CHAPTER 2

LITERATURE REVIEW

2.1 n-3 Polyunsaturated Fatty Acids (n-3 PUFAs)

Fatty acids are monobasic carboxylic acids consisting of a single carboxylic group attached to the end of a straight hydrocarbon chain. With some exceptions, most fatty acids that occur in nature are straight chain acids which contain an even number of carbon atoms (Sonntag, 1979). The fatty acids that contain double bond are termed unsaturated fatty acids. The term PUFAs, consisting more than one double bond, covers a wide range of acids of 18, 20 and 22 carbon chain length with two to six methylene interrupted double bonds (FAO, 1980). The PUFAs are classified into two groups. One is essential fatty acids and another is non-essential fatty acids. The two series of essential fatty acids, n-3 and n-6, are defined by the position of the first double bond closer to the methyl group of the fatty acids (Lauritzen *et al*., 2001). The n-3 fatty acids have their first double bond at the third carbon atom, while n-6 fatty acids have their first double bond at the sixth carbon atom (Garcia, 1998).

2.1.1 Source of n-3 PUFAs

Two of the important n-3 long-chain PUFAs are eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Adult humans could derive the n-3 long-chain PUFAs, from alpha linolenic acid (ALA, C18:3n-3) by chain elongation and desaturation. However, only a small amount of ALA is converted to DHA (less than 9%)(Burdge *et al*., 2002;

Burdge and Wootton, 2002). Rather these fatty acids are synthesized mainly by both uni- and multi- cellular marine plants such as phytoplankton and algae (Shahidi and Wanasundara, 1998). They are eventually transferred through the food web and are incorporated into lipids of aquatic species such as fish and marine-animals thus fish oil and marine oils are plentiful in n-3 PUFAs (Harris, 2004). Generally speaking, the "oilier" the fish, the more EPA and DHA are present. Fish that tend to have high concentrations of oil include tuna, sardines, salmon, mackerel, and herring (Table 1).

Table 1 Amount of EPA and DHA in fish, crustacean* and fish oils

	EPA+DHA content, g/3-oz Serving Fish (Edible Portion) or g/g Oil
Fish	
Tuna	
Light, canned in water, drained	0.26 0.73
White, canned in water, drained	0.24-1.28 0.98-1.70
Fresh	
Sardines	0.68
Salmon	0.68
Chum	1.09
Sockeye	1.48
Pink	1.09-1.83
Chinook	0.90-1.56
Atlantic, farmed	0.34-1.57
Atlantic, wild	
Mackerel	1.81
Herring	1.71
Pacific	

Atlantic	0.98
Trout, rainbow	0.84
Farmed	0.40-1.00
Wild	
Halibut	0.13
Cod	0.24
Pacific	0.20
Atlantic	
Haddock	0.15
Catfish	0.20
Farmed	0.42
Wild	
Flounder/Sole	

Table 1 (Continued)

	EPA+DHA content, g/3-oz Serving Fish (Edible Portion) or g/g Oil
Crustacean	
Oyster	
Pacific	1.17
Eastern	0.47
Farmed	0.37
Lobster	0.07-0.41
Crab, Alaskan King	0.35
Shrimp, mixed species	0.27
Clam	0.24
Scallop	0.17
Fish Oil	
Cod liver oil**	0.19
Standard fish body oil	0.30
Omega-3 fatty acid concentrate	0.50

Source: Adapted from Kris-Etherton *et al*. (2002)

*The intakes of fish given above are very rough estimates because oil content can vary markedly (>300%) with species, season, diet, and packaging and cooking methods.
**This intake of cod liver oil would provide approximately the Recommended Dietary Allowance of vitamins A and D.

2.1.2 Health Benefits of n-3 PUFAs

- Cardiovascular and heart disease effects

Interest of fish oil and marine diet concerning health benefits came from the study of Arthaud (1970 by Furst and Kuhn, 2000) in native Alaskans more than 30 years ago, which had a lower than expected occurrence of death due to ischemic heart disease (IHD). The similar results also reported from Greenland Eskimos studies (Dyerberg *et al*., 1978), which indicating that even though these Eskimos had a diet very high in fat, they had a very low rate of IHD. The beneficial effects of n-3 PUFAs to reduce cardiovascular disease have been ascribed to their ability to lower serum lipids and cholesterol levels and enhance their excretion, to increase membrane fluidity (Kinsella, 1986; Kris-Etherton *et al*., 2003), to generate prostanoids and leukotrienes with anti-inflammatory actions, and to inhibit synthesis of cytokines and mitogens that augment the inflammation and promote plaque formation (Uauy and Valenzuela, 2000; Kris-Etherton *et al*., 2003). Studies in humans have shown that n-3 PUFAs (~4 g/d) decreased serum triglyceride concentrations by 25% to 30%, with accompanying increases in LDL cholesterol of 5% to 10% and in HDL cholesterol of 1% to 3% (Kris-Etherton *et al*., 2002). Because n-3 fatty acid treatment has failed consistently to stimulate postheparin lipoprotein lipase or hepatic lipase activity, it has been assumed that the primary mechanism by which n-3 fatty acids lower triglycerides is by lowering production, not by enhanced clearance (Uauy and Valenzuela, 2000). The anti-inflammatory effect, one benefits on cardiovascular disease, of n-3 fatty acids may due to they displace the n-6 fatty acid arachidonic acid in lipoxygenase and cyclooxygenase pathway

(the biosynthesis of thromboxane A_2, leukotriene B_4, and all 2-series of prostaglandins), which could reduce pro-inflammatory and pro-thrombotic (Furst and Kuhn, 2000; Uauy and Valenzuela, 2000; Harris, 2004).

- Tumor and cancer effects

The n-6, arachidonic acid, can be converted to eicosanoid substance (2- series prostaglandins) by cyclooxygenase and lipoxygenase that associated with tumor growth (Kinsella, 1986). Therefore, the factors which inhibit prostaglandins synthesis can inhibit mammary cancer. It was known that n-3 PUFAs was a significant inhibition of n-6 fatty acid derived eicosanoid biosynthesis and thereby depression of tumor growth. For example, human breast carcinoma growth was suppressed in female athymic nude mice that consumed diet containing menhaden oil (Gonzalez *et al*., 1993). The risk of prostale cancer is also reduced with consumption of n-3 fatty acids (Norrish *et al*., 1999; Terry *et al*., 2001). Experiments have shown that the Swedish men who ate moderate or high amounts fish had a two-fold to three-fold lower frequency of prostate cancer than those who ate non (Terry *et al*., 2001).

- Visual function effect

DHA is efficiently retained in the retina as it is recycled via the pigment epithelium upon renewal of rod outer segment (ROS) disk membrane. DHA is most highly concentrated in rods and molecular investigating has focused on the importance of DHA in ROS membrane for the function of rhodopsin, the visual pigment of rods, (Lauritzen *et al*., 2001). A number of studies have suggested that the content of DHA had a positive influence on the function of cell membranes. Preterm infants receiving human milk or infant formula with DHA have a better visual acuity than those receiving infant formula without DHA (Jonsbo *et al*., 1995).

- Brain development effect

The adult brain contains approximately 50-60% of its dry weight as lipid, and approximately 35% of the lipids are

PUFAs, especially DHA (Lauritzen *et al*., 2001). High level of DHA are found in the more metabolically active areas of the brain, including the cerebral cortex, microchondria, synaptosomes, and synaptic vesicles (Morris *et al*., 2003). Within the mammalian brain, DHA containing phospholipids seem to correlate with development of the synapses and may play an important role in the survival of mammalian neuronal cells. Some studies indicate that there might be possible effects on receptor properties. Whereas other studies indicated that n-3 PUFAs content of membranes could exert an effect on receptor activity of the signal transduction pathway (Lauritzen *et al*., 2001). In humans, it may be possible for the nerve cells to transmit information to other nerve cells smoothly by growing "off shoots" called dendrites. Growth of dendrites can be triggered by any stimulation. DHA is a membrane component required for the growth of dendrites (Suzuki, 1993). A large number of animal studies have demonstrated that dietary n-3 fatty acids increased learning acquisition and memory performance (Jensen *et al*., 1996; Greiner *et al*., 1999; Carrie *et al*., 2000). For epidemiologic studies found that 131 persons of 815 sample participants were diagnosed as having incident Alzheimer disease, which less than 1% of the sample was taking n-3 fatty acids dietary supplements. Participants who consumed fish once per week or more had 60% less risk of Alzheimer disease compared with those who rarely or never ate fish in a model adjusted for age and other risk factors (Morris *et al*., 2003).

2.1.3 Intake of n-3 PUFAs

For general cardioprotection, the American Heart Association (AHA) recommends that all adults intake about 0.5 g of EPA and DHA per day or eat fish (particularly fatty fish) at least twice a week. For patients with known coronary heart disease, the AHA recommend about 1 g of EPA and DHA daily. Much higher intake, i.e., from 2 to 4 g per day, may be useful in

patient with hypertriglyceridemia to reduce triglyceride levels, and this should be done in consultation with a physician (Kris-Etherton *et al*., 2003; Harris, 2004). Generally, eating fish does not present a health hazard. However, some species of fish may contain significant level of methyl-mercury, polychlorinated biphenyls (PCBs), dioxins, and other environmental contaminants (Kris-Etherton *et al*., 2002). PCBs and methylmercury have long half-lives in the body and can accomulate in people who consume contaminated fish on a frequent basis. Mercury toxicity is mainly a concern for fetus and breast- feed infants (Harris, 2004). Two recent epidemiologic studies have reported conflicting finding about wheather there is an association between methylmercury exposer and cardiovascular heart disease (CHD), or which one shows a negative effect on CHD in adult men, whereas other reported no association between methylmercury exposure and CHD (Kris-Etherton *et al*., 2003). While further studies are needed to resolve this issue, fish oil capsules and enriching foods with n-3 fatty acids are alternative option for increasing consumption of these fatty acids. Both oils and powders (produced by microencapsulation technology) enriched with either EPA or DHA are available for nutritional products and for medical foods (Uauy and Valenzuela, 2000). The products containing fish oils or highly unsaturated fatty acids are susceptible to oxidation and may impart a fishy aroma or flavor, thus efforts to keep these products from oxidative rancidity during processing, cooking, and storage are necessary. Controlling oxidation and prevention of fishy aroma or flavor, are presently a major technological challenge for the industry.

2.2 Transesterification

Overconsumption of fish oils, especially fish liver oil can potentially lead to complications associated with vitamin A and D overdose, and increase intake of cholesterol and other saturated fatty acids. Therefore, concentrating or enriching fish

oil with n-3 PUFAs is currently interesting. Devoid of saturated and monosaturated fatty acids, fish oils can improve health function (Shahidi and Wanasundara, 1998). Fish oils consist primarily of triglyceride containing fatty acids in much greater variety of chain lengths and degree of unsaturation. Separation of individual fatty acids is difficult for production of highly concentrated n-3 components (Schlenk and Sand, 1967). Interesterification is a reaction that exchange carbonyl group of fatty acids within and between triglyceride molecule and it is used to modify the structure and composition of oils to improve their physical and nutrition properties of triglyceride (Basheer *et al*., 1995). Transesterification or ester interchange (interchange carbonyl group between oil and another oil) is one of three reactions associated with interesterification (Stirton, 1964; Marangoni and Rousseau, 1995).

2.2.1 Distinction between Enzymatic and Chemical Transesterification

Presently, there are two types of transesterification in use i.e. enzymatic and chemical transresterification. Usually, enzymatic transesterification is performed using lipase as catalysts. Lipase or acylglycerol ester hydrolases (E.C. 3.1.1.3) are enzymes capable to catalyze cleavage of carboxyl ester bound in tri-, di- and monoglyceride (Paiva *et al*., 2000). Enzymatic transesterification has many advantages such as milder processing condition and the possibility of regio-, stereo-, and fatty acid-specificity. Furthermore, the specificity that possible with enzymatic transformations permits structure unachievable by chemical means. However, enzymes are more expensive than chemical catalysts (Marangoni and Rousseau, 1995), and difficulties associated with process scale-up and control (Willis and Marangoni, 1999).

Chemical transesterification can be induced by chemical catalysts including alkali- metals, alcoholates, and sodium hydroxide with glycerol (Liu and Lampert, 1999). This reaction lacks specificity and offers little or no control over the

position distribution of fatty acids in the final product (Willis and Marangoni, 1999). Whereas the advantages of chemical transesterification over enzymatic transesterification include lower catalyst cost and also the existence and availability of industrial procedures and industrial equipment (Marangoni and Rousseau, 1995) that is easy to scale up (Willis and Marangoni, 1999).

For comparison of the change in triglyceride composition of oils such as sardine oil, it has been reported that the distribution of fatty acids in the triglyceride of chemically interesterified sardine oil was almost similar to that of the enzymatically interesterified oils. There were significant differences in the distribution of fatty acids between native and enzymatically interesterified sardine oils. Especially, the level of DHA in native sardine oil was decreased after enzymatic interesterification (Kimoto *et al*., 1994). The performance of lipase-catalyzed (Lipozyme IM 60 from *Mucor miehei*) and chemical catalyzed (sodium methoxide) transesterification, using canola oil and tricaprylin to produce the new structured triglyceride was investigated. The yield of new structured triglyceride for chemical tranesterification reaction (5h) was not significantly higher than for the lipase-catalyzed reaction (24 h). However, it must be kept in mind that reaction times were shorter than the lipase-catalyzed reaction (Willis and Marangoni, 1999).

2.2.2 Chemistry of Chemical Transesterification

There are two basic types of chemical transesterification; random and direct. In random transesterification, the reaction is initiated and completed at temperature above the melting point of the highest-melting triglyceride component in the mixture. Under such conditions, transesterification will rearrange all of the fatty acids into a

random pattern on the glycerol backbone after thermodynamic equilibrium is achieved. If transesterification is carried out at temperature below the melting point of the highest-melting triglyceride component, some fat with high melting point (usually a trisaturated triglyceride) are crystallized. The oils in the liquid phase will then reach a new thermodynamic equilibrium. The crystallized fats will not be involved in transesterification because they are separated from the reaction phase. This practice is known as direct transesterification that can be thought of as a combination of transesterification and fractionation (Marangoni and Rousseau, 1995; Liu and Lampert, 1999).

Chemical transesterification can be induced by chemical catalysts including alkali metals, alcoholates (Liu and Lampert, 1999), such as sodium or potassium alloy, metallic sodium, sodium methoxide and sodium hydroxide (Konishi *et al*., 1993). One of the most commonly used catalysts is sodium methoxide or sodium methylate. It is noted that the catalyst level, time and temperature of reaction vary widely (Table 2). This catalyst is easy to use, inexpensive, general active at relatively low temperature (50-90°C) and is required only in small amounts for catalysis. Furthermore, sodium methoxide catalyst is more active than other bases, metals or acid catalysts. However, it is extremely sensitive to moisture because water can react with the akylate to produce the corresponding alcohol, completely inactivating the catalyst. As even a trace amount of water will decrease the potency of the catalyst tremendously, oil should contain < 0.01% (w/w) water. Free fatty acid and peroxides also impair catalyst performance and their levels should be maintained as low as possible, preferably < 0.05% (w/w). Other factors that may influence transesterification onset include agitation intensity, catalyst particle size and temperature (Marangoni and Rousseau, 1995).

2.2.3 Mechanism of Chemical Transesterification

When sodium methylate powder is dispersed in previously dried oil maintained at about 60-80°C, white slurry is obtained. After heating for a while, the color of the mixture becomes brownish, indicating an onset of transesterification. This change of color is associated with the active catalyst formation, which is visualized as a reaction complex between the added catalyst and the triglyceride. Thus the methylate or any such added reagents act as initiators, and the true catalyst is the reaction complex (Sreenivasan, 1978). Two types of catalytic reaction mechanism have been suggested to explain chemical transeserification; the carbonyl addition mechanism and the enolate intermediate (Claisen condensation) mechanism (Figure 1). In the alkaline transesterification reaction, the catalyst (sodium methoxide) is a strong nucleophilic reagent that attacks the carbonyl carbon of a fatty acid-glycerol ester bond and forms a tetrahedral intermediate. The fatty acid methyl ester is then released, leaving behind a glycerylate anion that functions as the real catalyst and transfers acyl groups around the glyceride backbones. For the Claisen mechanism, the reaction starts when sodium methoxide attacks the α- hydrogen (acidic hydrogen) of an acyl group to form the enolate ion. This nucleophile will attack carbonyl groups, forming a β-keto ester and a glycerylate. The glycerylate is now free to attack other carbonyl carbons and exchange ester intra- and inter- molecule (Marangoni and Rousseau, 1995; Liu and Lampert, 1999).

Table 2 Reaction conditions for transesterification of sodium methoxide ($NaOCH_3$)

% $NaOCH_3$	Temperature (° C)	Time	References
0.1-0.5	50-92 under N_2 atmosphere	30-480 min	Haryati *et al*., 1999
0.2	60	5 hr	Willis and Marangoni, 1999

0.2	under vacuum		
	90	90 min	Petrauskaite *et al*.,
0.3	under vacuum		1998
	80-90	50 min	
0.5	under vacuum		Zeitoun *et al*., 1993
	80	5 hr	
0.5	under N_2		Kimoto *et al*., 1994
	atmosphere	30 min	
10	90		Schmidt *et al*., 1996
	under N_2	6 hr	
	atmosphere		Konishi *et al*., 1993
	30-60		
	under N2		
	atmosphere		

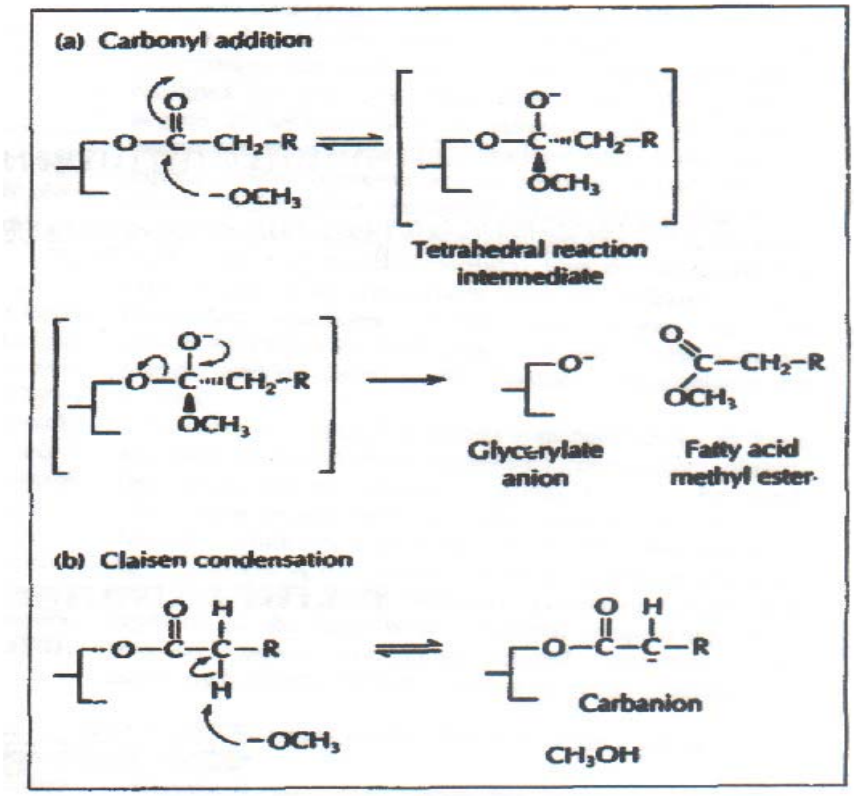

Figure 1 Proposed reaction mechanisms for chemical transesterification:
a), carbonyl addition and b), Claisen condensation.
Source: Marangoni and Rousseau (1995)

2.2.4 Applications of Chemical Transesterification in Food Lipids

Solid fats such as, margarine, are generally desired to crystallize in small, smoother, more palatable, β' crystals (Goh *et al*., 1993) since they aid in the incorporation of a large amount of air in the form of small air bubbles, giving rise to products of better plastic and creaming properties (Nawar, 1996). Whereas the larger, coarser, β crystals are usually avoided, specifically in the bakery and confectionary industries (Goh *et al*., 1993). It has been reported the effect on the physical properties of fat products formed by the transesterification of coconut oil with fully hydrogenated soybean oil. The blends before transesterification exhibited the β-form more than the β' -form, whereas after interesterification the β' -form was greater. That may be due to the randomization and rearrangement occurring during the transsterification reaction. Thus, the product-hard fat phase has the optimum texture and melting characteristics for use in margarine (Zeitoun *et al*., 1993).

Other uses of chemical transesterification include improving oxidative stability of fish oils such as skipjack oil based on peroxide value (Kimoto *et al*., 1994), and vegetable oils such as soybean oil transesterified with methyl stearate. Based on the peroxide value and volatile compounds content, soybean oil after transesterification was less susceptible to oxidation than the original soybean oil. The improved stability of the soybean oil was presumably due to incorporation of stearic acid into triglyceride that contained linoleic acid (Konishi *et al*., 1993).

Hydrogenation of lipids involves the addition of hydrogen to double bonds in the fatty acids chains. The process is of major importance in the fats and oils industries. During hydrogenation, not only some of the double bonds are saturated, but some may also be relocated and/or transformed from the

usual *cis* to the *trans* configuration. There is some evidence that dietary *trans* fatty acids, like most saturated fatty acids, raise levels of blood cholesterol (Nestel *et al*., 1992). Other researchers have warned the health risk of consuming *trans* fatty acids. There was the positive associations between intake of *trans* fatty acids and coronary heart disease (Asherio *et al*., 1994). With present consumer concerns about the *trans* fatty acids, chemical transesterification of liquid oils with an appropriate hardstock could open up new zero-*trans* plastic fats markets (Marangoni and Rousseau, 1995). Petrauskaite *et al*. (1998) used the oil blends to produce a zero-*trans* hard fat phase with melting characteristics similar to commercial fats. The transesterification did not produce *trans* isomer of unsaturated fatty acids. Therefore, *trans* fatty acid levels of transesterified blends were low (0.1%) compared to 1.3-12.1% in commercial food fats.

However, more currently interesting application is the incorporation of n-3 fatty acids into vegetable oils and the concentration of n-3 containing triglyceride in fish oil (Marangoni and Rousseau, 1995). For example, transesterification by sodium methoxide could enrich the EPA and DHA in cod liver oil from 9.1 and 6.9%mole to 10.7 and 7.7%mole, respectively (Kimoto *et al*., 1994).

2.3 Microencapsulation

Microencapsulation is used to transform liquid into dry and free flowing powders (Sheu and Rosenberg, 1995; Wagner and Warthesen, 1995; Dian *et al*., 1996; Pauletti and Amestoy, 1999). Microencapsulation is a process by which particles of sensitive or bioactive materials are packed into thin film of a coating material. The encapsulated materials such as fats and oils, aroma and flavor mixtures, etc. are called core materials, internal phase, or fill, and the films formed around the core are called the wall material or shell coating (Dziezak, 1988; Sheu and Rosenberg, 1995; Dian *et al*., 1996). The miniature

packages, called microcapsules, may range from submicrometer to several millimeters in size and have a multitude of different shapes, depending on the materials and methods used to prepare them (Pegg and Shahidi, 1999).

The food industry applies microencapsulation for a number of reasons: to stabilize and protect the core material from degradation by reducing its reactivity to its outside environment (e.g. heat, moisture, air, and light), to control the release of the core material (both the rate of release and the start of release), and to separate reactive or incompatible components of a formulation (Dziezak, 1988; Pegg and Shahidi, 1999). Moreover, encapsulation can modified the physical characteristics of the original material and made easier to handle. For example, a liquid component can be converted to solid particles; lumping can be prevented; hygroscopicity can be reduced; flowability and compression properties can be improved; dustiness can be reduced; and density can be modified (Pegg and Shahidi, 1999). The microcapsules offer the food processor a means to protect sensitive food components, ensure against nutritional loss, utilize otherwise sensitive ingredients, incorporate unusual or time-release mechanisms into the formulation, mask or preserve flavors and aromas, and transform liquids into easily handled solid ingredients (Dziezak, 1988). Various properties of microcapsules that may be changed to suit specific ingredient applications include composition, mechanism of release, particle size and final physical form (Pegg and Shahidi, 1999). The process of encapsulation generally consists of three stages including 1) establishment of a three phase system (material to be coated, liquid vehicle and coating material); 2) deposition of coating material over the product by emulsification; 3) solidification of coating material (Danviriyakul, 2001).

2.3.1 Corn Syrup Solids as Encapsulant

Encapsulants or coating substance or wall material or shell, which are basically film-forming materials, can be selected from a wide variety of natural or synthetic polymers, depending on the material to be coated and the characteristics desired in the final microcapsules. The composition of the coating material is the main determinant of the functional properties of the microcapsule and of how it may be used to improve the performance of a particular ingredient (Pegg and Shahidi, 1999).

Corn syrup solids are the products of starch hydrolysis that are produced by either heat and acid, or enzyme, or combined acid and enzymatic treatments (Chronakis, 1998). Major products of this reaction are D-glucose, maltose, and a series of oligosaccharides and polysaccharides such as maltose oligosaccharides, maltotriose, and maltotetraose mixtures. Classes of hydrolysates are divided according to their dextrose equivalent (DE) value indicating the reducing power relation to glucose of the same weight (as 100). Corn syrup solids by FDA (Food and Drug Administration) definition are the product of starch hydrolysis with DE equal or higher than 20. DE is a measurement of the degree of hydrolysis of the starch molecules that is inversely proportional to degree of polymerization (DP) or the number of anhydro β-D glucose units. For instance, molecules that are consisted of 20 glucose units have a corresponding DE of 5. The higher DE values, the smaller the molecular weight and the higher reducing power.

Originated from starch, corn syrup solids [$(C_6H_6O_6)_nH_2O$] consist of D-glucose units primary linked by alpha-1-4 bonds in linear chains and (1-4, 1-6) or (1-6) bonds at branched points. Their size ranges from oligomers to macromolecules (Chronakis, 1998). Different physical properties of corn syrup solids arise from the variation in their DE values. Viscosity and cohesiveness are enhanced by a decrease in DE. In contrast, hygroscopicity, solubility, osmolality, and an ability to reduce the freezing point increase as the DE increases.

Corn syrup solids have been used in encapsulated products to protect sensitive materials such as fat and oils, vitamins, and colorants from deteriorative reactions. Lin *et al*. (1995) studied the oxidative of microencapsulated squid oil in a wall system containing corn syrup solids. Polyene ratio of microencapsulated oil showed only a slight decrease after storage at 50°C up to 6 weeks. This suggested a reasonable protection of oil by wall materials.

Low DE corn syrup solids are non-hygroscopic providing free flowing properties of powders after drying. Higher DEs result in the increase of the hygroscopicity. Viscosity of corn syrup solids in a solution is relatively low (Danviriyakul, 2001). Thus they can be used at high concentration without exhibiting flow problem unlike other types of gums or carbohydrate polymers. This can greatly increase the economic efficiency of the production.

2.3.2 Emulsification

It is possible to form an emulsion by homogenizing pure oil and pure water together. Unfortunately, emulsions are thermodynamically unstable systems because of the unfavorable contact between oil and water phases and because the oil and water phases have different densities; hence, they will always break down overtime (Dickinson, 1992; Walstra, 1996; McClements, 1999). Emulsion destabilization may occur through a variety of different physicochemical processes (Figure 2), including gravitational separation (creaming and sedimentation), flocculation, coalescence, phase inversion and Ostwald ripening (McClements, 1999). For a particular emulsion-based product, the relative importance of these processes depends on the type of ingredients it contains, the way it was produced, and the environmental conditions it experiences during its manufacture, storage, and utilization.

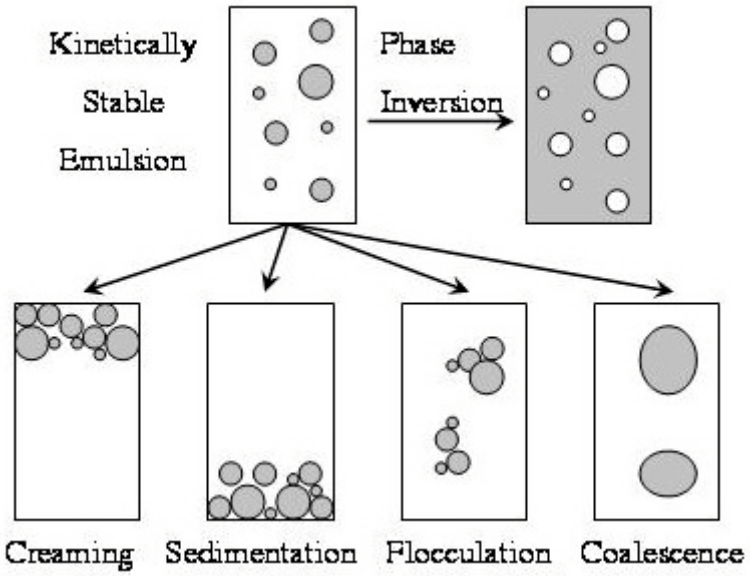

Figure 2 Instability of emulsion
Source: McClements, 1999

- Emulsifier

One of the most important and widely used methods of improving the stability of oil-in-water emulsions is to utilize emulsifiers (Dickinson, 1992). Emulsifiers are surface active ingredients that adsorb to the surface of fresh formed oil droplets during homogenization. One adsorbed, they facilitate further droplet disruption by lowering the interfacial tension, thereby reducing the size of the droplets produced during homogenization. Emulsifiers also reduce the tendency for droplets to aggregate by forming protective membranes and/or by generating repulsive forces between the droplets. A good emulsifier should rapidly adsorb to the surface of the oil droplets formed during homogenization, rapidly lower the interfacial tension by a significant amount, and protect the droplets against aggregation during emulsion processing, storage, and utilization (Dickinson, 1992; Walstra, 1996; McClements, 1999). A wide variety of different kinds of synthetic and natural emulsifier can be legally used in food emulsions, including small-molecule surfactants, phospholipid, proteins, and polysaccharides (Stauffer, 1999).

Lecithin is the commercial or popular name for a naturally occuring mixture of similar compounds more accurately identified as phosphatides or phospholipids. The principal components of the natural mixture are phosphatidyl-choline, phosphatidyl-ethanolamine, inositolphosphatides, and related phosphorus containing lipids. The chemical structures of the major phosphotides are given in Figure 3. Lecithin is nature's principal surface-active agent. It is found in all living cells of animal and vegetable origin. The major commercial source of lecithin is the soybean, which contain 0.3-0.6% phosphatides. The main function of phosphatides is to emulsify oils. The long-chain fatty acid moieties contribute hydrophobic properties; those properties are counter-balance by the polar or hydrophilic character of the phosphate moiety. In an oil-in-water emulsion the phosphatide components concentrate at the oil-water interface. The polar hydrophilic parts of the molecules are directed toward the aqueous phase and the non-polar, hydrophobic (or lipophilic) parts are directed toward the oil phase (Pomeranz, 1991).

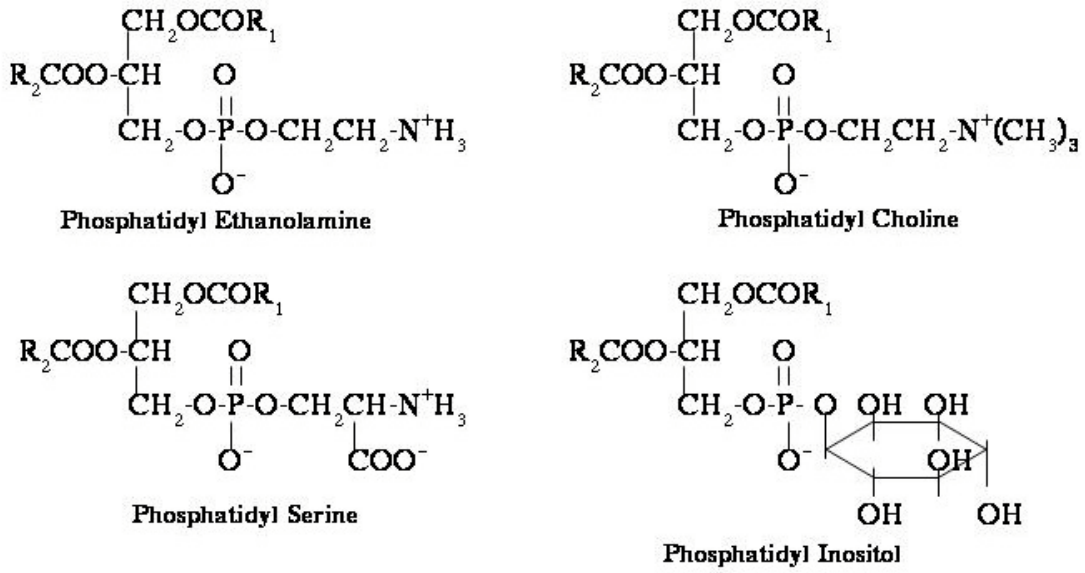

Figure 3 Molecular structural of major phospholipids; R = fatty acid residue.
Source: Adapted from Nawar (1996)

- Biopolymer-surfactants interaction

The droplets in many food emulsions have electrically charged surfaces because of the adsorbtion of emulsifiers which are either ionic or capable of being ionized (e.g., proteins, polysaccharides, and surfactants). The magnitude and sign of the electrical change on an emulsion droplet therefore depend on the type of emulsifier used to stabilize it, the concentration of the emulsifier at the interface, and the prevailing environmental conditions (e.g., pH, temperature, and ionic strength) (McClements, 1999).

The surface charge can be altered by the adsorption of surface-active ions; e.g. ionic emulsifiers and electrolyte ions due to electrostatic interactions (McClements, 1999). The charged droplet can also be adsorbed the opposite charge polymer to the droplet surface (Figure 4) (Goddard, 2002). Adsorbing polymers can cause bridging flocculation (Magdassi *et al*., 1997; Pinotti *et al*., 1997; Pinotti *et al*., 2001; Ogawa *et al*., 2003a). Bridging flocculation is restricted to low concentrations of a polymer with multiple anchore sites and a spatial extension beyond the range of the repulsive barrier between the colloid particles. Under these conditions the surfaces of the colloid particles are only partially covered and approaching particles easily become linked by polymer bridges, at distances that prevent any impact to the repulsive barrier (Magdassi *et al*., 1997; Syrbe *et al*., 1998). However, droplet flocculation in emulsions could be disrupt by homogenization or sonication, leading to the production of stable emulsion with relatively small particle diameter (Ogawa *et al*., 2003a).

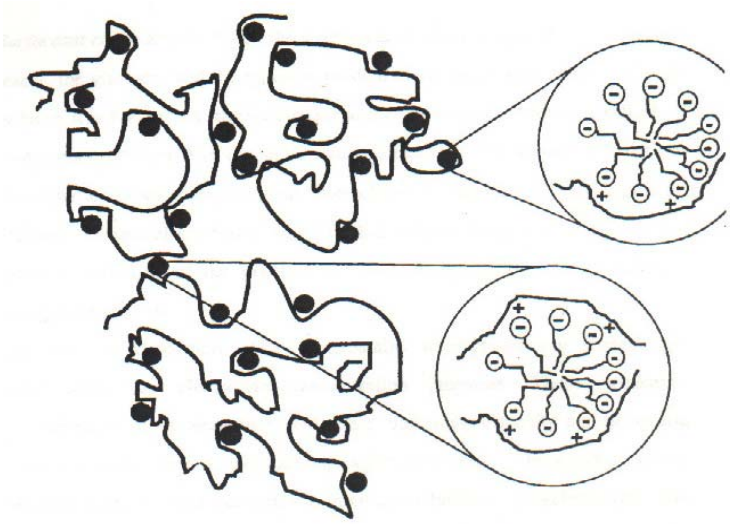

Figure 4 A schematic picture of the structure and the conformation of cationic starch/anionic surfactant complexes in dilute aqueous solutions.

Source: Merta *et al*. (1999)

Chitosan is the name used for low acetyl substituted form of chitin (Figure 5) and is composed primarily of glucosamine, 2-amino-2-deoxy-β-D-glucose, know as (1→ 4)-2-amino-2-deoxy-(D-glucose) (Shahidi et al., 1999). Chitosan is a hydrophilic, biocompatible, and biodegradable polysaccharide of low toxicity (He *et al*., 1999; Jumaa and Muller, 1999; Ilyina *et al*., 2000). This polymer offer a wide range of unique applications including bioconversion, for the production of value added food products (Shahidi and Synowiecki, 1991), preservation of food from microbial deterioration (Chen *et al*., 1998), formation of biodegradable films (Butler *et al*., 1996; Hoagland and Parris, 1996), recovery of waste material from food processing discards (Pinotti *et al*., 1997), purification of water (Muzzardli *et al*., 1989), and encapsulation and controlled release of nutraceuticals or drugs (Ribeiro *et al*., 1999; Hino *et al*., 2000). Further, its polycationic nature (pKa ~ 6.3-7) (Schulz *et al*., 1998), leads to strong interactions with oil droplets having the opposite charge. The cationic biopolymer (chitosan) adsorbs to the surface of the anionic droplets due to electrostatic attraction could be produced the positively charged droplets

(Faldt *et al*., 1993; Calvo *et al*., 1997; Magdassi *et al*., 1997; Pinotti *et al*., 2001; Ogawa *et al*., 2003a; 2003b; Aoki *et al*., 2005), which has a number of important potential advantages for many applications in food industry. For example, positively charged droplets are much less susceptible to destabilization by multivalent cations, such as calcium and iron (Kulmyrzaev *et al*., 2000; Silvestre *et al*., 2000). In addition, the lipids in positively charged droplets are much less susceptible to iron catalyzed oxidation because of the electrostatic repulsion between the droplet surface and iron (Mei *et al*., 1998a; 1998b; McClements and Decker, 2000). Finally, cationic droplets coated with anionic surfactants-chitosan membranes have also been shown to have better stability against flocculation and coalescence than droplets coated with anionic surfactants alone (Faldt *et al*., 1993; Calvo *et al*., 1997; Magdassi *et al*., 1997; Ogawa *et al*., 2003a; 2003b; Aoki *et al*., 2005). Chitosan recently is pending for "generally recognized as safe" (GRAS) status within the United States for general application in foods and beverages (FDA, 2005). The fact that chitosan can now be legally incorporated into food products means that novel chitosan based technologies developed in other industries can be applied to foods. Therefore, the utilization of the multilayer interfacial membrane emulsion system (lecithin-chitosan coated droplets) in combination with corn syrup solids may prove to be an effective means of improving the stability of microencapsulated oils.

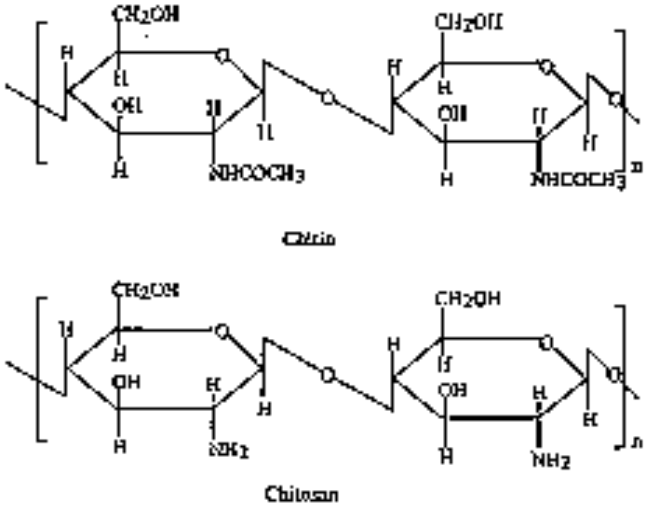

Figure 5 Structures of chitin and chitosan
Source: Majeti and Kumar (2000)

2.3.3 Spray Drying

Spray drying is a unique and important process in the production of dry powder (Figure 6). The liquid feed is pumped and atomized into spherical liquid droplets subsequently transformed into dry spherical particles under ideal circumstances. The particle size of the powder can be controlled to some extent (in the range of 10-500 microns) by controlling droplet size produced by atomization. Within a spray chamber, droplets have an residence time ranging from about 5 seconds (small pilot scale) to 50 seconds (large production scale) (Oakley, 1997). This short-residence-time drying is good for heat-sensitive materials. Furthermore, the droplets temperature during process remains at the wet bulb temperature of drying air. Therefore drying air at very high temperatures can be tolerated in a drier with a minimum of damage to the heat-sensitive components (Toledo, 1991). Taguchi *et al*. (1992, by Pegg and Shahidi, 1999) studied the oxidation stability of sardine oil embedded in spray drying egg white powder and use of the product as a source of n-3 PUFAs for fortification of cookies. They reported that use of microencapsulated sardine oil fortified cookies did not affect

their sensory quality. Lin *et al*. (1995) stabilized squid oil by spray-dried microencapsulation to provide a potential dietary supplement for n-3 fatty acids. The effect of hydrophilic macromolecules, gelatin, sodium caseinate, and maltodextrin, as wall materials in protecting squid oil microcapsules from oxygen was examined. They found that microencapsulation could effectively enhance oxidative and thermal stabilities of oils.

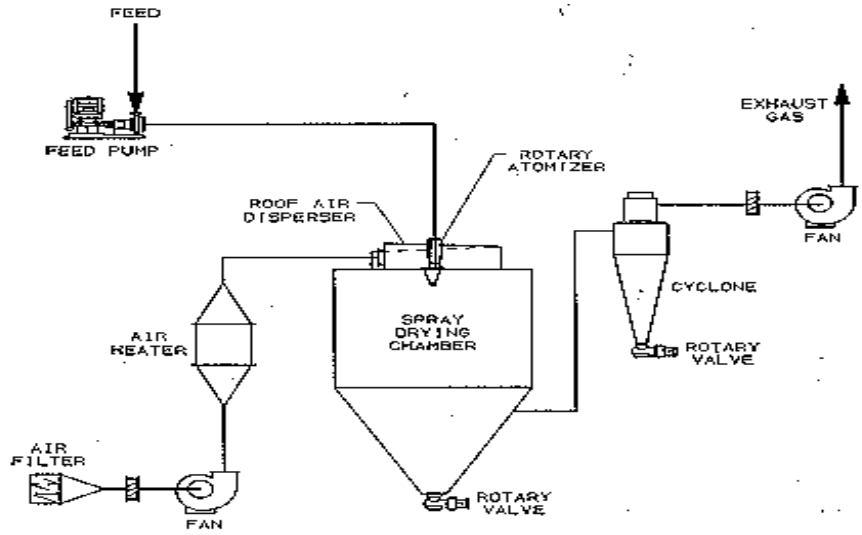

Figure 6 Spray drying system
Source: Dziezak (1988)

There are two mechanisms involving in the drying process:1) heat transfer from the external surroundings to the surface of the liquid droplets being dried (take place by a temperature difference between hot air and drying material) combined with heat transmission within the materials; and 2) mass transfer from inside to the surface of the material followed by external transport of moisture to the surroundings (Toledo, 1991; Crapiste and Rotstein, 1997). Moisture movement in food materials can be caused by a combination of different transport mechanisms (Crapiste and Rotstein, 1997) as follows:

1. Capillary flow due to gradients of capillary suction pressure.

2. Liquid diffusion due to concentration gradients.

3. Vapor diffusion due to partial vapor pressure gradients.

4. Viscous flow due to total pressure gradients, caused by external pressure or high temperatures.

As the wet droplets leave the atomizer, the surface rapidly loses water. The evaporation of water will cause the solute to be more concentrated at the surface. Because of this increased concentration, solids will come out of solution at the surface of the droplet, leading to the formation of a crust or skin around the particle (Oakley, 1997). The formation of the solid crust constitutes the constant stage of drying. When the crust becomes sufficiently thick to offer considerable resistance to movement of water toward the surface, the drying rate drops and the rate of drying is controlled by the rate of mass transfer. The temperature of particle increases, and the liquid trapped in the interior of the particle vaporizes and generates pressure. Eventually, a portion of the crust breaks and the vapor is released. Spray-dried particles consist of hollow spheres or fragments of spheres. This shape of the particles is responsible for the excellent rehydration properties of spray-dried powders (Toledo, 1991). The thickness of the crust will depend on the drying rate, the larger particles with thin shells and low density will be formed if droplets experience high initial drying rates whereas low initial drying rates will lead to smaller particles with thick shells and high density (Oakley, 1997). The particle model (droplet with the crust) during crust formation is shown schematically in Figure 7.

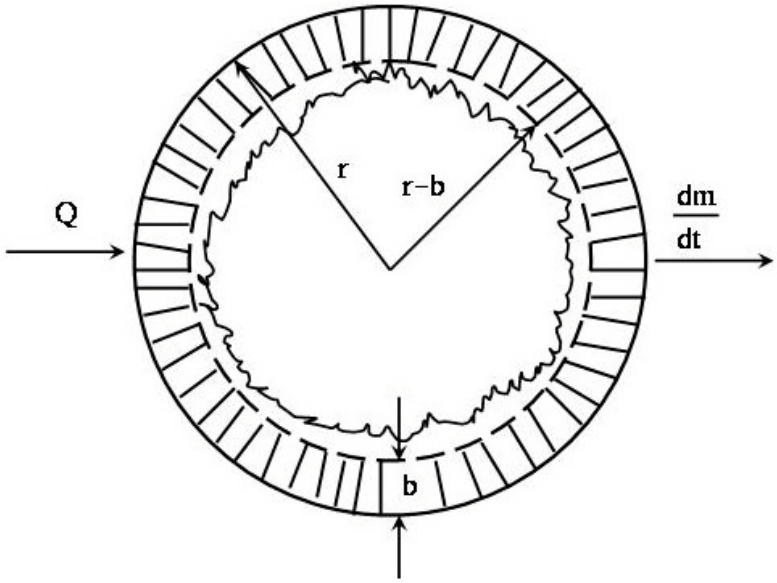

Figure 7 Schematic presentation of a droplet during drying in the crust formation period; Q = heat, b = crust thickness, r-b = radius of the liquid droplet, dm/dt = mass transfer
Source: Dolinsky, 2001

2.4 Changes in physicochemical properties of spray dried powder

2.4.1 Structural Collapse and Crystallization

The flow properties of powder containing oils will depend on properties of wall materials used in the system. The presence of un-encapsulated oils on the particle surface for example, could retard its flow (Onwulata *et al*., 1994). Powders containing a considerable amount of sugars and soluble materials are susceptible to caking or flowability loss upon exposure to a humid environment or elevated temperature (Peleg, 1993; Roos, 1995). Similar effects on the plasticization of food materials are obtained from both temperature and water. The physical characteristics of amorphous food materials may respond to the changes in the water content at a constant temperature the same as to temperature change at a constant water content. These physical changes are related to the glass transition of hydrophilic components (e.g. corn syrup solids, Table 3). Water presents even in a small amount can

significantly depress the glass transition temperature in food materials.

A transition of glassy amorphous materials into a rubbery state at temperature above Tg results in product stickiness, structure collapse, and crystallization (Roos and Karel, 1992). The sticky point of powder is defined as the temperature at which the stirring force of powder sample sharply increases (Danviriyakul, 2001). This can be used to indicate the conditions in which the powder undergoes drastic and abrupt physical changes. Caking, a result of structure collapse due to formation of permanent aggregates of sticky particles, leads to the loss of powder flowability (Peleg, 1993). Caking of sticky particles is observed if sufficient time is provided. A continue mass results from fusion of particles after liquid bridges are formed. The formation of liquid bridge is facilitated by 1) water adsorption; 2) melting of compounds (e.g. lipids); 3) chemical reactions that produce liquids (e.g. non-enzymatic browning); 4) excessive liquid ingredient; 5) water release due to crystallization of amorphous sugars; and 6) wetting of powder (Roos, 1995). To overcome the flow problem, anti-caking agents may be added. As a result, inter-particle forces are reduced while bulk density is increased (Onwulata *et al*., 1996). Crystallization of carbohydrate molecules is facilitated by a decrease in the viscosity due to plasticizing effect of water. The conversion of meta-stable to stable state is hindered by the free energy barriers to nucleation and to crystal growth (Danviriyakul, 2001). The free energy barrier to crystal growth can be overcome if the mobility is allowed to transfer nuclei to the crystal interface by either increasing water activity or temperature.

Changes in the physical properties of an amorphous material have complex influence on the properties of the dry powders. For instance, they may result in a release of the compound or have a protection effect for un-capsulated compounds via the reencapsulation process (Moreau and

Rosenberg, 1993; Ponginebbi *et al*., 2000). Loss of flavor in dehydrated foods occurred as the glassy food matrix is transformed into the rubbery state (Flink and Gejl-Hansen, 1972). This was found to increase with increasing water activity. Increasing water activity that leads to crystallization of encapsulating materials, e.g. lactose, resulted in a release or loss of encapsulated compounds (Shimada *et al*., 1991; Moreau and Rosenberg, 1993). However, oil may become reencapsulated if the changes lead to structural collapse, especially in the highly porous freeze-dried emulsion (Labrousse *et al*., 1992; Ponginebbi *et al*., 2000). As a sequence, their effects on the rate of reactions can be different. The evaluation of the effects of the physical properties on the quality of dry powders therefore becomes more complicated.

Table 3 Glass transition temperature of maltodextrins/corn syrup solids as a function of water activity.

	DE-10		DE-20		DE-36	
Water activity	X_{wo}	T_{go} (° C)	X_{wo}	T_{go} (° C)	X_{wo}	T_{go} (° C)
0.00	0.000	160	0.000	141	0.000	100
0.11	0.020	103	0.024	86	0.017	67
0.23	0.047	84	0.052	73	0.038	45
0.33	0.051	66	0.054	42	0.049	31
0.43	0.065	60	0.058	40	0.054	27
0.52	0.076	38	0.084	37	0.098	6
0.75	0.095	30	0.139	-9	0.170	-35
0.85	0.158	-6	0.208	-32	0.238	-52

Source: Danviriyakul (2001)
X_{wo} = water fraction
T_{go} = onset glass temperature

2.4.2 Maillard Reaction

Color is often the first sensory quality by which foods are judged, and it may also provide an indication of the

chemical changes suffered by them. In addition to natural pigments, the color of foods may be modified by a series of reactions among food constituents that produce the consequence commonly termed as food browning (Zamora and Hidalgo, 2005). The Maillard reaction is the nonenzymatic glycosidation of amino acids or proteins to form glycated products. It embraces a whole network of different reactions in which an extraordinary complex mixture of compounds are obtained in very different amounts. In foods, this reaction takes place essentially between the monosaccharides, glucose and fructose, or the disaccharides, maltose and lactose, as well as in some cases (e.g. meat) reducing pentoses, and amino acids and/or proteins. Although carbohydrate oxidation is also produced to some extent, the first step of the Maillard reaction is generally accepted to be the formation of N-substituted glycosylamine from an aldose (or ketose) reacting with a primary amino group of an amino acid, peptide, or protein. This glycosylamine then suffers an Amadori rearrangement type of reaction to yield 1-amino-1-deoxy-2-ketose. The next step of the reaction is the dehydration, fragmentation, or enolization of the sugar, or the Strecker degradation to produce both amino and nonamino compounds. Finally, the condensation of some of the products formed in this step is produced either among them or with amino compounds to form brown pigments and polymers (Figure 8) (BeMiller and Whistler, 1996; Zamora and Hidalgo, 2005).

Besides reducing sugars, other aldehydes and ketones present in foods can also take part in the carbonyl-amine reaction generated from the oxidation of lipids may react with amines, amino acids and proteins (Figure 8). Particularly, the reaction of lipid oxidation products (especially malonaldehyde) with amines, amino acids, and proteins has long been related to both the browning observed in many fatty foods during processing and storage (BeMiller and Whistler, 1996; Zamora and Hidalgo, 2005).

The extent of nonenzymatic browning (NEB) reaction could be determined using a tristimulus colorimeter. The method implies three color parameter the redness parameter (a-value) represent red/green; the yellowness parameter (b-value) represents yellow-blue; the L-value represents the lightness. In foods, such as milk powder mainly b-and L-value are determined. Other method performed colormetric measurements using the three values X, Y, and Z, and then they are used to calculate a browning index, which was found to be an adequate measure of nonenzymatic browning reactions (Thomas *et al*., 2004).

The rates of nonenzymatic browning closely depend on the temperature and water activity (Aw) of the system studied. When browning rates are representsed as a function of T-Tg (difference between storage and glass transition temperature), for a given experiment temperature, browning rate is increased with the increase of Aw. In samples stored at the same relative humidity, the browning rate increase with the increase of temperature (Roos and Himberg, 1994; Miao and Roos, 2004). As a result of the maillard reaction, the development of brown color, the formation of desirable or undesirable flavor, a change of texture, and a loss of nutritional value are usually brought about (Tanaka *et al*., 1993; Thomas *et al*., 2004). Further, this reaction implies food safety problems. Toxicological studies also have demonstrated the carcinogenic character of some Maillard degradation compounds (Thomas *et al*., 2004). However, many other Maillard reaction products are well documented to reduce lipid oxidation (Elizalde *et al*., 1991; Friedman, 1996).

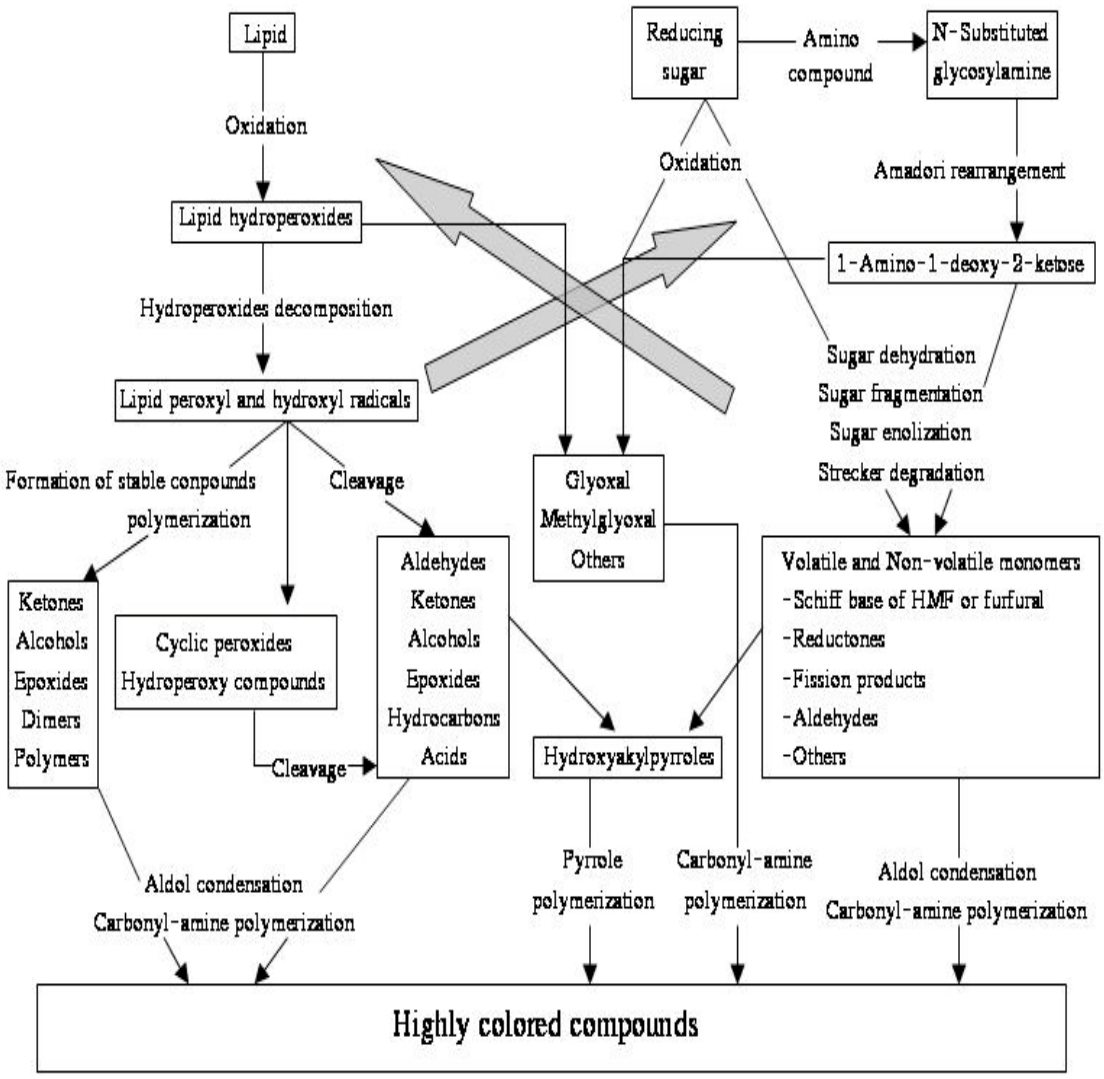

Figure 8 Interactions between Maillard reaction and lipid oxidation pathways in nonenzymatic browning development.

Source: Zamora and Hidalgo (2005)

2.5 Lipid Oxidation

Lipid oxidation is one of the major causes of food spoilage. It leads to the development, in edible oils and fat-containing foods, of various off flavors and off odors generally called rancid (oxidative rancidity), which render these foods less acceptable. In addition, oxidative reactions can decrease the nutritional quality of food, and certain oxidation products are potentially toxic. The autoxidation of lipids reaction occurs in 3

stages: induction, propagation, and termination (Figure 9). The production of the first few radicals in the initiation step is induced by hydroperoxide decomposition, metal catalysis, or light exposure. Odorless and colorless hydro-peroxides are the primarily initial products of the reaction. The compounds are relatively unstable and readily decompose to yield a wide variety of products including hydrocarbons, aldehydes, and acids (Frankel, 1984; Nawar, 1996).

2.5.1 Lipid Oxidation in Liquid Emulsion

Numerous foods contain lipids dispersed in water as membrane bilayers or emulsion droplets. These emulsions can be considered to contain three regions: the interior of a droplet, the continuous phase, and the interfacial membrane (Coupland and McClements, 1996; Mancuso *et al*., 1999). Hydroperoxides in emulsion droplets are often surface-active and therefore accumulate at the surface of the droplets, whereas many of the molecular species responsible for accelerating lipid oxidation originate in the aqueous phase; for example, transition metal or enzymes. Accelerated lipid oxidation may therefore require that the pro-oxidants come into close contact with the lipids at the droplet surface, which depends on the molecular characteristics of the various reactive species involved. Once free radicals have been formed at the droplet surface, they are able to interact with lipids in their immediate vicinity or within the droplet interior (McClements and Decker, 2000).

Antioxidants are substances that can delay the onset, slowing the rate of oxidation of autoxidizable materials. Numerous natural and synthesized compounds have been reported to exhibition antioxidant property. Due to their differences in molecular structure, various antioxidants exhibit substantially differ in the effectiveness when use in different types of oils and foods, and when used different processing and handling conditions (Nawar, 1996).

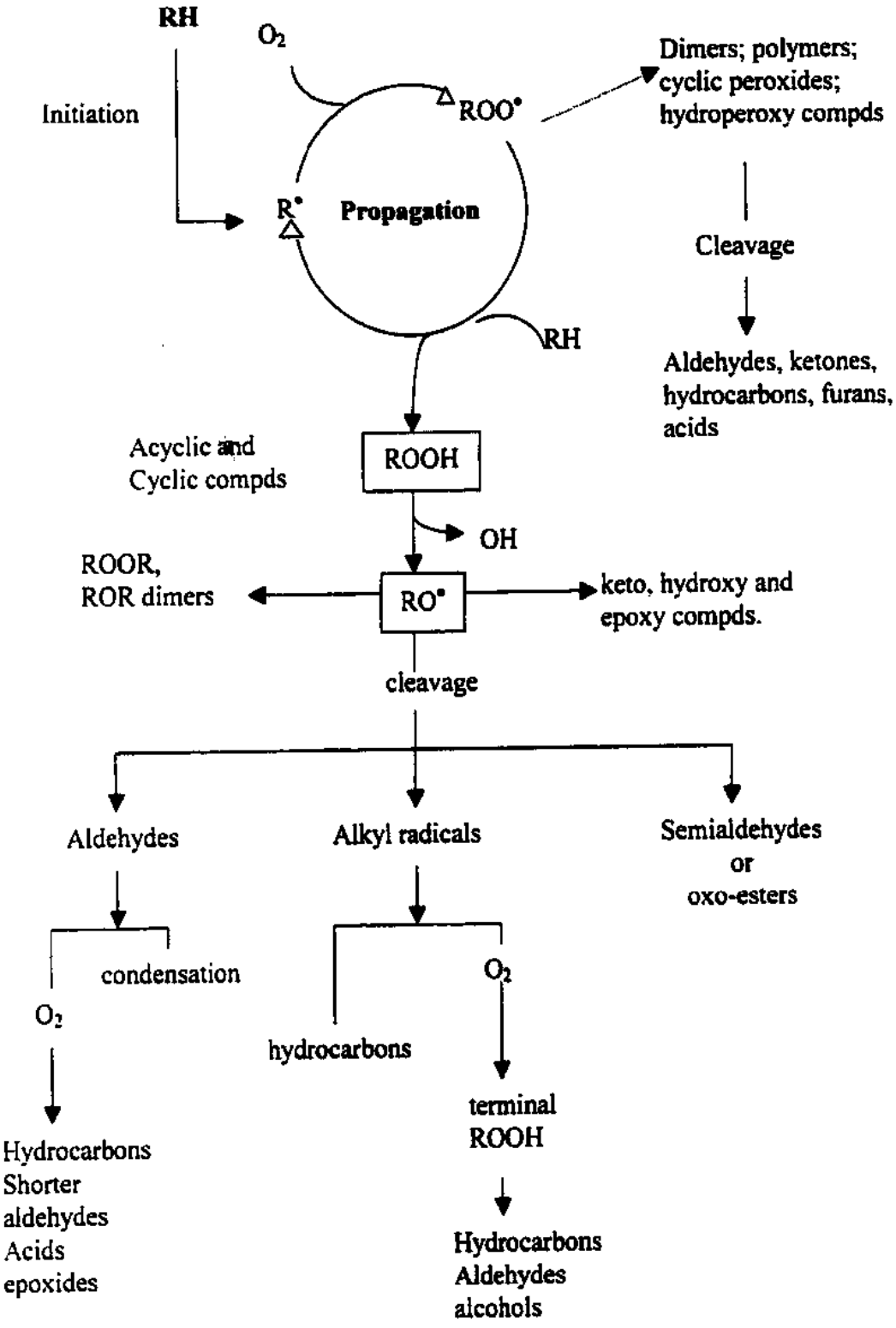

Figure 9 Generalized scheme for autoxidation of lipids.
Source: Nawar (1996)

In emulsions, antioxidants behavior is different from bulk oil system (Frankel *et al*., 1994; Coupland and McClements, 1996; McClements and Decker, 2000). The apparent activity of chain breaking antioxidants in multiphasic food systems such as emulsions is most prevarent (e.g. lipid vs water). In bulk oils, hydrophilic antioxidants preferentially locate at the oil-air interfaces and reverse micelles where lipid oxidation rates are high. Therefore hydrophilic antioxidants are more effective at protecting bulk lipids from oxidation than lipophilic antioxidants that are dispersed thoughout the oil phase. In oil-in-water emulsion, lipophilic antioxidants would concentrate in the oil droplets or at the oil-water interfaces and inhibit lipid oxidation more effectively than hydrophilic antioxidants that can partition into the water phase (Figure 10). For example, gallic acid and propyl gallate, the polar antioxidants, showed no activity or even functioned as pro-oxidants in emulsions, but exhibited high activity in bulk oil. In the contrary, the lipophilic antioxidant, α-tocopherol, had higher activity than hydrophilic antioxidant, Trolox, in corn oil or methyl linoleate emulsified with Tween 20 (Frankel *et al*., 1994).

In addition to chain breaking antioxidants, lipid oxidation in oil-in-water emulsions can be inhibited by many other machanisms. Previous reports suggested that transition metal-based catalysis is the dominant mechanism (McClements and Decker, 2000), therefore metal chelators such as EDTA are effective at inhibiting lipid oxidation in oil-in-water emulsions when present at concentration above the concentration of prooxidant metals (Mancuso *et al*., 1999; Frankel *et a*l., 2002). The oxidative stability of emulsified oil can also be increased by controlling emulsifier type, location, and concentration (Donnelly *et al*., 1998; Mancuso *et al*., 1999; Fomuso *et al*., 2002; Hu *et al*., 2003). For example, when oil-in-water emulsion droplets are surrounded by cationic emulsifiers, pro-oxidant metals are repelled and lipid oxidation rate decrease.

Because of the positively charge droplets inhibited iron-lipid interactions through electrostatic repulsive forces (Mei *et al*., 1998b; Mancuso *et al*., 1999). An additional method to inhibit lipid oxidation in oil-in-water emulsions is to produce thick interfacial emulsion droplet membranes that hinder interactions between water soluble pro-oxidants and lipids inside the emulsion droplets (Silvestre *et al*., 2000).

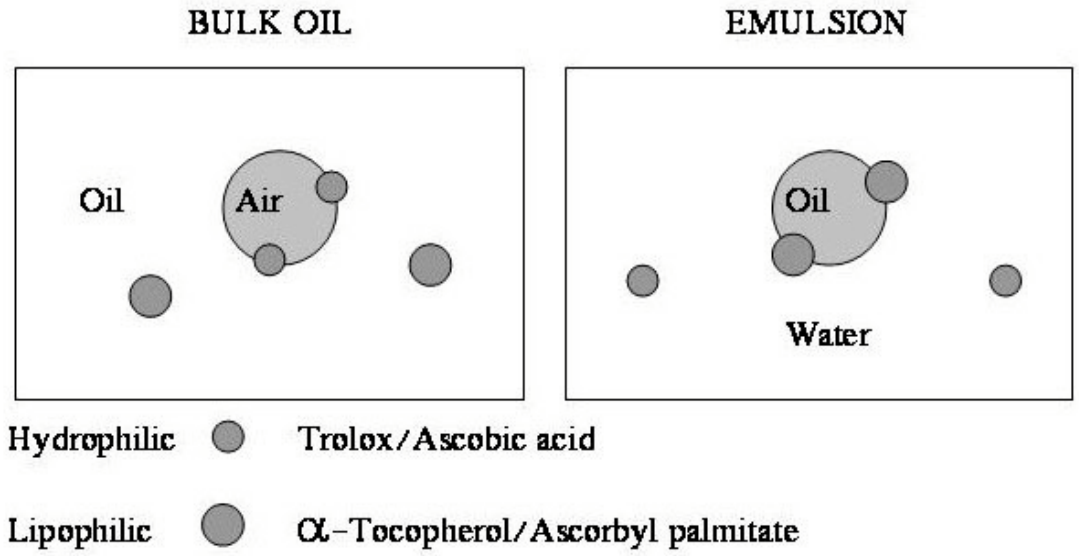

Figure 10 Interfacial phenomena to explain the action of antioxidants in bulk oil and oil-in-water emulsion systems.
Source: Frankel (1994)

2.5.2 Lipid Oxidation in Dried Emulsion

As a constituent of most food systems, water plays a particularly important role in lipid oxidation. Water activity or relative humidity of environment has been used to control lipid oxidation in susceptible food products and to explain the relationship between lipid oxidation rates and moisture content. The rate of lipid oxidation can vary as a function of water activity (Nelson and Labuza, 1992). In general, lipid oxidation

is lowest at water activity close to the water monolayer, which falls between 0.2 and 0.4 for most food. However, the rate of lipid oxidation increases rapidly when the water activity is either decrease below or increase above the monolayer (Nelson and Labuza, 1992; Velasco *et al*., 2003). At the monolayer, the binding of water to hydroperoxides upon the addition of water at low water activity range interferes with their decomposition, hindering the progress of oxidation. A decrease in the catalytic activity of transition metals due to water hydration may also be responsible for a reduction in the rate of oxidation (Danviriyakul, 2001). The rate of lipid oxidation which takes place in the oil phase is observed to increase as water activity is decreased below the monolayer (Figure 11, line b), can be explained by considering the rate of water in this reaction. Water can form a hydration sphere around metal catalysts such as Cu, Fe, Co and Cd. In the dry state the metal catalysts are most active. As water activity increases, the metals may hydrate which may reduce their catalytic action thus slowing the rate of lipid oxidation (Nelson and Labuza, 1992). At water activity above the monolayer value, the rate of lipid oxidation starts to increase again. This can be partially explained by the increased mobilization of the catalysts in the aqueous phase which would bring them to the lipid/water interface making them available to break down hydroperoxides into free radicals (Nelson and Labuza, 1992; Danviriyakul, 2001). The reduced viscosity of the aqueous phase may also allow for other materials to come to the lipid interface such that they can catalyze the reaction (Nelson and Labuza, 1992). The further addition of water (Aw > 0.7) may result in a decrease in the rate of oxidation, possibly as the result of the quenching of free radicals and singlet oxygen as well as a dilution effect on the catalyts (Danviriyakul, 2001). Numerous studies have suggested that this generalized view does apply to a number of systems (Rockland *et al*., 1961; Maloney *et al*., 1966; Quast and Karel, 1972). For example, Maloney *et al*. (1966) found that water had an antioxidant effect

on the rate of oxidation of methyl linoleate in a freeze dried model system. The rate of oxidation decreased with increasing water activity from the dry state up to a water activity of approximately 0.5. However, contradicting to the above findings also exist (Hardas *et al*., 2000; Ponginebbi *et al*., 2000; Baik *et al*., 2004). Ponginebbi *et al*. (2000) found that oxidation rate of freeze-dried linoleic acid emulsion was more rapid at the lower relative humidities (0 and 32%RH) as compared to 43 and 75%RH. Baik *et al*. (2004) found that the oxidation rate of microencapsulated fish oil measured by TBARS value was slowest at 11%RH and then increased with a further increase in RH to 43%.

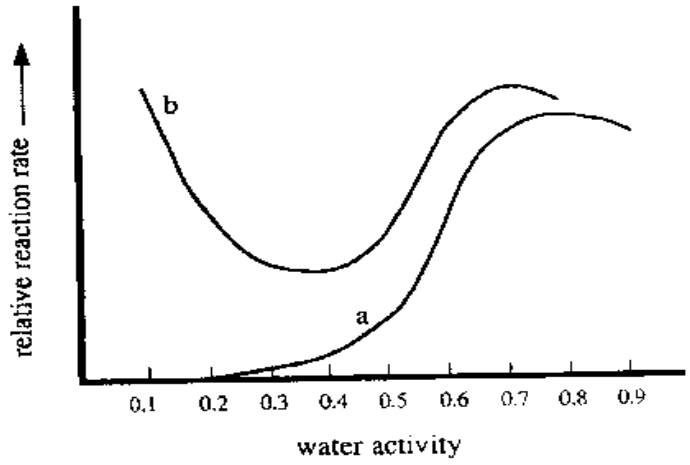

Figure 11 Relative rate of reaction as a function of water activity: a), nonenzymatic browning. b), lipid oxidation.
Source: Nelson and Labuza (1992)

After drying of oil-in-water emulsion a high viscosity solid matrix in the glassy amorphous state is obtained, giving relative protection to the encapsulated oil. However, when either moisture content or temperature increases the solid changes from the glassy state to another amorphous state, i.e. rubbery state, with a high molecular mobility. As molecular mobility increases by the plasticizing effect of water or by temperature, crystallization of sugars and/or the so called "collapse" may occur (Velasco *et al*., 2003). These physical changes are associated with the lost or partial release of

encapsulated oils and the releasing oils then become more susceptible to the oxidation (Shimada *et al*., 1991). However, contradicting reports also exist. Ponginebbi *et al*. (2000) found that at high moisture-induced sucrose crystallization was shown to lead to the reduced porosity, reduction of the surface oil and coalescence of oil droplets. These may be responsible for the decreased oxidation.

In general, the rate of oxidation in foods increase as the temperature is increased since molecular mobility is increased with temperature (McCluskey *et al*., 1997). Temperature also influences the relationship between rate and oxygen partial pressure. As temperature is increased, changes in oxygen partial pressure have a smaller influence on rate because oxygen becomes less soluble in lipids and water as the temperature is raised (Nawar, 1996). The increase of oxidation in whole milk powder is clearly observed with higher temperature (Liang, 2000). In the collapsed matrix where the molecular mobility is high, therefore, it may be difficult to separate the effects of two factors on the oxidation.