053 and Neobee M-5 contain both capric and caprylic acids, while Neobee 1095 is made up of only capric acid (Heydinger and Nakhasi, 1996). Neobee 1095 is a solid product. Therefore, this product may be suitable in certain applications which require solid fats. Neobee 1814 is an MCT derivative made by interesterification of MCT with butter oil (Babayan *et al.*, 1990); it contains half of the long-chain saturated fatty acids found in conventional butter oil and is suitable to replace butter oil in a variety of applications. Neobee 1814 may serve as a flavor carrier and functions as a textural component for low-fat food products (Heydinger and Nakhasi, 1996).

F. Modified Lipids in Health Promotion and Disease Risk Reduction

Various fatty acids (*n*-3 and *n*-6 series) may be incorporated into structured and modified lipids to promote health and nutrition. These fatty acids have shown health benefits related to various disease conditions such as arthritis, thrombosis, cardiovascular disease, diabetes, and cancer (Horrobin, 1990; Vartak *et al.*, 1997; Senanayake and Shahidi, 2000).

Diets rich in *n*-3 and *n*-6 fatty acids have shown beneficial effects in arthritic patients. Arthritic patients demonstrated a significant improvement in morning stiffness and number of tender joints when consuming EPA supplements compared to placebo in a double-blinded, crossover study (Kremer *et al.*, 1987).

Thrombosis is the formation of blood clots. Blood clotting involves the clumping together of platelets into large aggregates and is triggered when endothelial cells lining the artery walls are damaged. If the platelet membranes are rich in long-chain *n*-3 PUFAs, formation of certain eicosanoids such as prostacyclin I_3 and thromboxane A_3 is promoted. These do not trigger platelet aggregation as much as the corresponding eicosanoids, prostacyclin I_2 and thromboxane A_2, that are formed from *n*-6 PUFA. Therefore, long-chain *n*-3 PUFAs may help to reduce the tendency for blood to clot (Groom, 1993).

Inuits in Greenland have been shown to suffer from a lower incidence of cardiovascular disease compared to their Danish counterparts (Bang and Dyerberg, 1972, 1986). Dyerberg *et al.* (1975) suggested that the relatively high dietary *n*-3 PUFA intake of Inuits was related to their lower incidence of cardiovascular disease. Mori *et al.* (1997) suggested that *n*-3 fatty acid intake from fish consumption in conjunction with a low-fat diet was most beneficial in terms of reducing cardiovascular disease. Recent studies indicate that the *n*-3 fatty acids, especially EPA and DHA, may be effective in reducing the clinical risk of cardiovascular disease by favorably altering lipid and hemostatic factors such as bleeding time and platelet aggregation (Hornstra, 1989). Dietary supplementation of *n*-3 fatty acids has also been recommended for lowering the risk of cardiovascular disease and improving the overall health of humans, mainly due to lowering both the TAG levels in the plasma and the incidence of arrhythmia, among others.

Possible effects of *n*-3 PUFA on diabetic patients have been studied. The development of insulin resistance in normal rats fed a high-fat, safflower oil diet was found to be prevented by partial replacement of linoleic acid with EPA and DHA from fish oil (Storlien *et al.*, 1987). From human studies on diabetes, it is clear that *n*-3 PUFAs exert beneficial effects on lipid metabolism and may decrease the severity of cardiac disorder and hence lower the incidence of coronary artery disease (Bhathena, 1992).

Mitsuyoshi *et al.* (1992) studied the effect of structured lipid, containing caprylic and linoleic acids, as an energy substrate after hepatic resection in diabetic rats. The lipid sources used in this study were MCT, LCT, a simple physical mixture of MCT/LCT, and structured lipid. The blood ketone body ratio (acetoacetate/β-hydroxybutyrate) and the cumulative excretion of $^{14}CO_2$ in expired breath after [^{14}C]glucose administration were significantly higher in the structured lipid group than in the other groups. These findings suggest that structured lipids may be a superior energy substrate compared to other TAG preparations during the critical period after hepatectomy in diabetic patients.

Ling *et al.* (1991) demonstrated that tumor growth in mice was decreased when they were fed with a structured lipid made from fish oil and MCT. In another study, the tumor growth rate was reduced in rats fed with SL containing MCFAs and fish oil (Mendez *et al.*, 1992). In contrast to the tumor-promoting effects of diets high in fat, diets high in fish oil failed to promote tumor development in rats (Branden and Carroll, 1986). Reddy and Maruyama (1986) also showed that diets containing high levels of fish oil inhibit or suppress tumor growth in animal models. Dietary intake of fish oils was effective in destroying some cancer cells, but it is not known whether such results are reproducible with humans, or what potential side-effects exist (Haumann, 1997b). Although it is known that *n*-3 PUFAs play an important role in the growth of certain cells in the human body, the mechanisms involved in their effect on cancer treatment remains somewhat elusive.

Impact® (Novartis Nutrition) is another example of a structured lipid, produced by interesterifying a high-lauric oil with a high-linoleic acid oil. This product has been used for patients suffering from trauma, surgery, sepsis, or cancer (Haumann, 1997a). A structured lipid containing MCFA and linoleic acid is more effective in cystic fibrosis patients than safflower oil, which has about twice as much linoleic acid (McKenna *et al.*, 1985). The structured lipid diet, Impact, containing low levels of linoleic acid, resulted in decreased infection and decreased length of hospital stay compared to other enteral formulae. Bower *et al.* (1995) also demonstrated a decreased length of hospital stay and infection rate when using diets with a low level of linoleic acid and added fish oil.

G. New Developments in Production of Fatty Acid Conjugates and their Potential Health Effects

Recent studies have demonstrated unexpected potential benefits when conjugating *n*-3 fatty acids such as stearic acid, EPA, and DHA with other bioactive molecules such as the green tea polyphenol, epigallocatechin gallate (EGCG), and phytosterols. The EGCG−DHA products thus obtained displayed excellent bioactivities including antioxidant, anti-inflammatory, antiviral, and anticancer properties, some of which were greater than that of the EGCG or DHA alone (Shahidi and Zhong, 2010). The EGCG−fatty acid conjugates acted as radical scavengers

against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and peroxyl radicals and metal ion chelators which were more potent than or comparable to EGCG. The conjugates were able to inhibit lipid oxidation in food model systems (bulk oil, oil-in-water emulsion, and meat), suggesting their potential use as antioxidant preservatives in the food industry. They have also shown effectiveness in protecting various biological model systems from oxidative damage, including copper-induced low-density lipoprotein-cholesterol oxidation, radical-induced DNA scission, and ultraviolet-induced liposome photooxidation. These suggest their antiatherosclerotic, antimutagenic, and membrane protection properties, and hence their potential in preventing/treating cardiovascular diseases, cancer, and skin disorders, among other oxidation-mediated diseases. In addition, the EGCG−fatty acid conjugates exhibited antiviral activity by inhibiting NS3/4A protease and α-glucosidase, which are important enzymes for the infectivity of hepatitis C virus and human immunodeficiency virus, respectively. The antiviral effect was not found for EGCG itself. Moreover, the DPA conjugates of EGCG played an anti-inflammatory role in lipopolysaccharide-stimulated murine macrophages by suppressing the gene expression of inducible nitric oxide synthase and cyclooxygenase-2. The EGCG−DHA conjugates were found to be effective in inhibiting azoxymethane-induced colon tumorigenesis. Meanwhile, the docosahexaenoate esters of phytosterol effectively reduced plasma cholesterol levels and atherosclerotic lesions in mice (Tan *et al.*, 2012). Thus, conjugation of polyphenols or phytosterols with fatty acids, especially long-chain *n*-3 PUFAs, may be useful in developing novel bioactives with health-promoting properties as potential functional food ingredients and as natural health products.

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