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Physicochemical Properties of Flaxseed Oil-Fructooligosaccharide Emulsion and Its Application in Food Products

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Abstract

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Growing awareness about the role of diet and quest for human wellness has fuelled interest in 'functional foods' and functional attributes of many traditional foods that are being reinvented. Flaxseed continues to surge forward in its recognition as a functional food and has recently gained attention in the area of cardiovascular disease and anti-inflammation because it is the richest known source of α-linolenic acid (ALA). Because of the high content of this fatty acid, flaxseed oil is used as a food supplement, where enrichment with n-3 fatty acids (FA) is needed. The high content of ALA in flaxseed oil is, however, highly susceptible to oxidation, leading to

rapid deterioration of quality. Study regarding incorporation of flaxseed oil as a source of ALA in functional food formulations has been little conducted. Therefore, this study was conducted to develop physically and oxidatively stable emulsion using flaxseed oil, and its application in food products enriched with n-3 FA.

The objective of the first part of the study was to develop a practical and simple method to produce flaxseed oil emulsion with fructooligosaccharide (FOS). Effects of types and concentrations of emulsifiers and the ratio of dispersed phase to continuous phase in the emulsions on the physical properties (rheological property, particle size distribution, and emulsion stability) were tested. Among tested emulsifiers with HLB of 4.3, 8.6, 11.0, 14.5, and 15.0, the emulsion containing decaglycerol monolaurate (HLB 15) was the most stable. The concentrations of decaglycerol monolaurate above 2% (w/w) were able to form stable emulsions. Increase in emulsifier concentration increased viscosity and decreased particle size, showing pseudoplastic flow. All the tested ratios (3:7, 4:6, 5:5, 6:4, 7:3, and 8:2, weight basis) of the flaxseed oil and FOS except for 8:2 formed stable emulsions. The flaxseed oil emulsion using different ratios of flaxseed oil to FOS changed little flow behavior index. Oil in the emulsion was less oxidized than flaxseed oil itself, but was oxidized more than flaxseed oil added with TBHQ. These results suggest that the addition of FOS to the continuous phase of flaxseed oil emulsion may increase physical and

oxidative stability of the emulsion.

The objective of the second part was to determine the physicochemical properties and sensory characteristics of set and stirred yogurts added with flaxseed oil-fructooligosaccharide (FOS) emulsion. Descriptive analysis and preference test were carried out for six yogurts (set whole milk yogurt, set whole milk yogurt added with flaxseed oil-FOS emulsion, set non-fat milk yogurt added with flaxseed oil-FOS emulsion, stirred whole milk yogurt, stirred whole milk yogurt added with flaxseed oil-FOS emulsion, and stirred non-fat milk yogurt added with flaxseed oil-FOS emulsion). The addition of flaxseed oil-FOS emulsion little affected the physicochemical properties (pH, acidity, solid content, and color values) of the yogurts. The addition of flaxseed oil-FOS emulsion in the yogurts resulted in a large increase in ALA concentration. Fifteen trained descriptive panelists evaluated the six yogurts on a 16 cm structured scale. The descriptive analysis showed significant differences ($p < 0.05$) among the samples for 13 of 14 descriptive attributes. In descriptive analysis, the yogurts fortified with flaxseed oil-FOS emulsion were fishier than the yogurts without flaxseed oil-FOS emulsion regardless of the yogurt types ($p<0.05$), and the non-fat milk yogurts added with flaxseed oil-FOS emulsion were fishier than the whole milk yogurts added with flaxseed oil-FOS emulsion ($p<0.05$). Preference test indicated that the addition of the flaxseed oil-FOS emulsion did not have much impact on all attributes of the stirred whole milk yogurts ($p>0.05$).

The last part of this study was conducted to assess the effects of flaxseed oil and dried whitebait as a source of n-3 FA, which could be used to produce eggs enriched with n-3 FA, and FOS as a source of prebiotics on performance of hens (commercial Hy-Line® Brown laying hens), and FA composition, internal quality, and sensory characteristics of the eggs. Dietary FOS increased egg weight. The amounts of ALA, eicosapentaenoic (EPA), and docosahexaenoic acids (DHA) in the eggs from the hens fed the flaxseed oil alone or flaxseed oil + dried whitebait diets were higher than those of the control. Hedonic scores for off-flavor, fishy flavor, buttery taste, and overall acceptability of the eggs from the hens fed the diet containing flaxseed oil + dried whitebait were lower ($p<0.05$) than those of the control. Overall acceptability of the eggs from the hens that had been fed the diet containing soybean oil + dried whitebait was lower ($p<0.05$) than that of the control. However, all the sensory attributes of the eggs from the hens that had been fed the diet containing flaxseed oil, dried whitebait, and FOS were not significantly different from those of the control. These results confirmed that flaxseed oil in the diet for laying hens increases the ALA content in the laid eggs and a combination of flaxseed oil and dried whitebait in the diet increases EPA and DHA in the eggs. Of significance was that the addition of FOS to the flaxseed oil + dried whitebait diet improved the sensory characteristics of the eggs enriched with n-3 FA compared with the flaxseed oil or dried whitebait diet alone.

Key words: flaxseed oil, fructooligosacchride, sensory evaluation, emulsion, eggs, yogurt

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

ALA: α-linolenic acid

AV: anisidine value

CD: conjugated diene

DHA: docosahexaenoic acid

DPA: docosapentaenoic acid

EPA: eicosapentaenoic acid

FA: fatty acid(s)

FAME: fatty acid methyl ester(s)

FOS: fructooligosaccharide(s)

HLB: hydrophilic lipophilic balance

LA: linoleic acid

PV: peroxide value

PUFA: polyunsaturated fatty acid(s)

Chapter 1

Literature Review

1.1 Flaxseed and its oil

Flaxseed (*Linum usitatissimum*) is one of the most important oilseed crops for paint and coating industry as well as food and feed purposes. The Latin name of the flaxseed is *Linum usitatissimum*, which means “very useful.” It has been used for food and textile fiber for over 5,000 years (Oomah, 2001). The terms ‘flaxseed’ and ‘linseed’ are often used interchangeably. Flaxseed is used to describe flaxseed when eaten by humans while linseed is used to describe flax when it is used for industrial purposes (Morris, 2004). Every part of the linseed plant is utilized commercially, either directly or after processing. The stem yields good quality fiber with high strength and durability. The seed provides oil rich in n-3 FA, digestible proteins, and lignans (Morris, 2004).

Flaxseed has a hard shell that is smooth and shiny and the color ranges from deep amber to reddish brown depending upon whether the flaxseed is of the brown or golden variety (Fig. 1.1). The envelope or testa of the seed contains about 15% of mucilage. Flaxseed is rich in fat, protein, and dietary fiber. The composition of flaxseed can vary with genetics, growing environment, and method of seed processing (Daun et al., 2003). The composition of flaxseed is provided in Table 1.1 (Morris, 2004).

Fig. 1.1. Flaxseed (left: brown; and right: golden).

Table 1.1. Proximate composition of flaxseed and its oil.

	Energy (Kcal)	Total fat (g)	ALA (g)	Protein (g)	Total carbohydrate (g)	Total dietary fiber (g)
Whole seed (100 g, w/w wet basis)	450	41.0	23.0	20	29.0	28
Flaxseed oil (100 g)	884	100	57.0	-	-	-

Flaxseed is one of the most important oilseed crops of the world, cultivated in over 2.6 million ha. The important countries growing flaxseed are India, Canada, China, United States, and Ethiopia (Table 1.2). Canada is the world's largest producer of flax and accounts for nearly 80% of the global trade in flaxseeds (Oomah & Mazza, 1998). India ranks first in the world in respect of acreage accounting for 23.8% and third in flaxseed production contributing 10.2% (Morris, 2004).

Table 1.2. Area, production, and productivity of flaxseed in important countries (2004).

Country	Area (m ha)	Production (Million tonnes)	Productivity (kg/ha)
India	0.630	0.200	317.5
Canada	0.528	0.517	978.8
China	0.550	0.460	836.4
USA	0.200	0.266	1273.7
Ethiopia	0.150	0.077	514.0
Bangladesh	0.070	0.050	714.3
France	0.080	0.054	677.9
Unite Kingdom	0.031	0.054	1741.9
Russian Federation	0.074	0.058	778.5
World	2.645	1.956	739.5

(Source: FAO Production Yearbook, 2004).

In Fig. 1.2, FA composition of flaxseed oil is compared with other fats and oils. ALA constitutes 57% of the total FA in flaxseeds, making flaxseeds the richest source of ALA in the North American diet. ALA constitutes 63% of the total FA in perilla, which the richest source of ALA in the Korean diet. Linoleic acid constitutes 16% of the total FA in flaxseed oil. Flaxseed oil and canola oil have the lowest levels of the nutritionally undesirable saturated FA. The level of the desirable monounsaturates in flaxseed oil is modest (Morris, 2004).

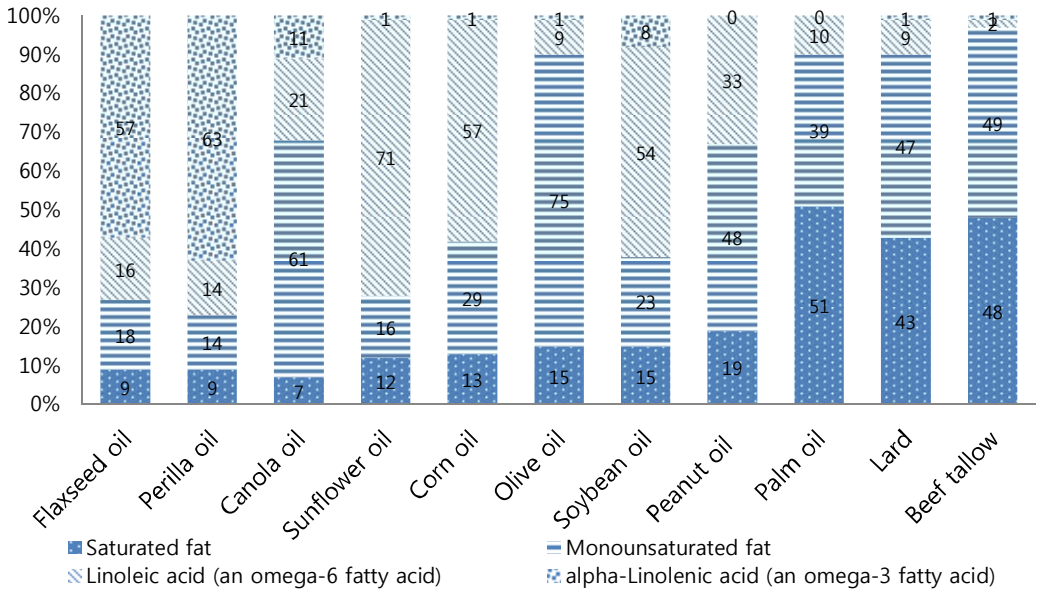


Fig. 1.2. Fatty acid composition of dietary fats and oils.

1.2 Essential fatty acids

There are two essential fatty acids (EFA) in human nutrition: ALA, an n-3 FA, and linoleic acid (LA), an n-6 FA. Humans must obtain EFA from foods because the human body cannot make them. EFA are required for the structure of cell membranes as they help keep membranes flexible since they are unsaturated. They are precursors of long-chain FA, some of which are converted to powerful compounds that affect many biological processes, including inflammation and cell signaling. EFA affects gene expression – that is, they turn genes on for the production of cell proteins (Sampath &

Ntambi, 2004; Toborek et al., 2002). EFAs also have antibacterial actions and are found in breast milk (Das, 2006).

1.2.1 n-3 and n-6 fatty acids

Fig. 1.3 shows the metabolic pathways of n-3 and n-6 FA. Note that ALA is the first or “parent” FA in the n-3 FA pathway, reflecting its position as an essential fatty acid. All the other n-3 FA in the pathway can be made from ALA within the body’s cells or obtained from foods (Burdge, 2006). Likewise, LA, being an essential fatty acid, is the parent FA in the n-6 pathway. All the other n-6 FA can be made from LA within the body’s cells or obtained from foods. Mammals cannot interconvert the n-3 and n-6 FA; they are two separate and distinct families. Furthermore, their metabolism requires the same enzymes, resulting in competition between the two families. An excess of one family of FA can interfere with the metabolism of the other, reducing its incorporation into tissue lipids and altering its biological effects (Burdge & Calder, 2005; Hussein et al., 2005).

1.2.2 Metabolism of ALA

About 96% of dietary ALA appears to be absorbed in the gut (Burdge, 2006). After absorption, ALA has several metabolic fates: 1) it can undergo

β-oxidation to produce energy; 2) it can be recycled to make other FA; 3) It can serve as a substrate for ketogenesis, the process of making ketone bodies; 4) it can be stored in adipose tissue for later use; 5) it can be incorporated into the phospholipids of cell membranes, where it affects membrane activities; and 6) It can be converted to long-chain n-3 FA like EPA, docosapentaenoic acid (DPA) and DHA, which have important functions in many types of cells and organs.

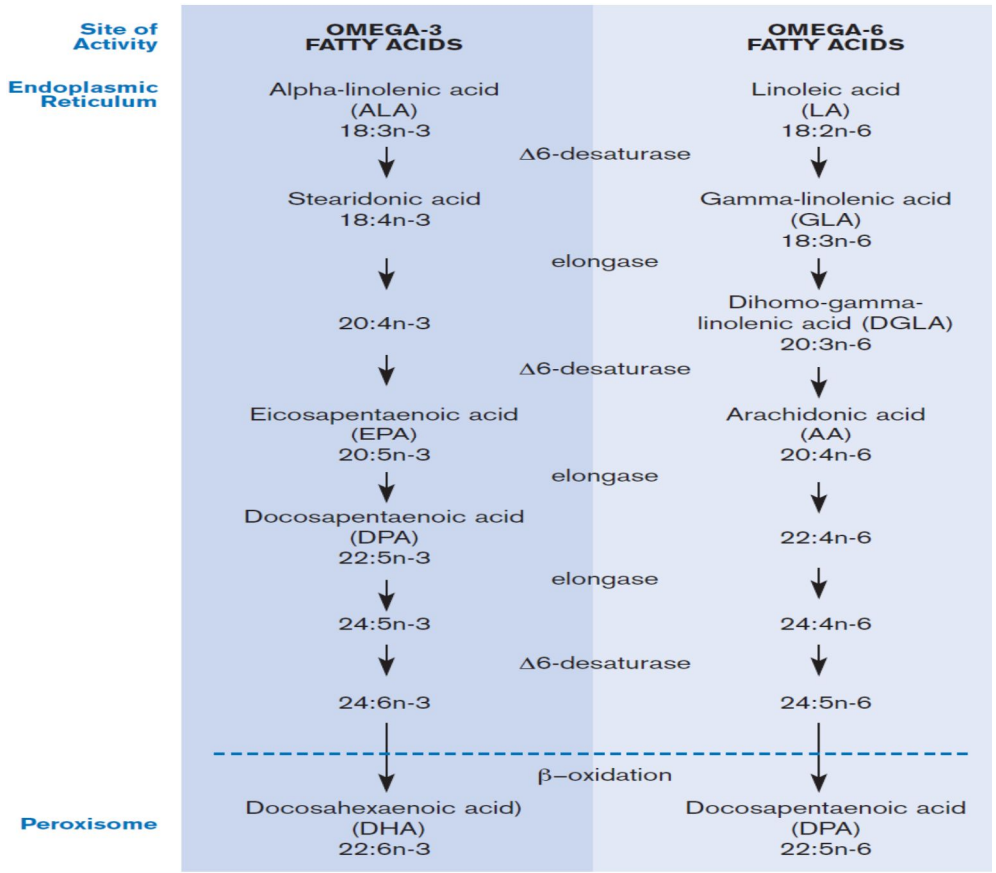


Fig. 1.3. Metabolic pathways of the n-3 and n-6 fatty acids (Morris, 2004).

1.2.3 Conversion of ALA to longer-chain n-3 fatty acids

ALA is converted to longer-chain n-3 FA by a series of alternating desaturations and elongations, as shown in Fig. 1.3. The desaturations add a double bond by removing hydrogen, while the elongations add two carbon atoms (Qiu, 2003). The main long-chain n-3 FA formed from the desaturation and elongation of ALA are EPA, DPA, and DHA. Estimates of the amount of ALA converted to EPA range from 0.2% to 8% (Burdge & Calder, 2005; Burdge et al., 2002), with young women showing a conversion rate as high as 21% (Burdge & Wootton, 2002). Conversion of ALA to DPA is estimated to range from 0.13% to 6% (Burdge, 2006). The conversion rate for young women is on the higher end (6%) (Burdge & Wootton, 2002). Conversion of ALA to DHA appears to be limited in humans, with most studies showing a conversion rate of about 0.05% (Burdge, 2006), although one study reported 4% (Emken et al., 1994). A conversion rate of 9% was reported in young women (Burdge & Wootton, 2002).

The large differences in the rates of ALA conversion reflect major differences in the studies' method. There is a need for more research to resolve the issue of how much ALA is converted to longer-chain n-3 FA in all age demographics.

1.2.4 Biological effects of ALA

ALA has several biological effects, which together contribute to its positive health effects:

1) Breast milk contains about 0.5-2.0% ALA and about 0.1-0.4% DHA (Innis, 2000) or roughly five times more ALA than DHA. ALA constitutes 75-80% of the total n-3 FA in breast milk (Bopp et al., 2005; Silva et al., 2005), supporting a role for ALA in the growth and development of infants.

2) ALA is required for maintaining the nervous system. In humans, a deficiency of ALA results in poor growth and physical problems such as numbness, weakness, pain in the legs, inability to walk, and blurring of vision (Holman et al., 1982). These clinical deficiency symptoms can be alleviated by adding ALA to the diet (Anderson & Connor, 1989).

3) ALA is the precursor of EPA, DPA, and DHA. Thus, ALA-rich diets increase the ALA, EPA, DPA, and total n-3 FA content of cell membrane phospholipids. In one study of 20 healthy men and women taking six flaxseed oil capsules a day (providing 3.5 g of ALA/day) for 8 weeks, the ALA content of red blood cell membranes increased 100%, the EPA content increased 33%, the DPA content increased 20%, and the DHA content was unchanged (Cao et al., 2006). In other studies, diets containing more than 4.5 g of ALA/day (contained in about 1/2 tbsp of

flaxseed oil or 2 heaping tbsp of milled flaxseed daily) increased the EPA content of plasma phospholipids between 33% and 37% and the DPA content between 5% and 50% (Burdge & Calder, 2005). The large ranges in the response of study volunteers to dietary ALA reflect differences in the amount of LA in their diets (Hussein et al., 2005). Increasing the n-3 FA content of cell membrane phospholipids also increases their flexibility and alters the way they behave in beneficial ways (Nair et al., 1997).

4) ALA dampens inflammatory reactions by blocking the formation of compounds that promote inflammation. Inflammation is a feature of many chronic diseases, such as heart disease, type 2 diabetes, metabolic syndrome, obesity, cancer, and Alzheimer's disease (Griffin, 2006; Greenberg & Obin, 2006).

1.3 Oxidative susceptibility of EPA and DHA

There is consensus that the longer-chain n-3 FA (EPA and DHA) is more effective in the attenuation of inflammation and CVD (cardiovascular disease) than the attenuation of moderate length n-3 FA (ALA). However, the use of these marine and algae oils is limited by their oxidative susceptibility and fishy, metallic off-flavors. It is a challenge in the food

industry to inhibit lipid oxidation of n-3 FA oils during processing, shipping, and storage (Djordjevic et al., 2004). Fats and oils are extremely prone to autoxidation and may potentially harm cells with the formation of free radicals. Autoxidation is a primary factor in the degradation of the quality of fats in foods (Sattar & deMan, 1975).

The oxidative stability of unsaturated FA decreases as its degree of unsaturation increases. In other words, the greater number of carbon double bonds present in the polyunsaturated fatty acid (PUFA), the more vulnerable it is to autoxidation (Kim & LaBella, 1987). Therefore, DHA is more readily oxidized than EPA and ALA. In the 18-carbon FA series, the relative oxidation rate of 18:0 is 1 time. The relative oxidation rates of 18:1, 18:2, and 18:3 are 100, 1200, and 2500 times, respectively, the oxidation rate of 18:0 (De Man, 1999).

It has been suggested that presence of the oil in an oil-in-water emulsion may increase the oxidative stability of the oil. The emulsion would consist of the oil being dispersed into a water-based food including beverages and dairy products (Djordjevic and others 2004). However, the incorporation of these long chain n-3 FA into a milk base still has issues with oxidative stability (Let et al., 2003; Venkateshwarlu et al., 2004). The combined unsaturated FA found in milk lipids, particularly the phospholipids, and the long chain n-3 FA added to the milk gives the enriched beverage a noted

vulnerability to oxidation producing an oxidized flavor. Alternatively, it has also been suggested that the use of n-3 FA in dairy products retards the lipid oxidation process because dairy products are generally refrigerated (Kolanowski and Weißbrodt, 2007). The lower temperatures help protect dairy products against oxidation during storage. In addition, the milk proteins provide an antioxidant property, also potentially aiding in the protection against lipid oxidation of the enriched dairy beverage (Packaged Facts. 2007).

1.4 Advantages of ALA over EPA and DHA

The recommendation to increase dietary EPA and DHA is limited to patterns of food choice and natural resources of fish to sustain a sufficient supply of fatty/oily fish (Burdge & Calder, 2005). For populations who do not consume seafood, muscle, or organ meat products, such as vegetarians, the longer-chain n-3 FA are only accessible from plant sources containing ALA (Garg et al., 2006).

The main role of ALA is to moderate the metabolism of LA and its subsequent eicosanoids by competing for available delta-6-desaturase. The affinity of delta-6-desaturase is greater for ALA; however, due to larger amounts of LA available in cellular pools, the metabolism of the n-6 family is

favored. The human body is capable of converting ALA into EPA and DHA through a series of desaturation and elongation steps. There is sound evidence of a modest conversion rate of ALA to EPA, varying from 0.2 to 15% depending on methods of measurement (Emken, 1994; Pawlosky et al., 2001). The Institute of Medicine (IOM) stated that ALA is not known to have any specific functions aside from being a precursor to EPA and DHA (Packaged Facts, 2007). Most n-3 FA research focuses on EPA and DHA, and due to a lack of research, it cannot be concluded that ALA does not serve more purpose than a precursor of longer-chain FA. Burdge and Calder (2005) reported that an increase in dietary ALA over a period of weeks to months can result in elevated levels of EPA in plasma lipids. However, increased dietary ALA did not enhance the levels of DHA, most likely due to the extra necessary elongation and desaturation steps. Overall, it is still important to increase the intake of ALA in the diet to compete with LA for the delta-6-desaturase enzyme, which will help attenuate the production of arachidonic acid (AA) and its inflammatory effects.

1.5 Formulations of functional foods using n-3 fatty acids

It has been strongly suggested by the American Heart Association (AHA) and the United Kingdom Scientific Advisory Committee on Nutrition to consume fish and fish products (including fish oil capsules) at least twice a

week to achieve a “healthy intake” of n-3 FA in the diet (Whelan & Rust, 2006). However modern Western societies seldom consume sufficient fish or fish products to fulfill this recommendation and fish oil capsules are not suitable for daily use (Mantzioris et al., 2000). Due to the small amount of n-3 FA in each fish oil capsule, a consumer would need to take at least 4-6 fish oil capsules in order to attain the large dose necessary for any beneficial effect. Consequently, for many consumers, it is unlikely that they will incorporate fish products and/or fish oil capsules into their diets due to high cost, aversion to fishy flavors, and inconvenience (Davidson et al., 1991). Formulation of n-3 FA enriched food products is a practical alternative to filling the void by increasing the dietary intake of n-3 FA and lowering the dietary n-6:n-3 FA ratio. Without dramatically altering a consumers’ dietary habits, it may be feasible to increase the intake of n-3 FA and benefit from its anti-inflammatory properties as well as protection against cardiovascular.

Previous efforts to incorporate n-3 FA into foods have been performed with various products such as margarine, salad dressing, mayonnaise, sausages, and confectionary Moghadasian, 2008). Despite significant achievements in this area, some limitations exist within quality, availability, and consumer acceptability of these enriched food products.

1.6 n-3 Fatty acid enriched animal products

There has been much effort to increase the n-3 FA content of animal products (i.e., milk, meat, and eggs) by the enrichment of animal feed. Animal feed enrichment has successfully increased the concentrations of ALA, EPA, and/or DHA in the tissues and products of the animals such as the muscles or the egg yolk (Kolanowski & Laufenberg, 2006). In addition, the FA profile of fluid milk has been modified to include greater amounts of unsaturated FA compared to normal bovine milk. However, the enrichment of animal products with n-3 FA raises concerns of whether significant organoleptic changes can occur.

Flaxseed as an animal feed has been limited until recently, although the benefits of feeding flaxseed to animals have been observed for nearly 100 years. Quantifying the benefits to animal health is needed. However, some studies have shown general health improvement along with enhancement to animal production and end product quality (Singh et al., 2011).

1.6.1 Milk

Attempts to modify the FA profile of cow's milk have been carried out. Milk fat is typically composed of 5% PUFA, 25% monounsaturated FA and 70% saturated FA. The Wisconsin Milk Board indicated at their 1988 Milk Fat

Roundtable that the ideal profile for milk fat should be composed of 10% PUFA, 82% monounsaturated FA and only 8% saturated FA (Grummer, 1991). Unfortunately, this cannot be accomplished solely with the alteration of cattle feed. Unsaturated FA are converted to saturated FA from rumen biohydration, resulting in a high content of saturated FA in milk fat despite the cattle's consumption of a diet rich in unsaturated FA (Maddock et al., 2005). There have been previous successes in lowering the saturated FA content of milk fat by various methods. The infusion of FA post-ruminally has been shown to decrease short and medium chain FA with an increase in oleic acid (monounsaturated FA) (Mansbridge & Blake, 1997). Another option is to encase and protect the lipid present in the feed. For example, the addition of a protective whey protein coating around a lipid core preserves the lipid content within, while being present in the rumen. The lipid core is released post-ruminally, and the unsaturated FA is available for absorption by the small intestine (Heguy et al., 2006). More innovative approaches to modify the fat composition of milk are still underway.

1.6.2 Poultry

Eggs have been described as the ideal model for the transfer of dietary lipids (Bourre, 2005). The intestinal physiology of hens allow for preservation of PUFA content in their feed, producing a consistent

enrichment of n-3 FA into the egg yolk (Bourre, 2005). Researchers have previously supplemented hen diets with EPA-rich marine algae (Nitsan et al.,1999), DHA-rich marine algae (Herber-McNeill &Van Elswyk, 1998), or flaxseed oil (Ferrier et al., 1995). All were able to successfully deposit the specific n-3 FA into the egg yolk.

Consumer acceptance tests were conducted with these nutritionally enriched eggs, without a unanimous conclusion. Some consumers indicated a detection of a significant fishy off-flavor (Jiang et al., 1992), where others found little to no difference in the flavor of the enriched eggs compared to control eggs (Van Elswyk, 1997; Farrell, 1998). Eggs were scrambled, hard-boiled, or poached, without additional seasonings. Consumers used various hedonic scales to indicate the palatability of egg dishes. The differences in palatability of the eggs may depend on source of n-3 FA as well as the amount added into the hens' diets.

Aside from eggs, n-3 FA-enriched animal feed has also been used to manipulate the white and dark meats of broiler chickens. The diets of broiler chickens were supplemented with various amounts of flaxseed oil and/or fish oil (Lopez-Ferrer et al., 2001; Rymer & Givens, 2006). Rymer and Givens (2006) compared the n-3 FA rich diets to a control diet high in vegetable oil (low in n-3 FA). Meat quality was analyzed for FA composition in thigh samples. The supplemented diets lowered the LA concentration in

poultry meat compared to the control diet (Rymer & Givens 2006). ALA concentration significantly increased in all of the supplemented diets, with the greatest increase in the higher concentrated ALA diet. The concentrations of the longer-chain FA also significantly increased; however, the fish oil diets deposited more than 3 times EPA and DHA than the flaxseed diets. This demonstrated the inefficiency of conversion of ALA to its longer-chain derivatives in poultry.

Sensory evaluation was performed only on thigh samples from chickens that were fed the fish oil enriched diets (Lopez-Ferrer et al., 2001). The highest dose of fish oil (4% by weight) produced an unpleasant odor and those samples were not evaluated. The thigh samples of chickens fed a combination of fish and flaxseed oils were most palatable, and consumers found it similar to the control (Pawlosky et al., 2001). However, sufficient details describing the sensory evaluation portion of the study were lacking. It is unknown whether the thigh samples of the chickens solely fed flaxseed oil had defects in its flavor, or how they compared to the evaluated thigh samples.

Lewis et al. (2000) estimated that an intake of three n-3 FA enriched eggs provided the equivalent amount of n-3 FA as one serving of fish. The replacement of standard eggs with n-3 FA enriched eggs may be a feasible option to increase the n-3 FA intake in the diet, especially for low fish-

consuming populations.

1.6.3 Beef cattle

Several researchers have looked at the use of flaxseed in beef cattle diets as a means to enhance n-3 composition in meat. The conversion or deposition of n-3 into milk and meat of cattle is far less efficient compared to poultry (Hall et al., 2006). Ruminal biohydrogenation of the unsaturated FA is thought to be the main reason for the poor deposition n-3 FA in the meat. However, feeding flaxseed did enhance ALA content in beef when compared to barley and corn based diets (Maddock, et al., 2006). The level of PUFA n-3 FA also increased in the meat of cattle fed a flaxseed diet. Maddock et al. (2006) reported that ALA, EPA, DPA, and DHA levels were higher in the phospholipid fraction of cattle fed 8% flaxseed diet compared to the control. LaBrune et al. (2008) also reported enhanced EPA and DHA in cattle fed a 5% flaxseed diet.

1.6.4 Health concerns

There is potential to replace animal products low in n-3 FA with its nutritionally enhanced alternatives to help increase dietary n-3 FA intake. However it is important to keep in mind that when increasing the amount of

animal products in the diet, there is an accompaniment of an increase in animal fat intake. Although the fats are not all composed of saturated FA (as in normal non-enriched meat products), consumption of such high amounts of animal products may raise health concerns such as excessive energy intake, weight gain, and obesity (Kris-Etherton et al., 2000).

Seafoods, especially fatty fish, are the best intrinsic food source of the long chain n-3 FA, EPA and DHA. There is concern that increased consumption of seafood may lead to a higher intake of adulterated marine oils. The uncertainty of the purity of the marine oils has raised apprehension about toxins such as parachlorobenzoic acid, dichloro-diphenyl-trichloroethane, dioxin, and mercury which may be present in the marine origin foods (Mahaffey, 2004; Melanson et al., 2005; Garg et al., 2006). It has not been determined whether the costs of animal feed enrichment are greater than fortifying food products to deliver n-3 FA. Even though n-3 FA enriched animal sources have been successful in increasing dietary intakes of n-3 FA, plant sources may provide a more optimal way to decrease the n-6:n-3 FA ratio. The use of plant derived n-3 FA products in diets is growing, as more formulations of novel food products are available.

1.7 Encapsulation of bioactives

Microencapsulation technology is well developed and commercialized in the chemical, pharmaceutical, cosmetic, and printing industries. In the food industry, it is used to mask unpleasant tastes and protect sensitive ingredients from degradation through reactions with its environment during storage and from other components when it is introduced into a food matrix (Gibbs et al., 1999)

Food bioactives are physiologically active components in food or dietary supplements of plant or animal origin that have a role in health beyond basic nutrition (Augustin & Sanguansri, 2008). The addition of bioactive components to foods, particularly those foods that are consumed as part of the normal diet of target populations, offers opportunities for improving the health of consumers. The interest of the food industry in these functional foods has resulted in the development of novel food products with enhanced levels of food components that have potential health benefits (Schmidl & Labuza, 2000). The delivery of bioactives through food is a major challenge. Many bioactives are susceptible to degradation, and thus there is a need to protect them during their storage as both an ingredient and in fortified food products, without compromising the sensory properties of the food (Augustin & Sanguansri, 2008). Food components considered as bioactives include vitamins, minerals, functional lipids, probiotics, amino acids, peptides and

proteins, phytosterols, phytochemicals, and antioxidants (Wildman, 2001). Their structure and function vary extensively and are important considerations when adding them to food.

Many bioactives are unstable. Regardless of the form they are added to food, it is essential that they be stabilized prior to addition to food, during the food manufacturing process, and throughout storage. When choosing the food vehicle for addition of a chosen bioactive, it is important to consider its solubility in the food matrix and its interactions with other ingredients in the food formulation (Augustin & Sanguansri, 2008). The incorporation of bioactives can alter flavor, odor, and texture of foods. As consumers only accept food products with good sensory appeal, the successful addition of bioactives into a range of functional food products must not compromise food quality. Furthermore, as the bioactive food component is selected for its specific physiological function, it is important that it is bioavailable when the food is consumed.

1.7.1 Considerations for design of the delivery system

The specific requirements of the encapsulant materials and the delivery system have to be considered. This includes protecting the bioactive throughout its storage as an ingredient, through the food manufacturing process, and during the storage of the food product. In order for the bioactive to survive food processing conditions, it is important to ensure that the core is protected from harsh processing environments and that the release of the core is not triggered (Schrooyen et al., 2001). The conditions during gastrointestinal transit of the food should also to be taken into account to deliver the bioactive to the desired site of the body to achieve its potential health benefits (Bell, 2001). The development of a successful encapsulation system for a target application requires knowledge about the stability of the chosen bioactive (core): the properties of the materials used for encapsulation (encapsulant) and the suitability of the delivery system (microcapsule) for its final application. Table 1.3 gives a summary of important considerations about delivery system (Augustin & Sanguansri, 2008).

Table 1.3. Properties of the core, encapsulant material, and microcapsule of importance in the design of encapsulated bioactives.

Component	Properties
Core	Bioactivity of the material, solubility (hydrophilicity/lipophilicity), stability to environmental conditions (e.g., moisture, heat, pH, salts, light, enzymes), taste, propensity to interact with other food components
Encapsulant material	Solubility, viscosity Stability to pH, salts, temperature, shear, enzyme degradation Film forming and emulsification properties Regulatory status for food application
Microcapsule	Format for delivery (i.e., liquid or powder) Storage stability Stability to different process conditions Release properties Particle size Payload (bioactive core loading) Cost of production

1.7.2 Encapsulant materials

Encapsulant materials can be selected from a wide range of natural or synthetic materials depending on the properties desired in the final microcapsule (Table 1.4) (Augustin & Sanguansri, 2008). The encapsulant materials in food are more restricted to natural food components (e.g., proteins, sugars, starches, gums, lipids, and cellulosic material) and other ingredients that have 'generally regarded as safe (GRAS)' status (e.g., cyclodextrin, chitosan, low-molecular weight emulsifiers such as Tweens, mineral salts, etc.). They can be used alone or in combination to achieve the desired functionality. The composition can significantly influence the functional properties of the final microcapsule as can the choice of processing technologies used. Cost also needs to be considered. Neutral taste and odor, low viscosity, good film forming, gelling and barrier properties are some of the desired characteristics of encapsulant materials (Koroleva & Yurtov, 2012). The functional properties of food biopolymers (water binding, gelling, and emulsifying) lend themselves to the development of a wide range of microstructures that can be used for delivery of bioactives. A good encapsulant protects the core from degradation during processing and storage and also masks any undesirable taste and odor associated with the bioactive core when added into a food product (Koroleva & Yurtov, 2012). Identification of the storage requirements and processing needs, as well as the mechanisms required for release of

the core material (e.g., pressure-based, dissolution-based, or melting-based triggers) are important. These are influenced by the mechanical strength of the capsule wall and its compatibility with the target food product. Nutritional value, sensory properties, and aesthetic properties are also important considerations (Brazel, 1999). Thus, the material properties of the capsule such as the permeability of the encapsulant, its resistance to conditions encountered (e.g., shear, heat, pH shift, light, enzyme, other ingredients) during processing, and gastrointestinal tract transit have to be designed to enable the desired control over the release of the bioactive (Champagne & Fustier, 2007).

Table 1.4. Materials used for encapsulation of bioactives for food applications.

Material class	Types of materials
Proteins	Milk proteins-caseins and whey proteins, soy proteins, wheat proteins, egg proteins, hydrolyzed proteins
Carbohydrates	Sugars (fructose, galactose, glucose, maltose, sucrose, oligosaccharide, corn syrup solids, dried glucose syrup) Starch and starch products (maltodextrins, dextrins, starches, resistant starch, modified starches) Gums (agar, alginates, carrageenan, gum acacia, gum arabic, pectin), carboxymethyl cellulose, chitosan, cyclodextrins
Lipids	Natural fats and oils, fractionated fats, mono- and diglycerides, phospholipids, glycolipids, waxes (beeswax, carnauba wax)

1.7.3 Encapsulated delivery systems for lipids

Many functional lipids including polyunsaturated lipids such as n-3 FA, conjugated linoleic acids and lipid-soluble bioactives such as carotenoids, coenzyme Q10, tocopherols, and some vitamins are sensitive to oxidation (Gibbs et al., 1999). If not adequately protected from the environment, they can degrade very rapidly. This results in the development of off-flavors and reduced bioactivity. Many types of materials have been used to encapsulate sensitive lipids to isolate them from oxygen and minimize their interactions with trace metal ions (e.g., Fe) that catalyze their oxidation. Lipids may be trapped in glassy matrices, stabilized in emulsion systems with low molecular weight surfactants or film forming biopolymers, or encapsulated in liposomes or cyclodextrin (Augustin & Sanguansri, 2008).

1.7.4 Glassy matrices

The ability of glassy matrices to encapsulate lipophilic bioactive molecules makes them useful for the delivery of oils and oil-soluble bioactives (Augustin & Sanguansri, 2008). However, the efficiency of encapsulation and the degree of protection offered varies with the formulation. Some authors suggest that increased molecular mobility as the temperature is raised above the glass transition temperature leads to an increase in the oxidation of encapsulated lipid components (Gejl-Hansen & Flink, 1977).

Shimada et al. (1991) found that above the glass transition temperature, encapsulated methyl linoleate was released from a lactose-gelatin matrix, resulting in its rapid oxidation. Labrousse et al. (1992) demonstrated that oxidation of methyl linoleate was arrested in sugar-gelatin glassy matrices, but that rapid oxidation occurred when the matrix collapsed. However, others have found that molecular mobility did not influence oxidation rates of flaxseed oil (Grattard et al., 2002). Others have shown that there was only partial protection of the oils as oxygen can permeate a glassy food matrix (25% sucrose, 45% maltodextrin, 5% gelatin, and 25% rapeseed oil), where the limiting factors were oxidation at low temperature and oxygen diffusion at higher temperatures (Orlien et al., 2006).

1.8 Oligosaccharides

Currently, the use of foods that encourage a state of well-being, better health and reduction of the risk of diseases have become popular as consumers are becoming more and more health conscious. In this sense, there has been a lot of attention paid to specific types of dietary carbohydrates, namely the non-digestible oligosaccharides. These compounds present important physicochemical and physiological properties beneficial to the health of consumers, and for this reason, their use as food ingredients has increased rapidly. Such properties include non-cariogenicity,

a low calorific value, and the ability to stimulate the growth of beneficial bacteria in the colon (Mussatto & Mancilha, 2007). They are also associated with a lower risk of infections and diarrhea, and an improvement of the immune system response. Moreover, due to the decrease in intestinal pH caused by their fermentation, oligosaccharides provoke a reduction of the pathogens flora, an increase of bifidobacteria population, and an increase of the availability of minerals (Verdonk et al., 2005). In the food industry, these compounds have great potential to improve the quality of many foods, providing modifications to food flavor and improving its physicochemical characteristics (Crittenden & Playne, 1996; Rivero-Urgell & Santamaria-Orleans, 2001). So that, the popularity of oligosaccharides as food ingredients has strongly increased, mainly in the last few years. As a consequence, several researches have been performed aiming the discovery of new oligosaccharides, as well as the development of new products containing these compounds.

1.8.1 Properties of oligosaccharides

The carbohydrates can be classified according to their molecular size or degree of polymerization (number of monosaccharide units combined), into monosaccharides, oligosaccharides, or polysaccharides. According to IUPAC nomenclature, oligosaccharides are defined as saccharides

containing between 3 and 10 sugar moieties. Other authorities classify saccharides including anyone from 3 to 19 monosaccharide units in this group (Mussatto & Mancilha, 2007). Thus, oligosaccharides are low molecular weight carbohydrates.

Oligosaccharides are water soluble and typically 0.3–0.6 times as sweet as sucrose. In fact, the sweetness depends on chemical structure, the degree of polymerization of the oligosaccharides present and the levels of mono- and disaccharides in the mixture (Crittenden & Playne, 1996; Voragen, 1998). According to Roberfroid and Slavin (2000) the sweetness decreases with longer the oligosaccharide chain length. This low sweetness intensity is quite useful in the various kinds of foods where the use of sucrose is restricted by its high sweetness property. They also provide a high moisture-retaining capacity, preventing excessive drying, and a low water activity, which is convenient in controlling microbial contamination (Crittenden & Playne, 1996). The caloric value of oligosaccharides has been estimated to be 1.5–2.0 kcal/g. This is approximately 40–50% of those of digestible carbohydrates such as sucrose (Sako et al., 1999).

Although oligosaccharides possess important physicochemical properties, most of the interest in their use as food ingredients is due to their many physiological properties beneficial for health. One of these is that unlike starch and simple sugars, the oligosaccharides are not utilized by mouth

microflora (Delzenne & Roberfroid, 1994). Therefore, the oligosaccharides can be used as low cariogenic sugar substitutes in products like confectionery, chewing gums, yoghurts, and drinks (Crittenden & Playne, 1996). Many oligosaccharides are not digested by humans because humans lack the enzymes required to hydrolyze the β-links formed among the units of some monosaccharides. Such compounds include carbohydrates, where fructose, galactose, glucose, and/or xylose are the monosaccharide units. This property makes the oligosaccharides suitable for use in sweet and low-caloric diet foods, and for consumption by individuals with diabetes (Crittenden & Playne, 1996; Rivero-Urgell & Santamaria-Orleans, 2001).

1.8.2 Applications of oligosaccharides

A number of oligosaccharides have been introduced as functional food ingredients during the last few decades, and their industrial applications are continuously increasing (Mussatto & Mancilha, 2007). Uses are focused in beverages (fruit drinks, coffee, cocoa, tea, soda, health drinks, and alcoholic beverages), dairy products (fermented milk, instant powders, powdered milk, and ice cream), probiotic yogurts (based on live microorganisms that exert beneficial effects for the host via improvement of the microbiological balance in the intestine) and synbiotic products (containing a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and

implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare) (Gibson & Roberfroid, 1995). Other current applications of oligosaccharides in the food industry include desserts such as jellies, puddings, and sherbets; confectionary products such as candy, cookies, biscuits, breakfast cereals; chocolate, and sweets; breads and pastries; table spreads and spreads such as jams and marmalades; and meat products such as fish paste and tofu (Voragen, 1998). Nevertheless, since the specific physicochemical and physiological properties of oligosaccharide products vary depending on the type of mixture prepared, the most appropriate oligosaccharide for a particular food application also varies (Crittenden & Playne, 1996).

In fact, nowadays, oligosaccharides are recognized as important food ingredients to keep and improve our health, and as many consumers depend on processed foods as the backbone of their diets, the increased oligosaccharides content of popular foods assist consumers in obtaining recommended levels of non-digestible carbohydrate. Moreover, as is known that the bifidobacterial number in the human gut tend to decrease with age, the ingestion of bifidobacteria containing preparations or foods, or food supplemented with substrates (bifidogenic factors or prebiotics) that specifically promote the growth of endogenous bifidobacteria in the gut, is

very useful for the solution of this problem (Flamm et al., 2001). For these reasons, synbiotic food products containing both probiotics and prebiotics are the more recent novelty in the food industry (Mussatto & Mancilha, 2007). The advantages provided to food industries by the physicochemical properties of oligosaccharides, combined with the growing consumer interest in health reinforce the expectations that the dietary supplement industry will continue to exhibit strong growth, and the oligosaccharides production and use will continue to expand.

Chapter 2

Physical and Oxidative Stability of Flaxseed Oil-Fructooligosaccharide Emulsion

(Study 1)

2.1 Introduction

Flaxseed or linseed (*Linum usitatissimum* L.), a member of the Linaceae family, is an important oilseed crop in the world. Flaxseed oil is highly unsaturated (about 90% of total FA), and particularly rich in ALA up to 60% of total FA (Angers et al., 1996; Nykter et al., 2006). A scientific literature suggests that regular consumption and/or dietary supplementation with long chain n-3 PUFA gives various health benefits, including the prevention of cardiovascular diseases and inflammatory diseases (Garg et al., 2006). Consuming flaxseed oil is one of the recommendable ways to increase n-3 FA in the diet as most of the society nowadays are known to consume too much n-6 FA and too little n-3 FA from the diet (Choo et al., 2007). However, the most critical problem in the quality of ALA-rich flaxseed oil is oxidation during storage or processing (Esterbauer et al., 1991). The high susceptibility of polyunsaturated lipids to oxidation has restricted their incorporation into food products.

Fructooligosaccharides (FOS) are linear polymers of fructosyl monomers linked through β (2→1) bonds (Azorin-Ortuno et al., 2009) with a polymerization degree more than ten (Akalin & Erisir, 2008). In addition to accuracy of their enzymatical production and their sweetness comparable to that of sucrose, their low caloric content is worth stressing. They also have high moisture-retaining capacity, preventing excessive drying, and

controlling microbial contamination (Crittenden & Playne, 1996). These compounds present important physicochemical and physiological properties beneficial to human health, and their use as food ingredients rapidly increases. Such properties include a low calorific value and the ability to stimulate the growth of beneficial bacteria in the colon as a source of prebiotics (Mussatto & Mancilha, 2007).

Incorporation of flaxseed oil and FOS into the food system has potential advantage for the food industry to provide EFA and prebiotics at the same time. Emulsion could be one of the promising processing options. To the best of our knowledge, however, emulsion using flaxseed oil and FOS has not been studied. Thus, the primary objective of this study was to develop a practical and simple method to produce a flaxseed oil emulsion with FOS. The mechanism of emulsification and behavior of emulsion components along with the knowledge of factors affecting emulsion properties during emulsification needed to be elucidated. Development of emulsion with desirable nutritional and physical attributes depends on the availability of improved methods of controlling their physical and oxidative stability, which in turn relies on thorough understanding of the mechanisms of physical properties and lipid oxidation in emulsion. In order to achieve the objective, (i) a suitable emulsifier to produce flaxseed oil emulsion with FOS, (ii) effects of concentrations of emulsifiers and ratio of dispersed phase to continuous phase on rheological properties and particle size distribution of

flaxseed oil-FOS emulsion, and (iii) their physical and oxidative stability were investigated.

2.2 Materials and methods

2.2.1 Materials

Flaxseed oil, which had been prepared from seeds by a screw press, was provided by Haitnimnara Co. (Hwaseong, Korea). The oil was stored in a refrigerator (4°C) until the preparation of emulsions. FOS containing 55% FOS, 17.5% fructose, 17.5% glucose, and 10% sucrose on dry basis (based on the manufacturer's information) was obtained from Samyang Genex Co. (Seoul, Korea). FOS used in this study was in liquid form, containing about 10% moisture. Glycerol monostearate (hydrophilic lipophilic balance (HLB) 4.3), sorbitan monolaurate (HLB 8.6), sucrose monostearate (HLB 11.0), decaglycerol monooleate (HLB 14.5), and decaglycerol monolaurate (HLB 15.0) were supplied by Ilshin Wells (Seoul, Korea). Food grade ingredients were used in this study.

2.2.2 Emulsion preparation

Flaxseed oil emulsions were prepared using glycerol monostearate,

sorbitan monolaurate, sucrose monostearate, decaglycerol monooleate, and decaglycerol monolaurate as emulsifiers (flaxseed oil:FOS:emulsifier= 55:40:5, weight basis). Among the tested emulsifiers, decaglycerol monolaurate, an emulsifier with the most stable emulsion forming ability, was selected and used in the following experiments. Emulsions with decaglycerol monolaurate at the concentrations of 0.5, 1, 2, 3, 4, and 5% (w/w, replacing FOS) were made in a mixture of 55% flaxseed oil and 45% FOS to determine optimum level of emulsifier to make a stable emulsion. Emulsions with different ratios of flaxseed oil to FOS (3:7, 4:6, 5:5, 6:4, 7:3, and 8:2, weight basis) using 3% (w/w) decaglycerol monolaurate were also tested. The emulsions mentioned above were made as follows: The emulsifiers were dissolved in FOS (liquid form) and heated to 60°C to make a continuous phase. Flaxseed oil was gradually added into the continuous phase and homogenized using a homogenizer (HG-15A, Daihan Scientific Co., Wonju, Korea) equipped with an HT1018 dispersion tool (rotor Ø12.7, stator Ø18.0) for 5 min at 3,000 rpm at 60±1°C. The viscosity and particle size distribution were measured immediately after they were prepared. Three sets of emulsions were made for each emulsion group.

2.2.3 Emulsion stability

Relative stability of the emulsions was evaluated immediately after they

were prepared. Ten mL of each emulsion was transferred into a cylindrical polystyrene tube (internal diameter, 17 mm; height, 120 mm), sealed with a screw cap and stored at room temperature for 7 weeks. Emulsion stability of the emulsions was determined by visually measuring the extent of oil layer, and expressed by the emulsion stability index (ESI) as $\{1-(H_o/H_t)\}$ x100, where H_o is the volume of oil layer and H_t is the total volume of oil in the emulsion sample (Cho, Shim, & Park, 2003).

2.2.4 Viscosity and rheological behavior

Viscosity of the emulsions was measured by a rotational programmable viscometer (Model LVDV-II Pro, Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) at 35°C using cone-plate geometry. Approximately 3 mL of an emulsion was placed in a concentric cylinder of the device, applying shear rate ranging from 10.3 to 1030/s. Rheological parameters (shear stress, shear rate, and apparent viscosity) were obtained. Flow behavior of the emulsions was described by fitting the experimentally measured shear stress–shear rate data to Herschel–Bulkley model: $\sigma = \sigma_0 + K\dot{\gamma}^n$, where σ is shear stress (Pa), $\dot{\gamma}$ is shear rate (1/s), *K* is consistency coefficient (Pa s^n), and *n* is the flow behavior index. Tests were carried out in triplicate.

2.2.5 Emulsion particle size

The emulsion (0.1 g) was dispersed in distilled water (10 mL) by gentle stirring. The particle size distribution of the dispersed sample was determined using a particle size analyzer (Analysette 22, Compact Laser Particle Sizer, Fritsch, Germany). Tests were carried out in duplicate.

2.2.6 Optical microscopy

Emulsions, which were diluted 10-fold in 10 mM sodium phosphate buffer (pH 7.0) at room temperature, were observed employing an optical microscope (KH-8700, Hiroxkorea Co., Ltd, Gyunggi-do, Korea) with differential interference contrast mode at 2,000 magnification.

2.2.7 Oxidative stability

Oxidative stability of flaxseed oil emulsions was evaluated under controlled storage condition at 35°C for 72 days. Emulsions were made mixing 55% (w/w) flaxseed oil and 42% FOS with 3% (w/w) decaglycerol monolaurate. Emulsions (40 g) were stored in loosely sealed polypropylene tubes (50 mL) at 35°C in an oven in the dark. Oxidative stability of flaxseed oil emulsion was measured by monitoring peroxide value (PV), conjugated diene (CD), and anisidine value (AV). Flaxseed oil without TBHQ was used

as control.

For these analyses, the oil from the emulsions was extracted as described by Folch et al. (1957) with some modification. One hundred grams of emulsion were homogenized consecutively adding 200 mL chloroform and then100 mL methanol stirring for 2 min and 1 min, respectively. One hundred mL 0.88% KCl was added to the mixture, followed by homogenizing for more than 1 min. The mixture was centrifuged at 1,300 x g for 10 min at 4°C to separate into two layers and then the lower (chloroform with the oil) phase was collected. The chloroform was evaporated in a rotary evaporator at 35°C.

PV was determined according to the AOCS (1990) with slight modification. Sample (5 g) was dissolved in a blended solution of 30 mL chloroform–glacial acetic acid (3:2, v/v), followed by adding saturated KI solution (0.5 mL). The mixture was shaken by hand for 0.5 min and kept in the dark for another 5 min. After addition of 30 mL distilled water, the mixture was titrated with sodium thiosulphate (0.1 M) until the yellow color almost disappeared. About 2 mL of starch indicator (1%) solution was added. Titration ended when the blue color just disappeared. A blank was also measured under the same condition. CD (Ti la-64) and AV (Cd 18-90) were determined according to the AOCS (1990) method. Tests were carried out in triplicate.

2.3 Results and discussion

2.3.1 Emulsion stability

ESI can provide indirect information about the extent of droplet aggregation in an emulsion: the more the aggregation, the larger flocs form and the faster creaming appears. ESI of flaxseed oil emulsions with 5 different emulsifiers during 7 weeks of storage at room temperature are presented in Table 2.1. The effects of emulsifiers with different HLB values on emulsion forming properties between flaxseed oil and FOS were determined in this study.

Decaglycerol monolaurate with HLB 15 provided complete stabilization in the flaxseed oil-FOS emulsion without oil phase separation during 7 weeks of storage, while the other emulsifiers showed oil phase separations from FOS so that it floated on the top in a single, continuous layer shortly after its preparation. Decaglycerol monolaurate was suitable to produce flaxseed oil-FOS emulsion with particle size ranging from 1 to 2 μm.

ESI of flaxseed oil emulsions with different concentrations of decaglycerol monolaurate stored for 7 weeks at room temperature are presented in Table 2.1. Emulsions prepared with decaglycerol monolaurate at the concentrations more than 2% were stable with no separation of oil. However, oil separation was observed after 7 weeks of storage in the emulsion

containing 1% decaglycerol monolaurate. Increasing decaglycerol monolaurate concentration increased the surface coverage of oil droplets against flocculation, resulting in decreased creaming.

ESI of the flaxseed oil emulsions with different ratios of flaxseed oil to FOS during 7 weeks of storage at room temperature are presented in Table 2.1. All of the emulsions except for the ratio of 8:2 (flaxseed oil:FOS) were stable with no oil phase separation at the 0 and 7th weeks. In Bancroft's rule (Bancroft, 1913) an emulsion's hydrophile colloid will tend to make water the dispersed phase while a hydrophobe colloid will tend to make water the dispersed phase, the phase which contains most of the surfactant becomes the continuous phase. Even in an emulsion formula with 60% oil and 40% water, if the emulsifier chosen is more soluble in water, it will create an oil-in-water system. Although there are some exceptions to Bancroft's rule, it is a very useful rule of thumb for most emulsion systems. The hydrophilic-lipophilic balance (HLB) of a surfactant can be used in order to determine whether it is a good choice for the desired emulsion or not. In this study phase inversion did not occur in the emulsion with the ratio of 7:3 (flaxseed oil:FOS).

These results indicate that type and concentration of emulsifiers and the ratio of dispersed and continuous phases played important roles in the stability of flaxseed emulsion with FOS. McClements (2004) explained that

the excellent physical stability in the emulsions containing polysaccharides is likely due to the ability of a polysaccharide to increase the viscosity of the continuous phase, which decreases droplet collisions, thus decreasing flocculation and coalescence. The use of FOS as continuous phase in this study may make the emulsions reasonably stable mainly due to increase in viscosity of continuous phase and slowing down the movement of dispersed droplets. Higher levels of FOS may also make them form a protective layer around emulsion droplets and reduce the rate of destabilizing phenomena such as coalescence, Ostwald ripening, flocculation, etc., by increasing emulsion stability.

Table 2.1. Emulsion stability index (ESI) of flaxseed oil-fructooligosaccharide (FOS) emulsions.

		ESI (%)	
		0 week	7 weeks
Emulsifier (HLB) [1)]	Glycerol monostearate (4.3)	20±0.6	0±0.0
	Sorbitan monolaurate (8.6)	70±0.6	56±1.0
	Sucrose monostearate (11.0)	12±0.6	10±0.0
	Decaglycerol monooleate (14.5)	50±0.0	40±0.6
	Decaglycerol monolaurate (15.0)	100±0.0	100±0.0
Concentration of decaglycerol monolaurate (%, w/w)[2)]	0.5	10±0.6	10±0.0
	1.0	100±0.0	95±0.0
	2.0	100±0.0	100±0.0
	3.0	100±0.0	100±0.0
	4.0	100±0.0	100±0.0
	5.0	100±0.0	100±0.0
Ratio of flaxseed oil to FOS (weight basis)[3)]	3:7	100±0.0	100±0.0
	4:6	100±0.0	100±0.0
	5:5	100±0.0	100±0.0
	6:4	100±0.0	100±0.0
	7:3	100±0.0	100±0.0
	8:2	10±0.0	10±0.0

[1)]Flaxseed oil:FOS:emulsifier=55:40:5.

[2)]Flaxseed oil-fructooligosaccharide (FOS) emulsions using different

concentrations of decaglycerol monolaurate (replacing FOS) in a mixture of 55% (w/w) flaxseed oil and 45% FOS.

[3)]Flaxseed oil-fructooligosaccharide (FOS) emulsions with 3% (w/w) decaglycerol monolaurate. Values with standard deviations from triplicate experiments.

2.3.2 Rheological properties

Fig. 2.1 shows the apparent viscosity of flaxseed oil emulsions containing different concentrations of decaglycerol monolaurate. The addition of the lower concentrations (0.5 and 1%, w/w) of the emulsifier resulted in lower viscosity with dilatant flow. The increase in the concentrations (2, 3, and 4%, w/w) of the emulsifier increased viscosity, showing pseudoplastic flow. As shown in Fig. 2.1, viscosity of the emulsions with 2, 3, and 4 % emulsifier decreased with increasing shear rate. Viscosity of the emulsifier with the concentration of 5% emulsifier was not measurable because the viscosity was too high. As shear rate sufficiently increases to overcome Brownian motion, the emulsion droplets become more ordered along the flow field and offer less resistance to flow, hence lowering the viscosity (Sun et al., 2007). Fig. 2.2 shows the apparent viscosity of flaxseed oil emulsions made with different ratios of flaxseed oil to FOS. The increase of flaxseed oil in the

emulsions increased viscosity and all of the emulsions showing pseudoplastic flow. Viscosity was not measurable when the ratio was 7:3 (flaxseed oil:FOS) because the viscosity was too high and no emulsion was formed when the ratio was 8:2.

Shear stress compared with shear rate of flaxseed oil emulsions at different concentrations of the emulsifier and different ratios of flaxseed oil to FOS was calculated using Herschel–Bulkley model. The flow behavior index indicates shear-thinning (pseudoplastic) ($n < 1$), dilatant ($n > 1$), or Newtonian ($n = 1$) behavior (Anton et al., 2001). The flaxseed oil emulsions containing the lower concentrations (0.5 and 1.0%) of decaglycerol monolaurate showed dilatant behavior with an *n* value more than 1 (Table 2.2). When shearing a concentrated stabilized solution at a relatively low shear rate, the repulsive particle-particle interactions keep the particles in an ordered, layered, and equilibrium structure. However, at shear rates elevated above the critical shear rate, the shear forces pushing the particles together overcome the repulsive particle-particle interactions, forcing the particles out of their equilibrium positions (Boersma et al., 1990). This leads to a disordered structure, causing an increase in viscosity. The flaxseed oil emulsions containing the higher concentrations (2, 3, and 4%, w/w) of decaglycerol monolaurate showed pseudoplastic behavior with an *n* value less than 1. The flaxseed oil emulsion using different ratios of flaxseed oil to FOS reflected the pseudoplastic behavior with an *n* value less than 1 (Table

2.2). The difference in the ratios of flaxseed oil to FOS changed little of the flow behavior index.

Consistency index is an indicator of viscous behavior and was observed to be drastically increased when the emulsifier concentration increased from 1.0% to 2.0%. Increase in the volume of flaxseed oil increased consistency index. Increase in emulsifier concentration in emulsions caused consistency index to increase, which was in a positive correlation with apparent viscosity of emulsions. Decrease in the emulsion flow behavior index, which suggests the extent of non-Newtonian behavior of emulsions, was influenced by increasing emulsifier concentration (Krstonosic et al., 2009).

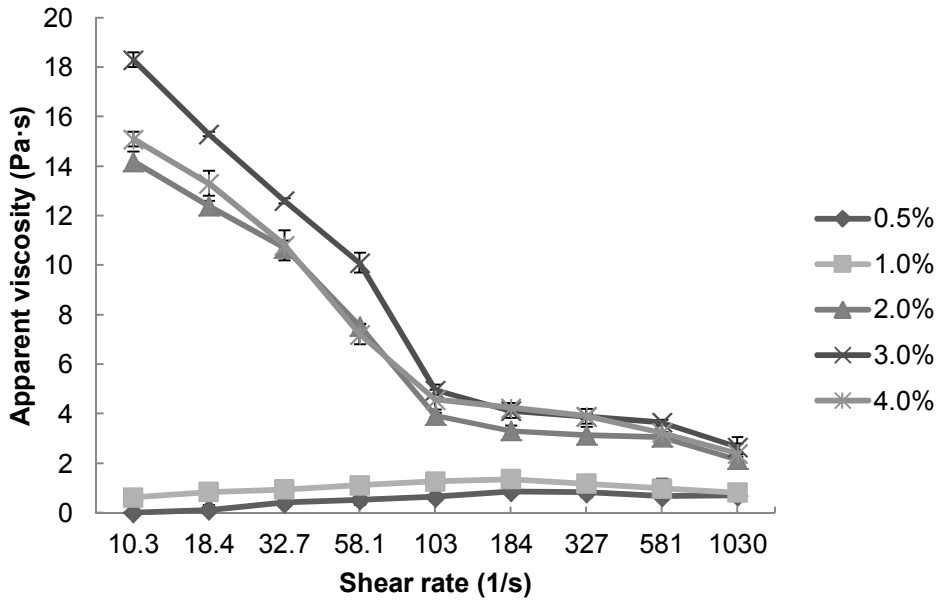


Fig. 2.1. Viscosity of flaxseed oil-fructooligosaccharide (FOS) emulsions with different concentrations of decaglycerol monolaurate (replacing FOS) in a mixture of 55% (w/w) flaxseed oil and 45% FOS. Values with standard deviations from triplicate experiments.

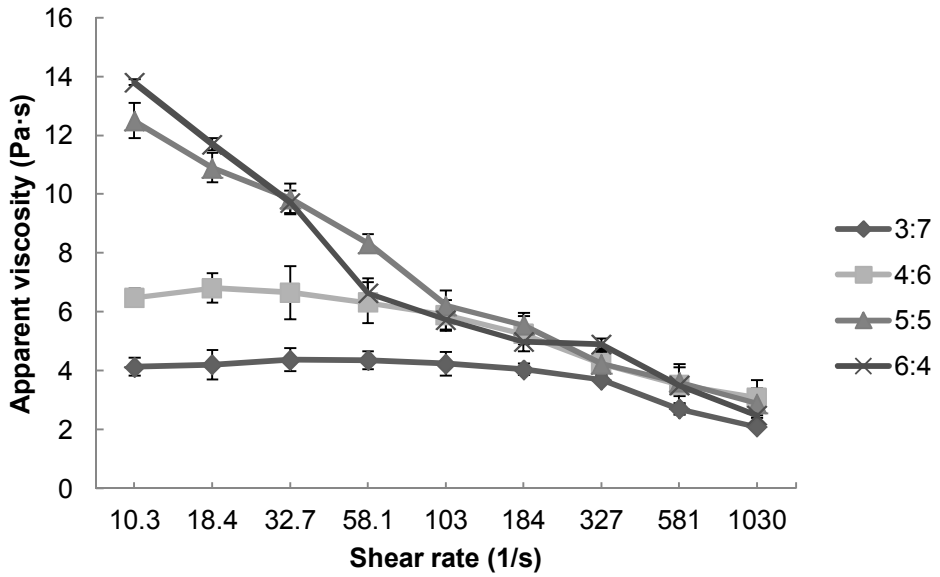


Fig. 2.2. Viscosity of flaxseed oil-fructooligosaccharide (FOS) emulsions with different ratios of flaxseed oil to FOS with 3% (w/w) decaglycerol monolaurate. 3:7, 4:6, 5:5, and 6:4:ratios of flaxseed oil to FOS (weight basis). Values with standard deviations from triplicate experiments.

Table 2.2. Rheological parameters of flaxseed oil-fructooligosaccharide (FOS) emulsions calculated using Herschel-Bulkley model.

		K (Pa s^n)	n	R^2
Concentration of decaglycerol monolaurate (%, w/w)[1)]	0.5	0.054	1.445	0.958
	1.0	0.732	1.063	0.961
	2.0	23.329	0.649	0.977
	3.0	24.536	0.673	0.982
	4.0	19.413	0.701	0.990
Ratio of flaxseed oil to FOS (weight basis)[2)]	3:7	6.659	0.871	0.962
	4:6	11.614	0.825	0.995
	5:5	22.527	0.718	0.997
	6:4	20.917	0.721	0.969

[1)]Flaxseed oil-fructooligosaccharide (FOS) emulsions with different concentrations of decaglycerol monolaurate (replacing FOS) in a mixture of 55% (w/w) flaxseed oil and 45% FOS.

[2)]Flaxseed oil-fructooligosaccharide (FOS) emulsionss with 3% (w/w) decaglycerol monolaurate.

K: consistency coefficient; and *n*: flow behavior index

2.3.3 Particle size and optical microscopy

Particle size distributions of flaxseed oil emulsions made with different concentrations of the emulsifier are presented in Table 2.3. Increase in the concentration of decaglycerol monolaurate led to decrease in average particle size of the flaxseed oil emulsions. This result agreed with the study reported by Krstonošić et al. (2009), where increase in Tween 80 concentration led to decrease in average droplet diameter of investigated emulsions. Particle size drastically decreased when the emulsifier concentration increased from 1.0% to 2.0%. Particle size distributions of the flaxseed oil emulsions with different ratios of flaxseed oil to FOS are presented in Table 2.3. Particle size increased with increasing oil phase volume.

Increase in emulsifier concentration enhanced emulsifier adsorption and surface coverage of oil droplets, which effectively inhibited droplet aggregation. The larger particle size of the emulsion containing 1% emulsifier and the 7:3 ratio of flaxseed oil to FOS suggested that the emulsifier content may not be enough to cover the oil droplets and form sufficiently dense absorption layer. This result agreed with the study previously reported by Sun and Gunasekaran (2009). Thus, on the basis of these results it may be concluded that droplet size of the flaxseed emulsion with FOS is likely to decrease with increase of emulsifier concentration and

decrease of oil volume.

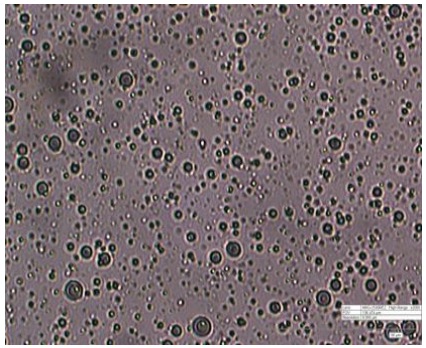
Microscopic images of emulsions are represented in Fig. 2.3. The microscopic images showed that flaxseed oil emulsions with FOS had homogeneously distributed with no sign of flocculation in the individual droplets. Larger droplets were observed in the emulsions with 1% emulsifier and with the 7:3 ratio of flaxseed oil to FOS. These results are in accordance with those obtained from particle size distribution of flaxseed oil emulsion (Table 2.3). Particle size and microscopic images were not measured because no emulsion was formed when the concentration of emulsifier was 0.5% and the ratio was 8:2 (flaxseed oil:FOS).

Table 2.3. Particle size (μm) distribution of flaxseed oil-fructooligosaccharide (FOS) emulsions.

		Cumulative volume		
		10%	50%	90%
Concentration of decaglycerol monolaurate (%, w/w)[1)]	1	1.05±0.01	2.52±0.01	8.76±0.10
	2	0.63±0.00	1.46±0.00	2.99±0.01
	3	0.40±0.01	1.26±0.01	2.92±0.01
	4	0.22±0.04	1.00±0.11	2.57±0.19
	5	0.19±0.01	0.94±0.02	2.58±0.11
Ratio of flaxseed oil to FOS (weight basis)[2)]	3:7	0.35±0.02	0.91±0.02	1.82±0.00
	4:6	0.13±0.00	0.74±0.00	2.26±0.01
	5:5	0.13±0.00	0.78±0.00	2.37±0.00
	6:4	0.44±0.01	1.37±0.00	2.98±0.00
	7:3	0.56±0.31	2.65±0.08	9.03±1.08

[1)]Flaxseed oil-fructooligosaccharide (FOS) emulsions with different concentrations of decaglycerol monolaurate (replacing FOS) in a mixture of 55% (w/w) flaxseed oil and 45% FOS.

[2)]Flaxseed oil-fructooligosaccharide (FOS) emulsions with 3% (w/w) decaglycerol monolaurate. Values with standard deviations from triplicate experiments.



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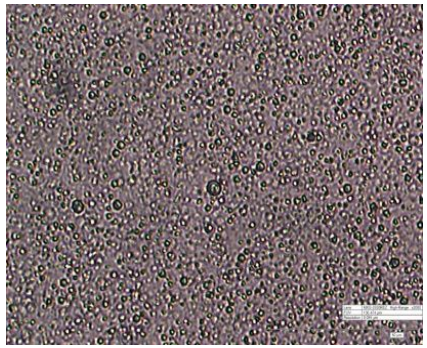

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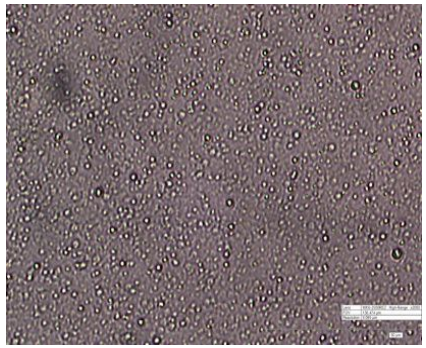

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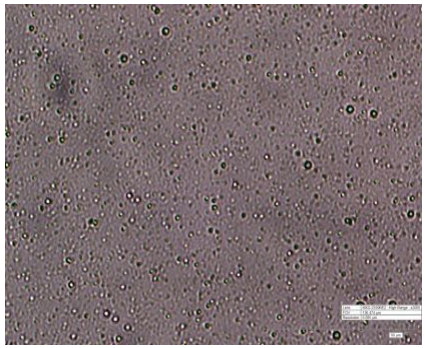

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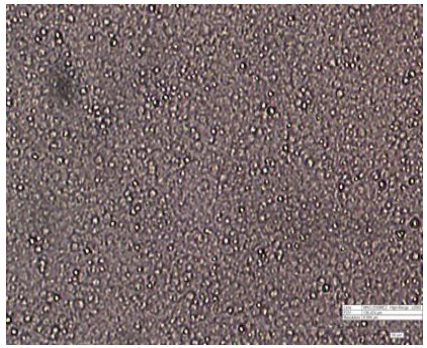

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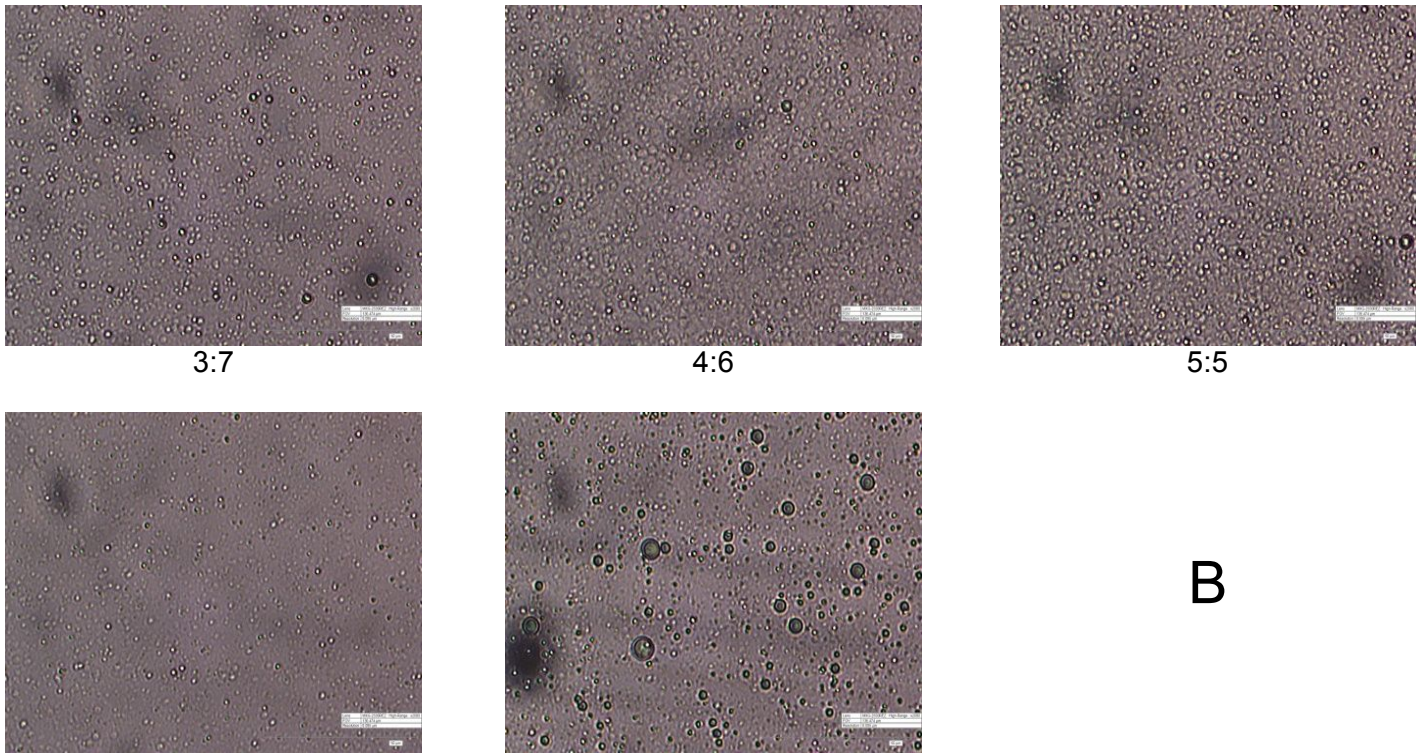

Fig. 2.3. Optical images of flaxseed oil-fructooligosaccharide (FOS) emulsions. A: emulsions using different concentrations of decaglycerol monolaurate (replacing FOS) in a mixture of 55% flaxseed oil and 45% FOS (w/w). B: emulsions using different ratios of flaxseed oil to FOS (w/w) with 3% decaglycerol monolaurate.

2.3.4 Oxidative stability

PV, CD, and AV in the emulsion and flaxseed oil, except the flaxseed oil added with TBHQ, increased during storage (Fig. 2.4). PV of the samples were initially 0.58 meq peroxides/kg oil. It increased to 15.05 meq peroxides/kg oil in the flaxseed oil-FOS emulsion and to 56.52 meq peroxides/kg oil in the flaxseed oil during 72 days of storage. Flaxseed oil also had more secondary oxidation products (CD and AV) than the flaxseed oil-FOS emulsion on the 72nd day. The flaxseed oil added with TBHQ had less CD and AV than the emulsion. These results indicate that the flaxseed oil emulsion was less oxidized than flaxseed oil, but more than the flaxseed oil added with TBHQ. Lipid oxidation can be accelerated by reactions that take place on the surface of emulsion droplets (McClements & Decker, 2000). Therefore, the rate of lipid oxidation increases as the droplet size decreases because smaller droplets lead to larger surface area per unit volume to the pro-oxidants at the interface (McClements & Decker, 2000). Oxidation is generally considered to occur faster in emulsions than in bulk oils (Frankel et al., 2002). Nevertheless, previous studies have shown controversial results, with some studies confirming the general hypothesis (Frankel et al., 2002) and some showing that droplet size might not be as important as the emulsifier composition at the interface (Let et al., 2007; Sørensen et al., 2007).

Chen et al. (2010) reported that oxidative stability of menhaden oil emulsion increased when alginate, carrageenan, and pectin (anionic polysaccharides) were used as a continuous phase. Sucrose has also been shown to reduce lipid oxidation of linoleic acid in oil-in-water emulsions stabilized by a non-ionic surfactant (Tween 20) (Ponginebbi et al., 1999). A number of mechanisms were proposed to account for the ability of sucrose to retard lipid oxidation in emulsions (McClements & Decker, 2000). FOS containing 10% sucrose (dry basis) was used for the preparation of all emulsions in my study. Sucrose decreases the concentration of oxygen dissolved in the aqueous phase, increases the viscosity of the aqueous phase (thereby decreasing the diffusion of reactive species to the droplet surface), and acts as a free radical scavenger (McClements & Decker, 2000).

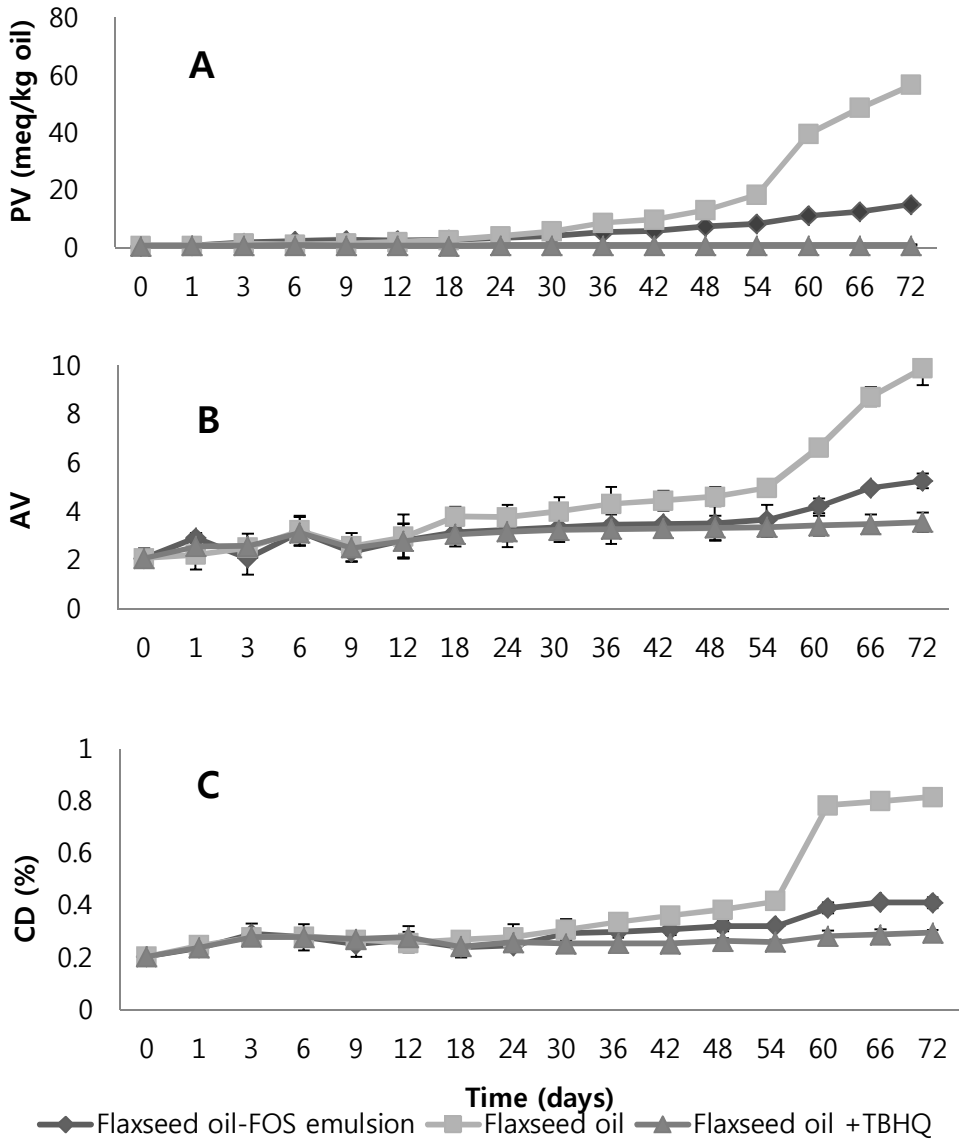

Fig. 2.4. Peroxide value (A), anisidine value (B), and conjugated diene (C) in flaxseed oil-fructooligosaccharide (FOS) emulsion, flaxseed oil, and flaxseed oil added with *tert*-butylhydroquinone (TBHQ). Values with standard deviations from triplicate experiments.

Chapter 3

Physicochemical and Sensory Characteristics of Yogurt Fortified with Flaxseed Oil-Fructooligosaccharide Emulsion

(Study 2)

3.1 Introduction

Yogurt is one of the most widely recognized dairy products consumed around the world, and it has been recognized as a healthy food due to the benefit from its high levels of proteins and calcium as well as a supply of probiotics. Traditionally produced yogurts contain 3-4% fat, which increases to 9-10% in concentrated yogurts, but they have been produced using skim milk in developed countries due to concern about the fat level (Tamime & Robinson, 1999). Yogurt is a good candidate for n-3 fatty acid (FA) fortification because of their high frequency of consumption and storage under refrigerated conditions (Kolanowski & Weißbrodt, 2007).

It has been proposed that dietary supplementation with long chain n-3 polyunsaturated fatty acid (PUFA) provides various health benefits, including prevention of cardiovascular diseases, inflammatory diseases, and depression (Garg, Wood, Singh, & Moughan, 2006). Consuming flaxseed oil is one of the recommendable approaches to increase the n-3 FA in the diet as the majority of the societies nowadays are known to consume an excessive amount of n-6 FA and too little n-3 FA from the diet (Choo, Birch, & Dufour, 2007).

An extensive variety of dairy and nondairy foods has been fortified with n-3 FA. Primary approaches to deliver these FA to foods are direct addition of fish oil or algal oil and bio-delivery through meat and poultry products

(Whelan & Rust, 2006). Most attempts to add DHA or EPA directly to foods have been unsuccessful because these FA are unstable, particularly readily susceptible to oxidation resulting in fishy flavor to make foods unpalatable. Using flaxseed oil as a functional food ingredient in a yogurt for delivery of n-3 FA may also help avoid fishy and metallic off-flavors associated with marine-derived sources.

One methodology to reduce lipid instability throughout the production of n-3 FA fortified foods is to prepare a stabilized emulsion of n-3 FA, which is then added to a finished or semi-finished food product without additional emulsification. Addition of n-3 FA oil at the latest possible stage can reduce the exposure to light, heat, and oxygen. In addition, contact between n-3 FA and potential prooxidants in a food product during processing is reduced by adding the oil in an already stabilized emulsion as the final step of the processing, because of the interfacial membrane surrounding the oil in the stabilized emulsion. Also, a stabilized oil-in-water emulsion is an effective carrier for an n-3 FA oil because it can be easily incorporated into an aqueous food. To make oil-in-water emulsion, fructooligosaccharide (FOS) was used as continuous phase in this study. FOS presents important physicochemical and physiological properties valuable to human health and their utilization as food ingredients expands. Such properties include a low calorific value and the capability to stimulate the growth of beneficial bacteria in the colon as a source of prebiotics (Mussatto & Mancilha, 2007).

To our knowledge, yogurt fortified with flaxseed oil-FOS emulsion has not been studied. The purpose of this study was to determine the physicochemical and sensory characteristics of yogurts added with the flaxseed oil-FOS emulsion directly before (set type) and after (stirred type) fermentation in whole and non-fat milk.

3.2 Materials and methods

3.2.1 Materials

Milk and non-fat milk powder (Seoul Milk Co., Seoul, Korea) were purchased from a local market. Flaxseed (*Linum usitatissimum*, Brown*)* oil was obtained from Haitnimnara Co. (Hwaseong, Korea). Fructooligosaccharide (FOS) containing 55% FOS, 17.5% fructose, 17.5% glucose, and 10% sucrose (based on the manufacturer's information) on a dry basis was obtained from Samyang Genex Co. (Seoul, Korea). FOS used in this study was in liquid form and contained about 10% moisture. Freeze-dried commercial yogurt starter culture combining *Lactobacillus acidophilus*, *Bifidobacterium longum*, *Lactobacillus casei*, and *Streptococcus thermophiles* (ABCT-HK, Culture Systems, Inc., Indiana, USA) was obtained from Samik Dairy & Food Co., Ltd (Seoul, Korea).

3.2.2 Preparation of emulsion

Flaxseed oil emulsions were prepared using decaglycerol monolaurate as emulsifier (flaxseed oil:FOS:emulsifier=50:47:3). The emulsifier was dissolved in FOS and heated to 60°C to make a continuous phase. Flaxseed oil was gradually added into the continuous phase and homogenized using a homogenizer (HG-15A, Daihan Scientific Co., Wonju, Korea) equipped with an HT1018 dispersion tool (rotor Ø12.7, stator Ø18.0) for 5 min at 3,000 rpm at 60±1°C.

3.2.3 Preparation of yogurt

The experimental protocols used for making set and stirred yogurts are shown in Fig. 1. Milk and non-fat milk powder were used as main dairy ingredients of the yogurts. Reconstituted non-fat milk (12.0%, w/w) was prepared by dissolving non-fat milk powder into distilled water. The flaxseed oil-FOS emulsion was added either before (set yogurt) or after (stirred yogurt) fermentation. Set yogurt was made as follows: The whole or non-fat milk was mixed thoroughly with 1.0% flaxseed oil-FOS emulsion using a hand blender to obtain a homogeneous yogurt mix. Containers with the mixture were placed in a water bath and heated to 85°C for 30 min. They were cooled in an ice water bath to inoculation temperature of 43°C. After inoculation, the yogurt mixture was agitated for 10 min to keep the

temperature at 43°C. The milk was inoculated with the starter culture at a level of 0.02% (w/v). The inoculated milk was transferred into 120 mL transparent plastic cups with lids and incubated at 43°C until the pH reached 4.50. The products were removed from the incubator and stored at 4°C until used. Stirred yogurt was made as follows: The whole or non-fat milk in a glass bottle (1 L) was placed in a water bath and heated to 85°C for 30 min. Milk was cooled in an ice water bath at 43°C. The milk was inoculated with the starter culture at a level of 0.02% (w/v). After inoculation, the yogurt mix was agitated for 10 min at 43°C. The mixture was placed in an incubator for fermentation at 43°C until pH reached 4.5. After fermentation, the mixture was agitated using a 3-blade stirring shaft at 500 rpm for 1 min at 43°C. During agitation, the flaxseed oil-FOS emulsion was added to the yogurt at a level of 1.0 % (w/v). Six types of yogurts were prepared: set whole milk yogurt (Set-W), set whole milk yogurt added with flaxseed oil-FOS emulsion (Set-WE), set non-fat milk yogurt added with flaxseed oil-FOS emulsion (Set-NFE), stirred whole milk yogurt (Stir-W), stirred whole milk yogurt added with flaxseed oil-FOS emulsion (Stir-WE), and stirred non-fat milk yogurt added with flaxseed oil-FOS emulsion (Stir-NFE). They were fermented for approximately 5 h. Fresh batches were made for the descriptive and preference tests and evaluated within 1 day after making.

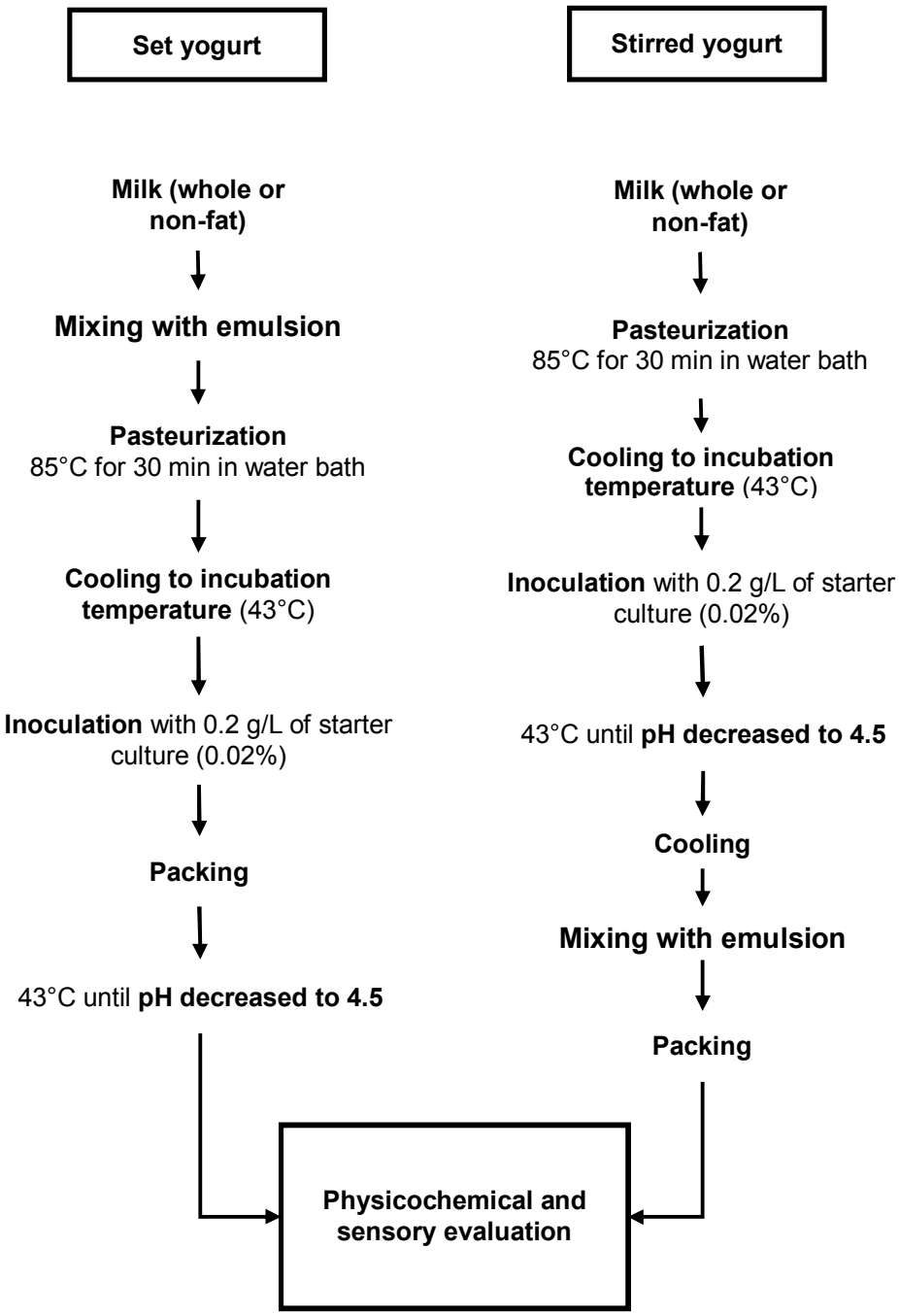

Fig. 3.1. Process of making set and stirred yogurts.

3.2.4 Physicochemical properties

3.2.4.1 pH and titratable acidity (TA)

pH of yogurt was measured using a digital pH meter (Thermo Fisher Scientific Inc., Waltham, MA, USA). TA (g/100 g as lactic acid) was measured using the method of Marshall (1992).

3.2.4.2 Total solids, crude proteins, and crude fat

Total solids, proteins and fat were determined according to AOAC (1995) methods.

3.2.4.3 Viscosity

Apparent viscosity of yogurt sample was measured at room temperature using a Brookfield DV I Prime Viscometer (Brookfield Engineering Laboratory Inc., Stoughton, MA, USA) with the spindle no. 64 at a speed of 5 rpm. The spindle was selected based on the torque measurement between 10 and 100%, as suggested by the manufacturer. Yogurt samples were stirred for 30 s before analysis and results were recorded in centipoises (cP) after 40 s of shearing.

3.2.4.4 Firmness

Firmness of the yoghurt was defined as the maximum force used in compression using TA-XT.2 Texture Analyzer (Stable Micro System, Goaldming, UK) with a cylindrical plunger (25 mm in diameter) and 50 kg load cell. The ratio of diameter of yogurt container to diameter of probe was 3.5:1. According to Amatayakul, Halmos, Sherkat, and Shah (2006), it is generally accepted that the boundary or wall effects diminishes when the diameter of sample is at least 3 times greater than the diameter of the probe. Yogurt samples were compressed at a constant penetration speed of 2 mm/s to a depth of 25 mm (75% of sample height). Firmness was defined as the maximum force used in penetration (Amatayakul et al., 2006). The test was carried out immediately after removing the samples from the refrigerator (4°C).

3.2.4.5 Color

Hunter L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) values of the yogurt samples were measured using a color difference meter (CM-3500d, Minolta Co., Ltd., Osaka, Japan). A white tile was used for standardization.

3.2.4.6 Analysis of fatty acid composition

FA composition of the lipids in the yogurts was determined using an *in situ* transesterification procedure (Park & Goins, 1994), which was a modification of the AOCS Official Methods (AOCS, 1980) for fatty acid methyl ester (FAME) preparation from fats and oils. Yogurt (100 μL) was transferred into a test tube. Methylene chloride (100 μL) and 1 mL 0.5 N NaOH in methanol were added to each sample. After being flushed with nitrogen and capped with a Teflon-lined screw cap, the test tube was heated in a water bath at 90°C for 10 min. The test tube was removed from the water bath and allowed to cool briefly before addition of 1 mL 14% BF_3 in methanol. After nitrogen flushing and screw-capping, it was continuously heated in a water bath at 90°C for 10 min. The test tube was cooled to room temperature. One mL distilled water and 500 μL hexane were added to the test tube and then FAME were extracted by vigorous shaking for about 1 min. Following centrifugation (1,000xg for 10 min), the top layer was transferred into a vial for GC analysis. FA composition was determined using Agilent 6890 gas chromatograph (Agilent Technologies, Paolo Alto, CA, USA) equipped with a capillary column (DB-23, 30 m x 0.25 mm x 0.25 μm, J&W Scientific, Folsom, CA, USA). The oven was programmed at 50°C for 1 min, to 175°C at 25°C/min, to 230°C at 4°C/min, held for 5 min, and to 250°C at 25°C/min, held for 3 min. The temperatures of the injector and detector (flame ionization detector) were 250 and 280°C, respectively.

Helium was used as the carrier gas with an injection split ratio of 10:1 (v/v). Peak identification and quantification were performed with reference to the retention times and peak areas as weight percent of a mixture of standard FAME (Supelco 37-component FAME mix, Supelco, Bellafonte, PA, USA). Individual FA were calculated by comparing the areas of FA to those of reference mixtures (GLC 3C, 4C, 7C, 8C, 12C, and 20A; Nu Chek Prep, Inc., Elysian, MN, USA). Results were expressed as % of total FA.

3.2.5 Sensory evaluation

3.2.5.1 Descriptive analysis

For descriptive analysis of yogurts 15 graduate students (six male and nine female, from 20 to 40 ages years old) were recruited as panelists based on their motivation, interest, and availability to pursue a three month study with two sessions per week. The panelists were trained in nine sessions of 30-40 min each. The first three sessions were used to develop the lexicon of descriptors and the next three sessions were devoted to the development of the ballot, establishing references for each attribute and exposing the panel to the scaling instrument. Table 1 illustrates the final terms, definitions, references, and reference intensities used by the panel for evaluation of the yogurts. The final three sessions were used for a trial run to make sure the panelists knew how to use the ballot and score the

products for each attribute. The samples were served to the panelists in 120 mL transparent plastic cups with lids labeled with a 3 digit randomized numbers. All six samples were served at each session, and the evaluations were repeated with freshly made samples. The panelists were provided with water for cleansing their mouths. A 16 cm structured scale was used by the panel to measure the attributes. The descriptive test was done in triplicate.

Table 3.1. Descriptive analysis attributes for the set and stirred yogurts fortified with flaxseed oil-fructooligosaccharide (FOS) emulsion.

Categories	Attribute	Abbreviation	Definition	Reference product [intensity of the reference[1]]
Appearance	Thick	Thick	The viscosity or readiness to flow as seen visually	Heavy whipped cream [5] (Seoul Milk, Seoul, Korea)
	Translucent	Trans	Degree of translucentness	Heavy whipped cream [12] (Seoul Milk, Seoul, Korea)
Aroma	Sour	SourA	Aroma associated with plain yogurt	Plain yogurt [9] (Denmark Milk, Dowon Dairy Food, Seoil, Korea)
	Butyric	ButyA	Aroma associated with typical cheese, butyric acid odor	Emmental Cheese [12] (Fromages Ermitage, Bulgneville, France)
	Fishy	FishA	Aroma associated with flaxseed oil	Flaxseed oil [13] (Haitnimnara Co., Hwaseong, Korea)
Taste	Sour	SourT	Taste on the tongue stimulated by acids such as citric acid	Citric acid [9] (ESFood Co., Anayang, Korea)
	Sweet	SweT	Taste on the tongue stimulated by sweeteners such as oligosaccharide	Fructooligosaccharide [9] (Samyang Genex Co., Seoul, Korea)
	Cheesy	CheT	Taste associated with typical cheese	Emmental Cheese [10] (Fromages Ermitage,

				Bulgneville, France)
	Fishy	FishT	Taste associated with flaxseed oil	Flaxseed oil [13] (Haitnimnara Co., Hwaseong, Korea)
Mouthfeel	Astringent	AstM	Complex mouthfeel associated with drying, roughness, and lingering residual	Black tea [8] (Twinings, wales, UK)
	Viscous	SmoM	The instantaneous thickness of heavy cream in mouth	Heavy whipped cream [3] (Seoul Milk, Seoul, Korea)
	Mouthcoat	MouM	The degree of coating on the tongue and palate during consumption	Light whipped cream [9] (Land O'Lakes, MN. USA)
Aftertaste	Sour	SourAT	Aftertaste on the tongue stimulated by acids such as lactic acid	Lactic acid [11] (ESFood Co., Anayang, Korea)
	Fishy	FishAT	Taste associated with flaxseed oil	Flaxseed oil [12] (Haitnimnara Co., Hwaseong, Korea)

[1]References were evaluated 3 times to generate a reference intensity and averaged 3 times of evaluations to give a mean value for the reference on a 16-cm structured scale.

3.2.5.2 Preference test

Preference test was conducted with 40 consumers (30 females and 10 males) ranging from 20 to 40 years old. Each consumer rated the six yogurts on a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely) for six liking attributes (appearance, color, aroma, taste, mouthfeel, and overall acceptability). The six yogurts were served to the consumers in 120 mL transparent plastic cups with lids. Water was provided for cleansing their mouths. Each sample was labeled with 3 digit randomized numbers, and served in a random order to each consumer. Fresh batches of the six yogurts were prepared for the preference test. The research for sensory evaluation (descriptive analysis and preference test) was reviewed and approved by Institutional Review Board at Seoul National University (IRB number: 1312/001-017). Each panelist signed written and informed consents to participate in the study.

3.2.6 Statistical analysis

Three separate batches of yogurt were freshly prepared for the physicochemical analysis and descriptive analysis. All analyses were performed in three times for each batch. Analysis of variance (ANOVA) with post-hoc mean difference using Duncan's multiple range test (α=0.05) was carried out for physicochemical data, descriptive analysis, and preference

test data, using SPSS package (SPSS Version 12.0K for Windows, SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) biplots were constructed using XLSTAT version 2013 (Addinsoft, NY, USA) for descriptive analysis data. To understand the relationship between descriptive analysis and preference test data, external preference mapping (PREFMAP) by partial least square regression was also done using XLSTAT (Addinsoft, NY, USA). Both of the data sets (descriptive analysis and preference test) were standardized before analysis (Thybo, Kuhn, & Martens, 2004).

3.3 Results and discussion

3.3.1 Physicochemical properties of yogurt

3.3.1.1 pH and TA

pH, TA, solid content, protein, fat, viscosity, firmness, and color of the yogurts fortified with the flaxseed oil-FOS emulsion are presented in Table 3.2. There was no significant difference ($p>0.05$) between the pH values of the set and stirred yogurts. Also, no significant difference was noted between the whole and non-fat milk yogurts ($p>0.05$). These values are within the normal ranges of set and stirred yogurts. TA of the samples ranged from 0.8 to 0.9 g/100 g (%, lactic acid). Fortification with the flaxseed oil-FOS emulsion had no significant effect on TA of the yogurts. The set and

stirred non-fat milk yogurts had higher TA than the corresponding whole milk yogurts ($p<0.05$).

3.3.1.2 Total solids, crude proteins, and crude fat

The averages of total solids, proteins, and fat in the six yogurts are shown in Table 3.2. The Set-WE and Stir-WE yogurts had higher solid contents than the others ($p<0.05$). Crude proteins were not significantly different between the set and stirred yogurts ($p>0.05$), but, significantly different between the whole and non-fat milk yogurts ($p<0.05$).

3.3.1.3 Viscosity and firmness

Apparent viscosity and firmness of the set yogurts were higher than those of the stirred yogurts ($p<0.05$) (Table 3.2) It was observed that the Set-NFE yogurt had a higher viscosity than the Set-WE yogurt. Yogurt supplementation with an increase in the total solid content, particularly protein content, brings about stronger texture and less whey separation (Peng, Serra, Horne, & Lucey, 2009). Perhaps the protein network of the non-fat milk yogurt was less disrupted by the addition of flaxseed oil-FOS emulsion as compared to the whole milk yogurt, because the non-fat milk yogurt was higher in protein content and lower in fat content. This has been

also observed in non-fat milk yogurts with addition of fruit preparations, concluding that polymerization of caseins may keep prevent protein network from disrupting completely (Lubbers, Decourcelle, Vallet, & Guichard, 2004). However, there was no significant difference among the stirred yogurts ($p>0.01$).

3.3.1.4 Color

The Set-NFE and Stir-NFE yogurts had significantly ($p<0.05$) lower 'a' value and higher 'b' value in comparison with the other samples (Table 3.2). However, 'a' and 'b' values were not significantly different ($p>0.05$) between the set and stirred yogurts. There was no significant difference ($p>0.05$) among the 'L' values of all the samples. This result suggests that color parameters may not be affected by the addition of flaxseed oil-FOS emulsion. Yellow appearance was significantly higher ($p<0.05$) in the non-fat milk yogurts (Set-NFE and Stir-NFE) than in the whole milk yogurts (Set-W, Set-WE, Stir-W, and Stir-WE). This result demonstrates that fat in the whole milk yogurts decreased the intensity of yellow color probably because of the scattering of light by the fat globules (Fox & McSweeney, 1998).

Table 3.2. Physicochemical properties of set and stirred yogurts fortified with flaxseed oil-fructooligosaccharide (FOS) emulsion.

		pH	Acidity (%, lactic acid)	Solid content (g/100 g)	Crude protein (g/100 g)	Crude fat (g/100 g)	Viscosity (Pa·s)	Firmness (g)	Color		
									L	a	b
Set yogurt	W[1]	4.50±0.1[2]	0.8±0.1[b]	12.1±0.1[b]	3.1±0.1[b]	3.9±0.1[b]	39.5±5.0[b]	73.1±3.1[b]	93.6±0.7	-1.4±0.2[a]	8.1±0.1[b]
	WE	4.49±0.1	0.8±0.0[b]	12.8±0.0[a]	3.0±0.1[b]	4.5±0.1[a]	42.2±8.0[b]	76.0±2.6[b]	93.6±0.5	-1.2±0.3[a]	8.3±0.4[b]
	NFE	4.60±0.1	0.9±0.1[a]	12.3±0.1[b]	4.1±0.1[a]	0.7±0.0[c]	54.1±2.8[a]	103.8±7.9[a]	92.3±1.4	-2.4±0.0[b]	9.4±1.3[a]
Stirred yogurt	W	4.50±0.1	0.8±0.0[b]	12.0±0.1[b]	3.1±0.1[b]	3.9±0.1[b]	1.5±0.1[c]	24.5±0.5[c]	93.7±0.5	-1.2±0.2[a]	8.2±0.2[b]
	WE	4.60±0.1	0.8±0.0[b]	12.8±0.1[a]	3.0±0.1[b]	4.4±0.1[a]	1.1±0.1[c]	25.5±1.2[c]	93.5±0.6	-1.2±0.3[a]	8.4±0.3[b]
	NFE	4.60±0.1	0.9±0.1[a]	12.1±0.0[b]	4.1±0.1[a]	0.7±0.0[c]	1.8±0.1[c]	25.7±0.5[c]	92.3±0.4	-2.5±0.3[b]	9.8±0.3[a]

[1]W, whole milk; WE, whole mlik+flaxseed oil-FOS emulsion; and NFE, non-fat milk+flaxseed oil-FOS emulsion

[2]Data are reported as mean values±standard deviation of three replicates.

[a-c]Means with different superscripts in the same column are significantly different ($p<0.05$).

3.3.1.5 Fatty acid composition

FA compositions of the lipids in the yogurts fortified with flaxseed oil-FOS emulsion are shown in Table 3.3. Saturated fatty acids (SFA) in the Set-W and Stir-W yogurts accounted for 72.6% and 72.7% of the total FA, respectively, and myristic, palmitic, and stearic acids were predominant. ALA was not detected in the Set-W and Stir-W yogurts. The addition of flaxseed oil-FOS emulsion resulted in a large increase in ALA concentration in the yogurts. The yogurts made with addition of flaxseed oil-FOS emulsion to the whole and non-fat milk showed n-6/n-3 FA ratios of 0.3-0.6, which were significantly lower ($p<0.05$) than the controls (Set-W and Stir-W) (2.6). However, the n-6/n-3 FA ratio may not be affected by the yogurt types. Considering that the Set-WE and Stir-WE yogurts had a fat content of approximately 4.5%, a serving (200 g) of the Set-WE and Stir-WE yogurts for 1 day may contain 0.62-0.64 g of ALA. The recommendation of ALA as an essential fatty acid is 0.6 g/day (MFDS, 2011). The yogurts fortified with n-3 FA by the addition of flaxseed oil-FOS emulsion have been designed to meet nutrient recommendations for ALA. It can be noted that an increase in n-3 FA in the yogurt, especially added with flaxseed oil-FOS emulsion, resulted in a greater reduction in the n-6/n-3 FA ratio.

Table 3.3. Fatty acid content of set and stirred yogurts fortified with flaxseed oil-fructooligosaccharide (FOS) emulsion.

(g/100 g yogurt (% of total FA), w/w, wet basis)

	Set yogurt			Stirred yogurt		
	W[1]	WE	NFE	W	WE	NFE
Butyric acid (C4:0)	0.05 (1.4)[2]	0.05 (1.2)	-	0.05 (1.4)	0.05 (1.1)	-
Caproic acid (C6:0)	0.06 (1.4)	0.06 (1.3)	-	0.06 (1.5)	0.05 (1.2)	-
Caprylic acid (C8:0)	0.04 (1.1)	0.04 (0.9)	-	0.04 (1.1)	0.04 (0.9)	-
Capric acid (C10:0)	0.10 (2.5)	0.10 (2.2)	-	0.10 (2.5)	0.10 (2.2)	-
Lauric acid (C12:0)	0.20 (5.2)[a]	0.21 (4.6)[a]	0.04 (5.5)[b]	0.20 (5.2)[a]	0.20 (4.6)[a]	0.04 (5.8)[b]
Myristic acid (C14:0)	0.46 (11.7)[a]	0.46 (10.2)[a]	0.02 (2.2)[b]	0.45 (11.6)[a]	0.45 (10.2)[a]	0.02 (2.5)[b]
Myristoleic acid (C14:1)	0.03 (0.9)	0.03 (0.8)	-	0.03 (0.9)	0.03 (0.8)	-
Pentadecanoic acid (C15:0)	0.04 (0.9)	0.04 (0.8)	-	0.03 (0.9)	0.03 (0.8)	-
Palmitic acid (C16:0)	1.26 (32.3)[a]	1.28 (28.5)[a]	0.01 (14.2)[b]	1.25 (32.2)[a]	1.28 (29.1)[a]	0.10 (14.6)[b]
Palmitoleic acid (C16:1)	0.06 (1.4)	0.05 (1.1)	-	0.06 (1.4)	0.04 (0.9)	-
Heptadecanoic acid (C17:0)	0.02 (0.5)	0.02 (0.5)	-	0.02 (0.5)	0.02 (0.5)	-
Stearic acid (C18:0)	0.59 (15.1)[a]	0.60 (13.4)[a]	0.09 (13.0)[b]	0.59 (15.2)[a]	0.62 (14.2)[a]	0.10 (14.2)[b]
Oleic acid (C18:1c)	0.88 (22.5)[b]	1.03 (22.8)[a]	0.09 (13.3)[c]	0.88 (22.5)[b]	0.94 (21.4)[a]	0.09 (12.9)[c]

Linoleic acid (C18:2 *n*6c)	0.10 (2.6)[b]	0.19 (4.2)[a]	0.08 (12.6)[c]	0.10 (2.6)[b]	0.19 (4.3)[a]	0.08 (12.4)[c]
α-Linolenic acid (C18:3 *n*3)	-	0.32 (7.1)[a]	0.25 (37.0)[b]	-	0.31 (7.1)[a]	0.24 (35.4)[b]
Arachidic acid (C20:0)	0.02 (0.6)	0.02 (0.5)	0.01 (2.0)	0.02 (0.6)	0.03 (0.6)	0.01 (2.1)
Total n-3	-	0.32 (7.1)[a]	0.25 (37.0)[b]	-	0.31 (7.1)[a]	0.24 (35.4)[b]
Total n-6	0.10 (2.6)[b]	0.19 (4.2)[a]	0.08 (12.6)[c]	0.10 (2.6)[b]	0.19 (4.3)[a]	0.08 (12.4)[c]
Total-6/n-3	2.6[a]	0.6[b]	0.3[c]	2.6[a]	0.6[b]	0.4[c]
Total SFA	2.83 (72.6)[a]	2.88 (63.9)[a]	0.25 (37.0)[b]	2.83 (72.7)[a]	2.88 (65.5)[a]	0.27 (39.2)[b]

[1]W, whole milk; WE, whole mlik+flaxseed oil-FOS emulsion; and NFE, non-fat milk+flaxseed oil-FOS emulsion

[2]Data are reported as mean values±standard deviation of three replicates.

[a-c]Means with different superscripts in the same row are significantly different ($p<0.05$).

3.3.2 Sensory characteristics

3.3.2.1 Descriptive analysis

In descriptive analysis, 13 of the 14 descriptive sensory attributes were found to be significantly different when studying the characteristics of the set and stirred yogurts fortified with flaxseed oil-FOS emulsion (Table 3.4). Appearance terms (thick and translucent) were found to differ significantly ($p<0.05$) in the samples. The set yogurt was thicker than the stirred yogurt with the Set-W and Set-WE being the thickest. The yogurt containing flaxseed oil-FOS emulsion was fishier than the yogurt without flaxseed oil-FOS emulsion with the Set-NFE and Stir-NFE being fishier than the Set-WE and Stir-WE. When both of the yogurts produced from different milks (whole and non-fat milk) were compared, the whole milk yogurt was significantly lower ($p<0.05$) in fishy odor. This might be because of the presence of fat in the whole milk yogurts, which goes about as a flavor carrier or flavor discharge modulator because the liquid fat has the ability to absorb lipophilic volatiles (Roberts, Pollien, & Watzke, 2003). The types of the yogurts did not affect fishy odor of the yogurts.

All the taste terms (sour, sweet, cheesy, and fishy) were found to differ significantly ($p<0.05$) among the samples. An inverse relationship was observed between cheesy and fishy tastes. The whole milk yogurts tasted cheesier and less fishy than the non-fat milk yogurts. The emulsion added

yogurts were higher in ‘fishy taste’ than the other yogurts. Astringent mouthfeel was observed to be higher in the non-fat milk yogurts as compared to their whole milk counterparts of the stirred type ($p<0.05$). Folkenberg and Martens (2003) observed that an increase in fat content significantly reduced astringency and sour taste in plain stirred yogurts. Yogurt fortified with the flaxseed oil-FOS emulsion had a greater fishy aftertaste than the yogurt without the flaxseed oil-FOS emulsion in the set and stirred yogurts ($p<0.05$) and the Set-NFE and Stir-NFE had fishier aftertaste as compared to the Set-WE and Stir-WE ($p<0.05$).

The PCA biplot of the yogurts illustrates specific differences among the products (Fig. 3. 2). The biplot explains 80.9% of the total variation, with factor 1 on the x-axis explaining 52.2% of the data and factor 2 on the y-axis explaining 28.7%. Yogurts were discriminated along the factor 1 according to their fishy attributes and mouthfeel and their aroma and taste. Some attributes responsible for fishy attributes (aroma, taste, and aftertaste) and mouthfeel (smooth and astringent) were opposed to the aroma attributes (sour, sweet, and butyric) and taste attributes (sour, sweet, and cheesy). The factor 1 exhibited a difference of the yogurts made by whole and non-fat milk. Fishy attribute was the most intense in the Set-NFE yogurt. The yogurts appear to be separated into 2 well defined groups: moving up to down along the factor 2 of PCA, the set yogurts are separated from the stirred yogurts. The set yogurts had higher intensities in thickness,

translucent, and mouthfeel, whereas the stirred yogurts had higher intensities in sour attributes (aroma, taste, and aftertaste). The Stir-W and Stir-WE yogurts were sourer (aroma, taste, and aftertaste) than the other two yogurts (Set-W and Set-WE). Thickness and cheesy taste were the most intense in the Set-WE and Set-W yogurts, respectively.

Table 3.4. Descriptive analysis attributes of set and stirred yogurts fortified with flaxseed oil-fructooligosaccharide (FOS) emulsion.

Categories	Attributes[1]	Set yogurt			Stirred yogurt		
		W[2]	WE	NFE	W	WE	NFE
Appearance	Thick	11.1±0.4[a3]	11.1±0.1[a]	9.4±0.8[b]	4.8±1.1[c]	5.1±1.2[c]	4.6±1.0[c]
	Translucent	10.7±0.2[a]	11.0±0.4[a]	8.8±1.1b[c]	8.5±0.6[bc]	9.4±0.1[b]	8.0±0.5[c]
Aroma	Sour	7.2±0.4[ab]	6.1±0.7[b]	6.1±1.1[b]	8.0±0.7[a]	7.1±0.7[ab]	6.5±1.0[ab]
	Butyric	6.9±0.7	6.8±0.6	6.6±1.1	6.9±0.6	6.1±0.5	6.2±0.5
	Fishy	4.2±0.7[c]	7.3±0.9[b]	9.1±0.5[a]	4.1±0.4[c]	6.9±0.5[b]	8.6±0.3[a]
Taste	Sour	8.1±0.6[b]	6.8±0.4[d]	7.6±0.2[bc]	8.8±0.3[a]	7.3±0.5[cd]	8.0±0.3[b]
	Sweet	6.7±0.4[ab]	6.3±0.7[ab]	4.9±0.0[c]	7.2±0.7[a]	6.9±0.9[ab]	5.8±0.4[bc]
	Cheesy	8.6±0.5[a]	8.2±0.3[a]	6.7±0.4[b]	8.0±0.8[a]	8.2±0.2[a]	6.8±0.2[b]
	Fishy	3.9±0.5[c]	8.3±1.2[b]	11.0±0.2[a]	3.7±0.4[c]	7.5±0.4[b]	10.3±0.6[a]
Mouthfeel	Astringent	6.2±0.1[bc]	6.8±0.7[bc]	7.5±1.3[ab]	5.9±0.4[c]	6.0±0.7[c]	8.4±0.2[a]
	Viscous	5.5±0.6[ab]	5.8±0.2[ab]	7.6±0.7[a]	5.0±0.6[c]	4.8±0.5[c]	6.4±0.1[b]

	Mouthcoat	8.2±1.0[a]	7.9±0.3[ab]	7.3±0.7[ab]	7.0±0.4[ab]	6.8±0.3[b]	7.2±0.4[ab]
Aftertaste	Sour	7.9±0.8[ab]	6.7±0.5[c]	7.6±0.7[abc]	8.3±0.6[a]	7.1±0.0[bc]	8.1±0.6[ab]
	Fishy	3.9±0.6[d]	8.8±0.7[b]	11.0±0.3[a]	3.7±0.3[d]	7.7±0.3[c]	10.5±0.1[a]

[1]A 16-cm structured scale was used for the descriptive analysis.

[2]W, whole milk; WE, whole mlik+flaxseed oil-FOS emulsion; and NFE, non-fat milk+flaxseed oil-FOS emulsion

[3]Data are reported as mean values±standard deviation of three replicates.

[a-d]Means with different superscripts in the same row are significantly different ($p<0.05$).

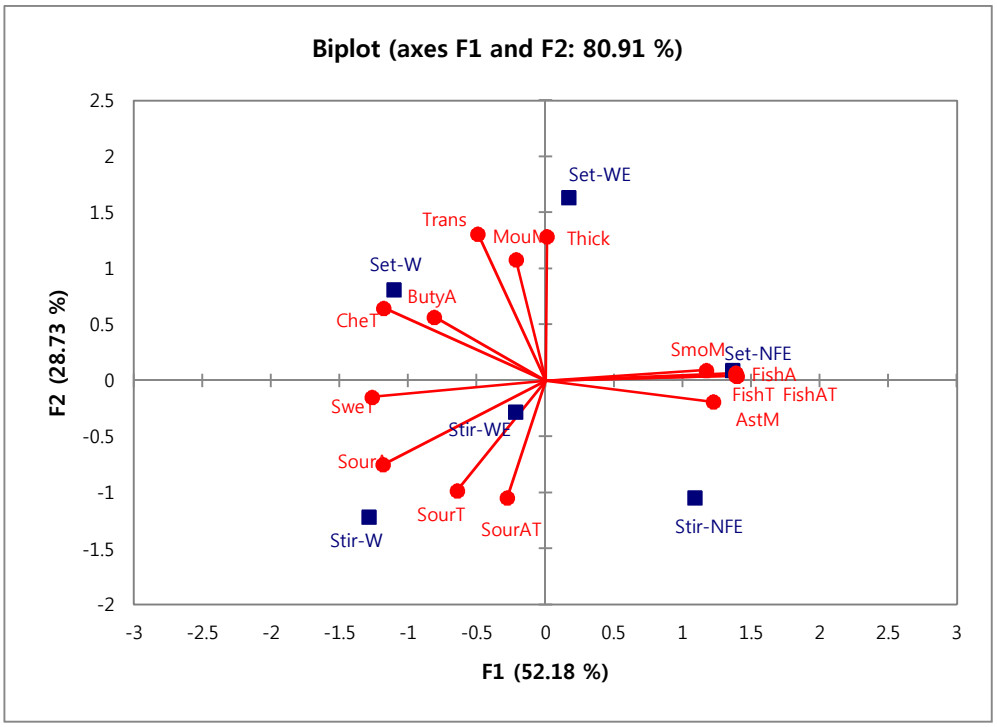

Fig. 3.2. Principal component analysis biplot on the descriptive quantitative analysis scores of set and stirred yogurts fortified with flaxseed oil-fructooligosaccharide (FOS) emulsion. Legends: yogurt samples (■: W, whole milk; WE, whole mlik+flaxseed oil-fructooligosaccharide (FOS) emulsion; and NFE, non-fat milk+flaxseed oil-fructooligosaccharide (FOS) emulsion) and descriptors (●: refer to Table 1 for abbreviations)

3.3.2.2 Preference test

The preference panelists observed significant differences in appearance, color, aroma, taste, mouthfeel, and overall acceptability of the set and stirred yogurts fortified with flaxseed oil-FOS emulsion (Table 3.5). The stirred yogurts were observed to appear better than the set yogurts ($p<0.05$). There were no differences ($p>0.05$) in color between the set and stirred yogurts made with whole milk added with flaxseed oil-FOS emulsion. Color parameters of a product are important for marketability and preference. Even though a functional food could provide several health benefits to consumers, they cannot be marketable without visual attraction to the consumers. Thus, the color of the supplemented products should ideally remain unaltered after production and throughout storage (Zare, Boye, Orsat, Champagne, & Simpson, 2011). Addition of the flaxseed oil-FOS emulsion did not affect aroma of the stirred whole milk yogurts. However, addition of the flaxseed oil-FOS emulsion had negative effect on aroma of the set whole milk yogurt. Taste was not significantly ($p>0.05$) different among the Set-W, Stir-W, and Stir-WE yogurts. Addition of the flaxseed oil-FOS emulsion did not have much effect on all the attributes of the stirred whole milk yogurts ($p>0.05$). When grouping the products by fat content, the whole milk yogurts were preferred over the non-fat milk yogurts. Salvador and Fiszman (2004) noted the same pattern in set type yogurts, where the consumers liked the whole milk samples more than the non-fat milk samples.

This demonstrates that the decreased fat content may have a negative effect on the acceptability of the yogurts. The overall acceptability for the yogurts by and large showed a similar tendency to their preference for aroma and taste, suggesting that overall acceptability may be basically influenced by preferences for aroma and taste.

External preference mapping (PREFMAP) was directed utilizing the vector model to correlate overall acceptability of the yogurts to the product profiling of the yogurts completed by the trained descriptive panel (Fig. 3.3). PREFMAP is a valuable tool that allows preference information from preference data to be mapped on a multidimensional space to develop a relationship to descriptive attributes (Hough & Sánchez, 1997). The assumption is that consumer preference for a product is determined to a certain extent by the product's sensory attributes (Faber, Mojet, & Poelman, 2003). The PREFMAP shows most of the preference panelists accepting the Stir-W and Stir-WE, probably because of their sweet taste and sour attribute, and perhaps a richer mouthfeel resulting from the higher milk fat content. Proportion of sweetness and sourness is a typical significant correlation factor for preference with yogurts (Barnes, Harper, Bodyfelt, & McDaniel, 1991). Similar to the results found in this study, yogurts which are characterized as being not excessively sweet with a consistency thicker than milk yet pourable are most desirable to consumers (Thompson, Lopetcharat, & Drake, 2007). The non-fat milk yogurts were not preferred by

the majority of the panelists probably because of their fishy attributes (aroma, taste, and aftertaste) and lower milk fat contents.

Table 3.5. Preference for the set and stirred yogurts fortified with flaxseed oil-fructooligosaccharide (FOS) emulsion.

	Set yogurt			Stirred yogurt		
	Set-W[1]	Set-WE	Set-NFE	Stir-W	Stir-WE	Stir-NFE
Appearance[2]	5.5±0.5^{b}[3]	6.1±1.3^{b}	2.2±0.1^{c}	6.9±0.3^{a}	7.1±0.6^{a}	5.8±1.2^{b}
Color	6.5±0.1^{ab}	6.6±0.5^{ab}	4.1±0.0^{c}	7.1±0.6^{a}	7.1±0.8^{a}	6.1±0.1^{b}
Aroma	6.8±0.0^{a}	5.1±0.4^{b}	2.9±0.2^{c}	7.1±0.5^{a}	6.6±0.7^{a}	5.5±0.6^{b}
Taste	5.7±0.5^{a}	2.8±0.6^{c}	2.3±0.8^{c}	5.9±0.7^{a}	6.0±0.0^{a}	4.5±0.2^{b}
Mouthfeel	6.3±0.1^{ab}	5.6±0.0^{ab}	3.3±0.6^{c}	6.2±0.1^{ab}	6.4±0.3^{a}	5.4±0.7^{b}
Overall acceptability	5.8±0.2^{a}	3.5±0.4^{c}	2.2±0.5^{d}	6.4±0.2^{a}	6.5±0.6^{a}	4.9±0.2^{b}

[1]W, whole milk; WE, whole mlik+flaxseed oil-FOS emulsion; and NFE, non-fat milk+flaxseed oil-FOS emulsion

[2]Samples were evaluated on 9-point hedonic scales (1 = dislike extremely; 9 = like extremely).

[3]Data are reported as mean values±standard deviation of three replicates.

[a-d]Means with different superscripts in the same row are significantly different ($p<0.05$).

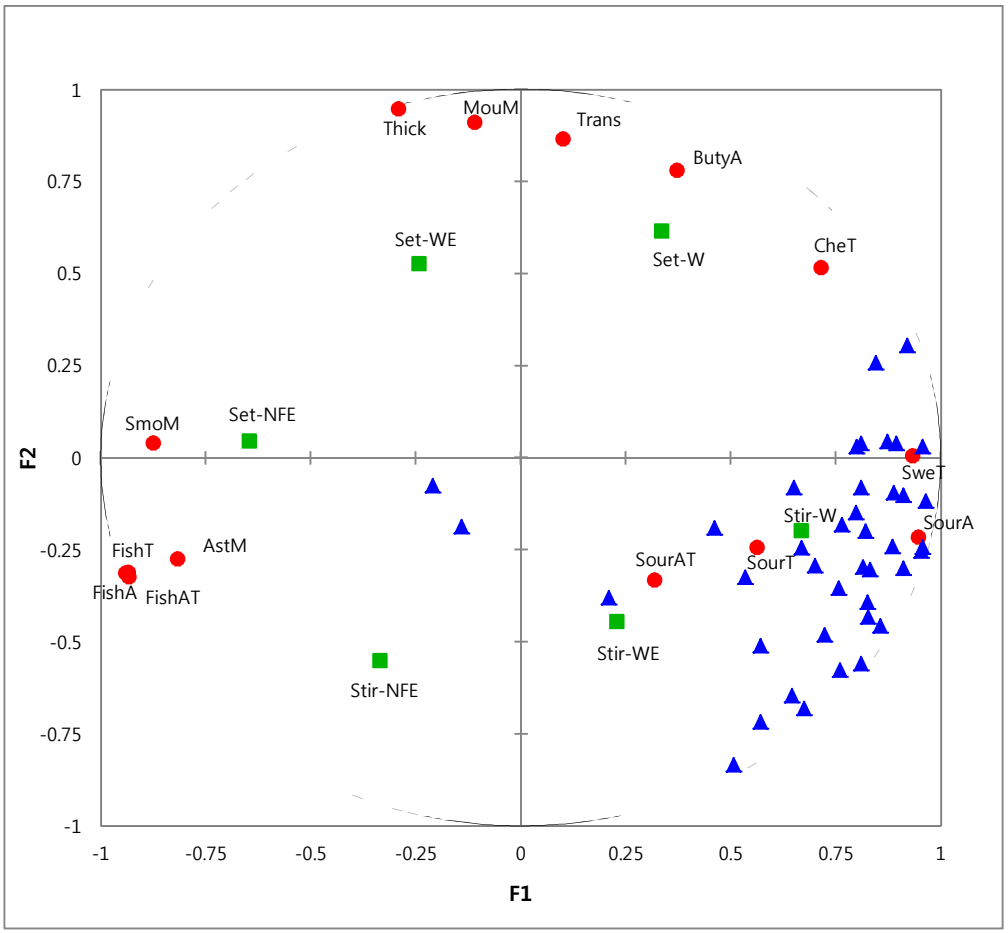

Fig. 3.3. An external preference map showing the interrelationships between descriptive analysis data and overall acceptability data. Legends: yogurt samples (■: W, whole milk; WE, whole mlik+flaxseed oil-fructooligosaccharide (FOS) emulsion; and NFE, non-fat milk+flaxseed oil-fructooligosaccharide (FOS) emulsion) and descriptors (●: refer to Table 1 for abbreviations); ▲, overall acceptability data of consumers.

Chapter 4

Fatty Acid Composition and Sensory Characteristics of Eggs Obtained from Hens Fed Flaxseed Oil, Dried Whitebait, and/or Fructooligosaccharide

(Study 3)

(Accepted in Asian Australasian Journal of Animal Science, Feb. 18, 2014)

4.1 Introduction

The lipid profile of eggs can be modified by the inclusion of specific oils such as fish oil and flaxseed oil in the diet of laying hens (Shapira et al., 2008; Souza et al., 2008). A proposal from the European Commission's Scientific Panel (European Food Safety Authority, 2005) established a minimal n-3 FA content of 300 mg/100 g egg weight to allow commercial eggs to be labeled as 'sources' of n-3 FA. The use of marine oils to generate eggs enriched with n-3 FA has been done successfully (Gonzalez-Esquerra & Leeson, 2001). However, the use of marine/fish meals and oils in laying hen's diets has raised concerns about 'fishy odors' and 'fishy off-flavors,' which are considered to be the major undesirable side-effects when including high n-3 FA levels in the diets fed to hens (Parpinello et al., 2006). Dietary additions of vitamin E and rosemary extracts as antioxidants have been tested with eggs enriched with n-3 FA to eliminate the undesirable flavors (Galobart et al., 2001; Scheideler et al., 1997).

Prebiotics are defined as non-digestible food/feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already residing in the intestines (Gibson & Roberfroid, 1995). Among prebiotics, FOS has been extensively studied for their ability to improve poultry health and performance. Chen et al. (2005) reported that dietary oligofructose and

inulin increased egg production and feed efficiency of layers without impairing egg quality. In addition, Chen and Chen (2004) observed that oligofructose or inulin promoted the bird's health and improves eggshell quality. The addition of oligosaccharides in animal feed is suggested to improve the sensory quality of the eggs by manipulating the composition of the intestinal flora, stimulating gut integrity, affecting nitrogen metabolism, and reducing offensive fecal odor (Flickinger et al., 2003). Fructans are considered to inhibit the production of protein-fermentative end-products (excreta odor components) (Gibson & Roberfroid, 1995). However, no information was available on the influence of supplementation with FOS on the sensory qualities of eggs enriched with n-3 FA.

Thus, the aim of this study was to examine the effects of flaxseed oil, dried whitebait and/or FOS supplementation in diets of hens on performance of hens, and FA composition, internal quality, and sensory characteristics of egg.

4.2 Materials and methods

4.2.1 Bird housing and experimental protocol

One hundred and forty 80-week-old brown laying hens (Hy-Line® Brown, Hy-Line International, Dallas Center, IA, USA) were used, and the

experiment was conducted for four weeks (August, 2012) in Happy Shinsun Farm (Sejong, Korea). Hens were assigned randomly to one of seven dietary treatments as described later and each group of 20 hens was kept in an open hen house (3 m x 5 m x 3 m height) with dirt floor and a roof. The hens had approximately 0.75 m^2/hen of floor space under natural environment with light from sunrise to sunset giving a photoperiod of about 14 h light and 10 h darkness. Feed was provided in a fixed amount (120 g/day/hen) and was fully consumed during the experimental period. Water was provided for ad libitum consumption. All the procedures for this study were approved by the Institutional Animal Care and Use Committee at Seoul National University (IACUC number: SNU-120914-1).

4.2.2 Diets

The basal diet was obtained from Nonghyup Feed Co. (Seoul, Korea) and was identical to the diet that the birds had been consuming prior to the experiments. The ingredients in the basal diet were not fully informed by the producer except that the main ingredients were corn, wheat meal and soybean meal. Some of the details of the diet can be found in Table 4.1. Flaxseed (Linum usitatissimum, Brown) oil was obtained from Haitnimnara Co. (Hwaseong, Korea). Soybean oil (Sajohaepyo Co., Seoul, Korea) and sun-dried whitebait (Salangichthys microdon, proximate composition is

shown in Table 4.1) (In-a Feed, Seocheon, Korea) were purchased from a local market. FOS containing 55% fructooligosaccharide, 17.5% fructose, 17.5% glucose and 10% sucrose (based on the manufacturer's analysis) on a dry basis was obtained from Samyang Genex Co. (Seoul, Korea). The diets were formulated to meet the nutrient requirements of laying hens, consuming 120 g of feed per day, which was based on suggestions from the National Research Council (NRC, 1994). The hens were fed a basal diet with 1.5% soybean oil (control, C), 1.5% flaxseed oil (T1), 1.5% soybean oil + 1.5% FOS (T2), 1.5% flaxseed oil + 1.5% FOS (T3), 1.5% soybean oil + 1.5% dried whitebait (T4), 1.5% flaxseed oil + 1.5% dried whitebait (T5) or 1.5% flaxseed oil + 1.5% dried whitebait + 1.5% FOS (T6). Flaxseed and soybean oil were used by emulsion with FOS. Flaxseed oil (soyben oil)-FOS emulsions were prepared using decaglycerol monolaurate as emulsifiers (oil:FOS:emulsifier=50:50:3).The emulsifiers were dissolved in FOS and heated to 60°C to make a continuous phase. Oil was gradually added into the continuous phase and homogenized using a homogenizer (HG-15A, Daihan Scientific Co., Wonju, Korea) equipped with an HT1018 dispersion tool (rotor Ø12.7, stator Ø18.0) for 5 min at 3,000 rpm at 60±1°C.

4.2.3 Diet and egg analysis

Feed samples (basal diet and dried whitebait) and egg samples were

analyzed for moisture (AOAC 920.36), crude fat (AOAC 920.39), crude protein (AOAC 984.13), crude fiber (AOAC 962.09) and crude ash (AOAC 942.05) according to AOAC methods (1995). The pH of the egg was measured after diluting the sample with 9 volumes of deionized distilled water. Total cholesterol of the egg samples were determined according to Fenton and Sim (1991) using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a capillary column (HP-1, 30 m x 0.22 mm x 0.33 μm, Hewlett-Packard, Waldbronn, Germany). The oven was programmed with an initial temperature of 260°C for 5 min, to 280°C at 4°C/min, held for 10 min. Helium was used as the carrier gas at a flow rate of 2 mL/min. A split injector with 50:1 (v/v) split ratio was used. The temperatures of the injector and detector (flame ionization detector) were 270 and 290°C, respectively. The quantity of the cholesterol was determined using standard cholesterol (Sigma-Aldrich, St. Louis, MO, USA) and squalene (Supelco, Bellafonte, PA, USA) was used as the internal standard for the cholesterol quantification.

The FA composition of the lipids of the egg yolks was determined using an in situ transesterification procedure (Park & Goins, 1994), which was a modification of the AOCS Official Methods (1980) for FA methyl ester (FAME) preparation from fats and oils. FA composition was determined using the Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a capillary column (DB-23, 30 m x 0.25 mm x 0.25 μm, J&W

Scientific, Folsom, CA, USA). The oven was programmed at an initial temperature of 50°C for 1 min, to 175°C at 25°C/min, to 230°C at 4°C/min, held for 5 min, and to 250°C at 25°C/min, held for 3 min. The temperatures of the injector and detector (flame ionization detector) were 250 and 280°C, respectively. Helium was used as the carrier gas at a flow rate of 2 mL/min with an injection split ratio of 30:1 (v/v). Peak identification and quantification were performed with reference to the retention times and peak areas as weight percent of a mixture of standard FAME (Supelco 37-component FAME mix, Supelco). The individual FA was identified by comparing the retention times of FA to those of external reference mixtures (GLC 3C, 4C, 7C, 8C, 12C and 20A; Nu Chek Prep, Inc., Elysian, MN, USA). The results were expressed as % of total FA. Prior to the analysis the feed samples and eggs were stored in the dark at 4°C.

4.2.4 Collection of eggs

Collecting eggs daily from the floor, the weight (g) of each egg and the total egg production (%) of each group was obtained. Daily egg mass (g/d) during the 4 wk was calculated as the average egg production (%) x average egg weight (g). Feed intake was recorded every day and the feed conversion ratio was calculated as feed intake/egg mass (g/g). All the data were expressed as the mean values for the entire experimental period.

During the final 3 days of the study, eggs were randomly collected for FA analysis, egg quality measurements and sensory evaluation. Sensory evaluation was completed within 7 days after the eggs had been laid. Prior to the sensory evaluation the eggs were stored in the dark at 4°C.

4.2.5 Egg quality

Albumen height was measured and the Haugh unit was calculated as 100 log (HA + 7.57 – 1.7 WE0.37), where HA is maximum albumen height and WE is egg weight (Haugh, 1937). Eggshell thickness was measured near the equator of the egg, using an electronic micrometer (QCT Device, TSS, York, England). Twenty eggs from each treatment were collected for the measurement of eggshell breaking strength, using an Instron Testing Machine (Model 5542, Instron Ltd., High Wycombe, England), equipped with a 500 Newton load cell (Hamilton, 1982). The egg was placed horizontally for measurements. The eggs were compressed at a constant crosshead speed of 10 mm/min and the breaking strength was determined at the moment of the eggshell fracture. A 35 mm diameter plate was used as a compression device.

4.2.6 Sensory evaluation

Sensory characteristics were evaluated by 15 graduate students (ages 20 to 30, 7 male and 8 female) in the laboratory who had taken a sensory evaluation course. Ten eggs per treatment produced during the final 3 days of the experimental period were randomly selected. They were placed in boiling water (10 L) for 15 min, and then cooled using running tap water. Shells were immediately removed and each egg was cut through the center into quarters, and then served. All the samples were evaluated immediately after preparation was completed.

Organoleptic characteristics of the eggs were recorded on a seven point hedonic scale (1 = dislike extremely; 7 = like extremely) with ascending ratings for the desired attributes of color, off-flavor, fishy flavor, buttery taste and overall acceptability. Off-flavor (the presence of something agreeable or disagreeable differing from the typical egg odor/taste) and fishy flavor (the presence of fishy odor/taste) were defined with the panelists and 'higher scores were assigned to the samples with the lower off-flavor and fishy flavor'. Egg samples were coded with a 3 digit randomized numbers. The samples were served to the panelists in individual booths on white plastic plates in a random order. A plate containing seven samples in a random order was presented to the panelists to evaluate. Cold water was also provided for rinsing their mouths before testing each sample. This procedure was done in duplicate with each panelist. They evaluated the eggs in two

different sessions. Thus, the trained panel was able to evaluate more complex factors but was still reflecting their personal opinion.

4.2.7 Statistical analysis

Analysis of variance (ANOVA) was done using the SPSS package (SPSS Version 12.0K for Windows, SPSS Inc., Chicago, IL, USA). Where significant differences ($p<0.05$) were detected, Duncan's multiple range test was used to compare treatment means.

4.3 Results and discussion

4.3.1 Diet and egg composition

Proximate composition of the basal diet and the dried whitebait are shown in Table 4.1. The crude fat contents of the basal diet and dried whitebait were 4.3% and 10.7%, respectively. Proximate composition, pH, and cholesterol of the eggs from the hens that were fed the diets of flaxseed oil, dried whitebait and/or FOS are shown in Table 4.2. No differences were observed in the proximate composition of the eggs from the hens fed the different diets ($p>0.05$). Egg cholesterol concentration was not significantly ($p>0.05$) affected by a diet containing flaxseed as previously reported for

other oilseeds (Milinsk et al., 2003). Frank (2005) reported that dietary inulin in layers reduced egg cholesterol. However, the cholesterol level in the eggs was not affected by the different diets in this study.

Table 4.1. Proximate composition of the basal diet and the dried whitebait.

(%, w/w)

	Basal diet	Dried whitebait
Moisture	11.9±0.8[1]	19.7±1.2
Crude fat	4.3±0.5	10.7±0.4
Crude protein	15.2±0.6	53.0±0.7
Crude fiber	2.0±0.1	0.3±0.1
Crude ash	7.9±0.4	13.8±0.3
Calcium[2]	3.3	-
Phosphorus[2]	1.0	-
Methionine + cysteine[2]	0.6	-
Metabolic energy (kcal/kg)[2]	2,740	-

[1] Data are reported as the mean values ± standard deviation of three replicates.

[2] Provided by the feed manufacturer.

Table 4.2. Proximate composition, pH, and total cholesterol of the eggs from hens fed diets with flaxseed oil, dried whitebait, and/or fructooligosaccharide (FOS).

	C[2]	T1	T2	T3	T4	T5	T6
Moisture (%)	77.0±1.3[1,3]	76.7±1.8	77.3±2.1	77.6±1.9	76.7±2.0	76.1±0.7	75.4±1.6
Crude fat (%)	6.3±1.0	6.7±0.5	6.3±0.8	7.3±1.1	7.6±0.5	7.6±0.4	7.7±0.7
Crude protein (%)	12.3±0.4	12.2±0.3	12.0±0.2	11.2±0.8	12.1±0.4	12.2±0.4	12.5±0.5
Ash (%)	0.8±0.0	0.8±0.1	0.8±0.0	0.8±0.1	0.8±0.1	0.9±0.0	0.9±0.1
pH	7.9±0.2	8.0±0.1	8.0±0.2	7.9±0.0	7.8±0.1	7.7±0.1	7.8±0.1
Total cholesterol (mg/100 g)	227±10.7	241±5.9	221±7.8	244±6.6	229±7.9	247±10.1	244±8.4

[1] Data are reported as mean values ± standard deviation of three replicates obtained during the final 3 days of the experiment (one sample per group = 3 pooled eggs).

[2] C: basal diet with 1.5% soybean oil; T1: basal diet with 1.5% flaxseed oil; T2: basal diet with 1.5% soybean oil + 1.5% FOS; T3: basal diet with 1.5% flaxseed oil + 1.5% FOS; T4: basal diet with 1.5% soybean oil + 1.5% dried whitebait; T5: basal diet with 1.5% flaxseed oil + 1.5% dried whitebait; and T6: basal diet with 1.5% flaxseed oil + 1.5% dried whitebait + 1.5% FOS.

[3] Not significant.

4.3.2 Laying performance and egg quality

No mortalities were observed during the experimental period. Laying performance and egg quality are presented in Table 4.3. The eggs from the hens fed the T6 diet had the heaviest weights ($P < 0.05$). Gonzalez-Esquerra and Leeson (2000) reported that hens fed a diet containing fish oil laid heavier eggs. In contrast, Scheideler and Froning (1996) reported that hens fed flaxseed diet laid lighter eggs. The eggs of the T3 and T6 diet were heavier than those of the C diet, suggesting that supplementation with FOS in the diet for the hens may increase egg weight. These results agree with a previous study (Chen et al., 2005) which reported that the addition of 1.0% oligofructose increased ($p<0.05$) the weekly total egg weight per hen.

Egg production was lower in the groups fed the T2 and T6 diets than in the other groups. T3 diet group had the highest egg production. Scheideler and Froning (1996) reported a positive effect on egg production after feeding flaxseed at the 5%, 10%, and 15% levels for 6 weeks. Aymond and Van Elswyk (1995) observed a decrease in egg production in hens fed a diet containing 15% flaxseed as early as 2 weeks after feeding. Chen et al. (2005) reported that adding oligofructose and inulin improved layer performance. Also, Park and Park (2012) reported that inulin oligosaccharides improved layer performance. On the other hand, Yildiz et al. (2006) reported that laying performance was not affected by

supplementation of Jerusalem artichoke as an inulin source.

The albumen height and Haugh unit of the eggs from the hens fed the T2 diet were significantly higher ($p<0.05$) than those of the eggs from the other treatment groups. Chen et al. (2005) reported that Haugh units of eggs were not ($p>0.05$) affected by oligofructose or inulin treatment. Eggshell thickness was significantly ($p<0.05$) different among the treatments. Eggshell thickness was highest in the T1 and lowest in the T3. However, eggshell breaking strength was not significantly ($p>0.05$) different among the treatments. Park and Park (2012) reported that the Haugh unit, eggshell thickness and eggshell breaking strength are higher in inulin oligosaccharide addition group (250 mg/kg diet) than those in the control group. In the present study, however, flaxseed oil and fish meal as well as FOS did not affect the eggshell thickness and eggshell breaking strength of the egg. One difference in this experiment was that the hens were in a late stage of the egg production cycle.

Table 4.3. Laying performance of the hens fed diets with flaxseed oil, dried whitebait, and/or fructooligosaccharide (FOS) and their egg quality.

	C[3]	T1	T2	T3	T4	T5	T6
Egg weight (g)[1]	62.5±1.3^{c}	63.8±1.5^{b}	63.8±0.9^{b}	64.8±1.7^{a}	65.4±1.7^{a}	63.9±1.9^{b}	65.6±1.9^{a}
Egg production (%)	88.2±5.6^{ab}	87.3±5.1^{ab}	80.5±9.9^{c}	90.6±7.3^{a}	85.6±6.3^{b}	89.0±5.7^{ab}	80.8±9.2^{c}
Egg mass (g/d)	55.1±3.8^{bc}	55.7±3.3^{bc}	51.4±6.5^{d}	58.8±5.4^{a}	56.0±4.4^{b}	56.9±4.4^{ab}	53.1±6.6^{cd}
Feed intake (g/d/h)	120±0.0	120±0.0	120±0.0	120±0.0	120±0.0	120±0.0	120±0.0
Feed conversion ratio (g feed/g egg)	2.2±0.2^{bc}	2.2±0.1^{c}	2.4±0.4^{a}	2.1±0.2^{c}	2.2±0.2^{c}	2.1±0.2^{c}	2.3±0.3^{ab}
Albumen height (mm)[2]	6.2±0.9^{c}	6.5±0.8^{bc}	7.1±0.8^{a}	6.2±0.7^{c}	7.0±0.7^{a}	6.6±1.0^{abc}	6.5±1.0^{bc}
Haugh units (HU)	77.9±6.3^{bc}	79.4±6.5^{bc}	83.7±5.3^{a}	76.9±5.1^{c}	82.9±4.2^{ab}	79.3±7.2^{bc}	78.8±7.1^{bc}
Eggshell thickness (mm)	0.34±0.0^{ab}	0.35±0.0^{a}	0.35±0.0^{a}	0.32±0.1^{b}	0.32±0.0^{b}	0.34±0.0^{ab}	0.34±0.0^{ab}
Eggshell breaking strength (g/cm^{2})	3587±747	3478±642	3386±705	3322±778	3205±802	3691±733	3264±854

[1] Data for laying performance are reported as mean values ± standard deviation obtained during the experiment.

[2] Data for egg quality are reported as mean values of twenty replicates obtained during the final 3 days of the experiment.

[3] C: basal diet with 1.5% soybean oil; T1: basal diet with 1.5% flaxseed oil; T2: basal diet with 1.5% soybean oil + 1.5% FOS; T3: basal diet with 1.5% flaxseed oil + 1.5% FOS; T4: basal diet with 1.5% soybean oil + 1.5% dried whitebait; T5: basal diet with 1.5% flaxseed oil + 1.5% dried whitebait; and T6: basal diet with 1.5% flaxseed oil + 1.5% dried whitebait + 1.5% FOS.

[a,b,c,d]Means in the same row with different superscripts are significantly different ($p<0.05$).

4.3.3 Fatty acid composition

FA compositions of the basal diet, soybean oil, flaxseed oil and dried whitebait used for this study are shown in Table 4.4. Flaxseed oil was rich in ALA (48.4 %) and dried whitebait was rich in Eicosapentaenoic (EPA) (20.9 %) and docosahexaenoic acids (DHA) (20.4 %), which were hardly present in the basal diet and soybean oil.

The FA compositions of the lipids in the eggs are shown in Table 4.5. ALA in the eggs from the hens fed the T1 and T5 diets were 6.8 and 5.9 times, higher than those of the control eggs, respectively. The flaxseed oil in the diet resulted in the large increase in ALA concentration in the eggs. ALA in the eggs from the hens fed the diet containing dried whitebait without flaxseed oil (T4) was as low as that of the C diet. Souza et al. (2008) reported similar results, where higher levels of this FA in the eggs from the hens fed flaxseed or its oil were observed compared to those with corn, soybean, canola and sunflower oils. ALA was significantly lower ($p<0.05$) in the eggs produced by the hens fed the diets of flaxseed oil and/or dried whitebait with FOS (T3 and T6) than in the eggs from the hens fed the diets without FOS (T1 and T5). However, the biological mechanism with respect to how FOS causes less ALA to be found in the eggs is not clear.

EPA and DHA were significantly higher ($p<0.05$) in the eggs produced by the hens fed the T1, T3, T4, T5 and T6 diets than in the eggs from the hens

fed the C diet. Flaxseed oil does not contain EPA or DHA, but it contains a large amount of ALA, which can be a precursor of EPA and DHA. EPA and DHA in the eggs might also come from the direct deposit of these FA in the diet (e.g., from the fish oil) or from synthesis from precursors such as ALA. EPA and DHA were the highest in the lipids of the eggs from the hens fed the T5 and T6 diets, respectively. The increase in these FA might be due to the addition of flaxseed oil and dried whitebait in the diet ($p<0.05$), while the FOS in the diet had no effect on the levels of these FA. These results confirm once again the theory of slight de novo synthesis of these long chain PUFA from their precursors (Yildiz et al., 2006).

The addition of flaxseed oil and dried whitebait to the diet of the hens gave a n-6/n-3 FA ratio (2.3 to 8.2) in the lipids of the eggs, which was significantly lower ($p<0.05$) than the control diet (13.5). However, the n-6/n-3 FA ratio did not seem to be affected by the addition of FOS. It can be noted that an increase in the n-3 FA in the diet, especially by combining these two feed sources, might result in a greater reduction in the n-6/n-3 FA ratio. Total n-3 FA content in the eggs from hens fed flaxseed oil, dried whitebait and/or FOS diets ranged from 93 to 487 mg/100 g egg weight in this study. Among them, the eggs from the hens fed the T1, T3, T5 and T6 diets contained at least 3 g n-3 FA/ kg egg weight and thereby meet the criteria suggested by the European Commission's Scientific Panel to be labeled as a source of n-3 FA (European Commission, 2005). In conclusions, the diets based on

dried whitebait promoted higher levels of DHA in the eggs of laying hens, while the addition of flaxseed oil increased the total n-3 FA in the eggs, mainly as ALA.

Table 4.4. Fatty acid composition of basal diet, soybean oil, flaxseed oil, and dried whitebait.

(% of total FA, w/w)

Fatty acids	Basal diet	Soybean oil	Flaxseed oil	Dried whitebait
Myristic acid (C14:0)	0.6±0.0[1]	0.1±0.0	-	1.7±0.0
Myristoleic acid (C14:1)	-	-	-	-
Pentadecanoic acid (C15:0)	0.1±0.0	-	-	0.4±0.0
Palmitic acid (C16:0)	16.6±0.1	10.7±0.0	4.7±0.0	22.0±0.1
Palmitoleic acid (C16:1)	0.9±0.0	0.1±0.0	0.1±0.0	4.6±0.0
Heptadecanoic acid (C17:0)	0.2±0.0	-	-	5.7±0.0
cis-10-Heptadecenoic acid (C17:1)	-	-	-	-
Stearic acid (C18:0)	4.9±0.0	4.6±0.0	5.1±0.0	5.7±0.0
Oleic acid (C18:1c)	31.5±0.1	25.2±0.0	25.0±0.1	7.9±0.0
Linoleaidic acid (C18:2 *n*6t)	-	1.2±0.0	0.6±0.0	-
Linoleic acid (C18:2 *n*6c)	42.5±0.1	51.7±0.1	15.9±0.0	1.3±0.0
α-Linolenic acid (C18:3 *n*3)	2.0±0.0	5.8±0.0	48.4±0.1	0.7±0.2

Arachidic acid (C20:0)	-	0.4±0.0	0.2±0.0	2.1±0.0
Eicosadienoic acid (C20:2)	0.4±0.0	0.2±0.0	0.2±0.0	-
Heneicosanoic acid (C21:0)	-	-	-	0.3±0.0
Arachidonic acid (C20:4 *n*6)	-	-	-	0.8±0.0
Eicosapentaenoic acid (C20:5 *n*3)	0.3±0.0	-	-	20.9±0.2
Behenic acid (C22:0)	-	0.5±0.0	-	-
Erucic acid (C22:1)	0.3±0.0	-	-	2.8±0.2
Tricosanoic acid (C23:0)	-	-	-	0.8±0.0
Lignoceric acid (C24:0)	-	-	-	1.1±0.0
Docosahexaenoic acid (C22:6 *n*3)	-	-	-	20.4±0.2
Nervonic acid (C24:1)	-	-	-	1.1±0.0

[1] Data are reported as mean values ± standard deviation of three replicates.

Table 4.5. Fatty acid content in the eggs from hens fed diets with flaxseed oil, dried whitebait, and/or fructooligosaccharide (FOS).

(g/100 g egg (% of total FA), w/w, wet basis)

Fatty acids	C[2]	T1	T2	T3	T4	T5	T6
Lauric acid (C12:0)	0.00 (0.0)[1]	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.01 (0.1)	0.00 (0.0)
Myristic acid (C14:0)	0.02 (0.4)^{a}	0.02 (0.3)^{ab}	0.02 (0.4)^{a}	0.03 (0.3)^{ab}	0.02 (0.3)^{ab}	0.02 (0.2)^{b}	0.03 (0.4)^{a}
Myristoleic acid (C14:1)	0.01 (0.1)^{a}	0.00 (0.1)^{ab}	0.01 (0.1)^{ab}	0.01 (0.1)^{ab}	0.01 (0.1)^{b}	0.01 (0.1)^{ab}	0.01 (0.1)^{ab}
Pentadecanoic acid (C15:0)	0.00 (0.1)	0.01 (0.1)	0.00 (0.1)	0.01 (0.1)	0.01 (0.1)	0.00 (0.1)	0.00 (0.1)
Palmitic acid (C16:0)	1.53 (24.5)^{a}	0.49 (22.3)^{c}	1.53 (24.3)^{a}	1.66 (22.7)^{c}	1.81 (23.8)^{ab}	1.75 (23.0)^{bc}	1.85 (24.0)^{ab}
Palmitoleic acid (C16:1)	0.18 (2.9)^{ab}	0.20 (3.0)^{ab}	0.18 (2.9)^{ab}	0.22 (3.0)^{ab}	0.20 (2.6)^{b}	0.25 (3.3)^{a}	0.26 (3.4)^{a}
Heptadecanoic acid (C17:0)	0.01 (0.2)^{ab}	0.01 (0.2)^{ab}	0.01 (0.2)^{ab}	0.01 (0.2)^{ab}	0.02 (0.2)^{a}	0.01 (0.2)^{b}	0.01 (0.2)^{ab}
cis-10-Heptadecenoic acid (C17:1)	0.01 (0.2)^{d}	0.01 (0.2)^{ab}	0.01 (0.2)^{bcd}	0.02 (0.2)^{a}	0.01 (0.2)^{cd}	0.01 (0.2)^{abc}	0.01 (0.2)^{bcd}
Stearic acid (C18:0)	0.51 (8.2)	0.54 (8.1)	0.51 (8.0)	0.57 (7.8)	0.62 (8.2)	0.59 (7.8)	0.60 (7.8)
Elaidic acid (C18:1t)	0.01 (0.2)	0.02 (0.2)	0.02 (0.4)	0.03 (0.3)	0.03 (0.3)	0.03 (0.3)	0.02 (0.3)
Oleic acid (C18:1c)	2.57 (41.1)^{b}	2.83 (42.6)^{ab}	2.60 (41.2)^{b}	3.16 (43.2)^{a}	3.13 (41.3)^{b}	3.26 (42.8)^{ab}	3.25 (42.1)^{ab}

Linoleaidic acid (C18:2 *n*6t)	0.01 (0.1)^{b}	0.01 (0.1)^{b}	0.02 (0.3)^{a}	0.02 (0.3)^{a}	0.02 (0.2)^{ab}	0.02 (0.2)^{ab}	0.02 (0.2)^{ab}
Linoleic acid (C18:2 *n*6c)	1.12 (17.9)^{a}	0.93 (13.9)^{b}	1.12 (17.7)^{a}	1.03 (14.2)^{b}	1.31 (17.3)^{a}	1.01 (13.2)^{b}	1.06 (13.7)^{b}
γ-Linolenic acid (C18:3 *n*6)	0.00 (0.1)^{b}	0.00 (0.1)^{b}	0.01 (0.1)^{a}	0.01 (0.1)^{ab}	0.01 (0.1)^{ab}	0.00 (0.1)^{b}	0.00 (0.1)^{b}
α-Linolenic acid (C18:3 *n*3)	0.04 (0.6)^{d}	0.29 (4.3)^{a}	0.04 (0.6)^{d}	0.21 (2.9)^{c}	0.04 (0.6)^{d}	0.29 (3.8)^{b}	0.20 (2.5)^{c}
11-Eicosenoic acid (C20:1)	0.01 (0.2)^{a}	0.01 (0.2)^{c}	0.01 (0.2)^{a}	0.01 (0.2)^{bc}	0.02 (0.2)^{a}	0.02 (0.2)^{ab}	0.02 (0.2)^{ab}
Eicosadienoic acid (C20:2)	0.01 (0.2)^{a}	0.01 (0.1)^{bc}	0.01 (0.2)^{a}	0.01 (0.2)^{bc}	0.02 (0.2)^{a}	0.01 (0.1)^{c}	0.01 (0.2)^{b}
cis-8,11,14-Eicosatrienoic acid (C20:3 *n*6)	0.01 (0.2)^{b}	0.01 (0.2)^{ab}	0.01 (0.2)^{ab}	0.01 (0.2)^{a}	0.01 (0.2)^{ab}	0.01 (0.2)^{ab}	0.01 (0.2)^{a}
Arachidonic acid (C20:4 *n*6)	0.12 (1.9)^{a}	0.08 (1.2)^{de}	0.12 (1.9)^{a}	0.10 (1.4)^{c}	0.13 (1.7)^{b}	0.08 (1.1)^{e}	0.09 (1.2)^{d}
cis-11,14,17-Eicosatrienoic acid (C20:3 *n*3)	0.00 (0.0)^{b}	0.01 (0.1)^{a}	0.00 (0.1)^{ab}	0.00 (0.1)^{ab}	0.00 (0.0)^{b}	0.01 (0.1)^{a}	0.00 (0.1)^{ab}
Eicosapentaenoic acid (C20:5 *n*3)	0.00 (0.0)^{c}	0.00 (0.1)^{b}	0.00 (0.0)^{c}	0.00 (0.1)^{b}	0.00 (0.1)^{b}	0.01 (0.1)^{a}	0.01 (0.1)^{a}
Docosadienoic acid (C22:2)	0.00 (0.0)^{c}	0.02 (0.2)^{b}	0.00 (0.0)^{c}	0.02 (0.2)^{b}	0.02 (0.3)^{b}	0.02 (0.2)^{b}	0.02 (0.3)^{a}
Lignoceric acid (C24:0)	0.01 (0.1)^{c}	0.02 (0.3)^{bc}	0.01 (0.1)^{c}	0.02 (0.3)^{b}	0.02 (0.2)^{c}	0.03 (0.3)^{bc}	0.03 (0.4)^{a}
Docosahexaenoic acid (C22:6 *n*3)	0.05 (0.9)^{d}	0.14 (2.1)^{b}	0.05 (0.8)^{d}	0.14 (2.0)^{bc}	0.13 (1.8)^{c}	0.18 (2.4)^{a}	0.19 (2.4)^{a}
Total saturated fatty acids	2.09 (33.4)^{a}	2.09 (31.4)^{b}	2.09 (33.1)^{a}	2.30 (31.4)^{b}	2.49 (32.9)^{a}	2.41 (31.7)^{b}	2.53 (32.8)^{a}

Total *n*-3 fatty acids	0.09 (1.5)[d]	0.44 (6.6)[a]	0.09 (1.5)[d]	0.37 (5.0)[b]	0.18 (2.4)[c]	0.49 (6.4)[a]	0.40 (5.1)[b]
Total *n*-6 fatty acids	1.26 (20.1)[a]	1.03 (15.4)[b]	1.28 (20.3)[a]	1.18 (16.1)[b]	1.48 (19.5)[a]	1.12 (14.7)[b]	1.18 (15.3)[b]
n-6/*n*-3 fatty acids ratio	13.5[a]	2.4[c]	13.5[a]	3.2[c]	8.2[b]	2.3[c]	3.0[c]

[1] Data are reported as mean values of three replicates obtained during the final 3 days of the experiment (one sample per group = 3 pooled eggs for each group).

[2] C: basal diet with 1.5% soybean oil; T1: basal diet with 1.5% flaxseed oil; T2: basal diet with 1.5% soybean oil + 1.5% FOS; T3: basal diet with 1.5% flaxseed oil + 1.5% FOS; T4: basal diet with 1.5% soybean oil + 1.5% dried whitebait; T5: basal diet with 1.5% flaxseed oil + 1.5% dried whitebait; and T6: basal diet with 1.5% flaxseed oil + 1.5% dried whitebait + 1.5% FOS.

[a-e]Means in the same row with different superscripts are significantly different ($p<0.05$).

4.3.4 Sensory characteristics

The panelists observed significant differences in off-flavor, fishy flavor, buttery taste and overall acceptability of the boiled eggs (Table 4.6). Hedonic scores for all attributes, except color, of the eggs from the hens fed the T5 diet (which contained flaxseed oil and dried whitebait) were lower ($p<0.05$) than those of the eggs from the hens fed the C diet. There were no differences in all attributes between the eggs from the hens fed the C diet and the diet with the addition of flaxseed oil (T1). The addition of dried whitebait (T4 and 5) resulted in decreased overall acceptability ($p<0.05$) compared to the control (C). However, there were no differences in off-flavor, fishy flavor and buttery taste between the T4 and C diets ($p>0.05$). There were no differences ($p>0.05$) in all attributes between the eggs from the hens fed the diets without FOS (C and T1) and the diets with FOS (T2 and T3). Hedonic scores for off-flavor, fishy flavor, buttery taste and overall acceptability were significantly higher ($p<0.05$) in the eggs produced by the hens fed the C diets than that those of the eggs of the hens fed the T5 diet. However, there was no difference ($p>0.05$) between the eggs from the hens fed the C and T6 (containing flaxseed oil, dried whitebait and FOS) diets.

The organoleptic quality of the n-3 eggs tends to be similar to regular table eggs although in some cases panelists are able to detect off-flavors (Milinsk et al., 2003). Koehler and Bearse (1975) first reported lower flavor

ratings for eggs produced from hens fed 5% fish meal, as well as a very slight off-flavor in eggs from hens fed a 6% menhaden oil diet. This remains a problem associated with the commercial production of this type of product. It has been suggested that the use of combinations of anti-oxidants in the hen's diet could help to suppress these off-flavors (Farrell, 1998). However, the sensory evaluation of eggs from hens fed FOS has not been reported before.

Traditionally, feed and feed additives have been thought to be the cause of egg flavor problems (Maga, 1982). The ’fishy egg‘ aroma compound was identified by Hobson-Frohock et al. (1973) to be trimethylamine (TMA). Feed can affect egg taint either by increased dietary TMA levels, by providing precursors for TMA formation by the intestinal microflora or by provision of inhibitors of the endogenous TMA oxidase (Zentek, 2003). The eggs produced by the hens fed fish meal may carry TMA because fish meal contains TMA ranging from 0.04 to 0.07 g/kg and TMA oxide ranging from 4.3 to 4.9 g/kg (Fenwick et al., 1981; Pearson et al., 1983).

Stewart et al. (1993) summarized the potentially beneficial effects of prebiotics such as leading to the antagonism of pathogens, competition with pathogens, stimulation of enzyme reactions, decreases in ammonia and phenol production, and increased colonization resistance. The two main types of fermentation that are carried out in the gut are saccharolytic and

proteolytic. Saccharolytic fermentation is more favorable than a proteolytic fermentation due to the acid nature of the resulting products (Fuller & Perdigón, 2003). The main end-products of saccharolytic fermentation are the short chain FA: acetate, propionate and butyrate. The end-products of proteolytic fermentation, on the other hand, include nitrogenous metabolites such as phenolic compounds, amines, and ammonia (Fuller and Perdigón, 2003). An aim of using prebiotics is to increase the amount of carbohydrates that reach to colon. This would increase the amount of saccharolytic fermentation throughout the gut and reduce proteolysis (Fuller & Perdigón, 2003). Thus, when there is saccharolytic fermentation, those bacteria do not use as much protein for energy. This may result in less amines, ammonia, and branched chain FA (Hahati & Rezaei, 2010). The current results suggest that it may be possible to include flaxseed oil and dried whitebait of high quality-when supplemented with FOS-without encountering major negative differences in consumer preferences compared to normal commercial eggs.

Table 4.6. Sensory evaluation of the eggs from hens fed diets with flaxseed oil, dried whitebait, and/or fructooligosaccharide (FOS).

	C[2]	T1	T2	T3	T4	T5	T6
Color	5.0±1.5[1]	4.6±1.4	4.5±1.4	4.6±1.1	5.0±1.6	4.7±1.3	5.3±1.3
Off-flavor	4.4±1.4[a]	4.4±1.2[a]	4.5±1.3[a]	4.2±1.1[ab]	3.9±1.4[ab]	3.6±1.3[b]	4.3±1.3[a]
Fishy flavor	4.6±1.4 [a]	4.5±1.5[ab]	4.7±1.3[a]	4.4±1.2[ab]	4.2±1.3[ab]	3.9±1.3[b]	4.2±1.5[ab]
Buttery taste	4.5±1.2[a]	4.4±1.3[ab]	4.4±1.2[ab]	4.4±1.3[ab]	4.1±1.0[ab]	3.8±1.3[b]	4.4±1.5[ab]
Overall acceptability	4.7±1.4[a]	4.2±1.6[abc]	4.4±1.1[ab]	4.4±1.2[ab]	3.9±1.0[bc]	3.7±1.3[c]	4.2±1.4[abc]

Samples were evaluated on 7-point hedonic scales (1 = dislike extremely; 7 = like extremely).

[1] Data are reported as mean values ± standard deviation of fifteen panelists evaluating eggs obtained during the final 3 days of the experiment. Each panelist tasted each egg twice. The four samples from each egg were given to the same four panelists.

[2] C: basal diet with 1.5% soybean oil; T1: basal diet with 1.5% flaxseed oil; T2: basal diet with 1.5% soybean oil + 1.5% FOS; T3: basal diet with 1.5% flaxseed oil + 1.5% FOS; T4: basal diet with 1.5% soybean oil + 1.5% dried whitebait;

T5: basal diet with 1.5% flaxseed oil + 1.5% dried whitebait; and T6: basal diet with 1.5% flaxseed oil + 1.5% dried whitebait + 1.5% FOS.

[a,b,c]Means in the same row with different superscripts are significantly different ($p<0.05$).

Chapter 5

Summary and Conclusions

A significant amount of effort has been placed on the promotion of adequate consumption of n-3 FA, due to their documented health benefits. Recent recommendations indicate a dietary ratio of n-6 to n-3 FA to be as low as two to one. It seems difficult to comply with such low dietary recommendations through the consumption of conventional food products. Therefore, a number of strategies have been employed to generate food products enriched with n-3 FA. Despite significant achievements in this area, there still exist some limitations with quality, availability, and the consumer acceptability of these enriched food products. Flaxseed oil is emerging as one of the key sources of phytochemicals in functional food products. α-Lnolenic acid (ALA) is attractive for the development of functional foods for specific health advantages such as protection against cardiovascular disease and anti-inflammation. The high content of ALA in flaxseed oil is, however, highly susceptible to oxidation, leading to rapid deterioration of quality. Study regarding incorporation of flaxseed oil as a source of ALA in functional food formulations has been little conducted. Therefore, this study was conducted to develop a physically and oxidatively stable emulsion using flaxseed oil, and its application in food products enriched with n-3 FA.

Study 1: In the first study, effects of type and concentration of emulsifiers, and the ratio of dispersed phase to continuous phase of the flaxseed oil-FOS emulsions on their rheological and physical properties (viscosity, particle size distribution, and emulsion stability index) were evaluated.

1) The presence of decaglycerol monolaurate (above 2%) in the emulsions increased the stability against oil separation. The flaxseed oil-FOS emulsions were highly viscous and showed strong shear-thinning.

2) It was possible to prepare emulsions with 50% flaxseed oil, which was physically and oxidatively stable for 72 days.

3) The flaxseed oil-FOS emulsion was less readily oxidized than flaxseed oil itself, whereas it was more oxidized than the flaxseed oil added with TBHQ.

Study 2: The second study was designed to develop yogurt fortified with flaxseed oil-FOS emulsion (as a source of n-3 FA) and to evaluate the effects of adding flaxseed oil-FOS emulsion on the physicochemical and sensory properties of the final products.

1) This study showed that it might be possible to fortify yogurts with n-3 FA by adding flaxseed oil-FOS emulsion at a marginal level.

2) The addition of flaxseed oil-FOS emulsion to yogurt did not greatly modify physicochemical properties such as pH, acidity, solid contents, and color values.

3) The yogurts containing flaxseed oil-FOS emulsion were more intense in fishy attributes than those without the emulsion.

4) Consumers liked the whole milk yogurts more than the non-fat milk yogurts, with the stirred whole milk yogurt containing the flaxseed oil-FOS emulsion being acceptable.

Study 3: In the final study, the production of eggs enriched with n-3 FA by addition of flaxseed oil and dried whitebait to laying hen diets has been designed to achieve the recommended total n-3 FA content of 300 mg/100 g egg weight.

1) The results of this study suggest that supplementation with FOS in the diet for the hens might increase egg weight without impairing egg quality such as eggshell breaking strength.

2) The results of this study also suggest that a diet with flaxseed oil and dried whitebait supplemented with FOS would be beneficial in terms of obtaining high levels of the various n-3 FA in eggs without significantly undesirable organoleptic characteristics.

In this study, to produce n-3 FA enriched food using flaxseed oil, physically and oxidatively stable flaxseed oil-FOS emulsion could be developed. The addition of flaxseed oil-FOS emulsion to yogurt before or after fermentation increased fishy attributes, and preference was better when the emulsion was added after the fermentation. Further research involving various dose and longer study period is needed to understand how oligosaccharide affected sensory properties of eggs enriched with n-3 FA.

Flaxseed oil-FOS emulsion could be applied to other food systems where enrichment with n-3 FA is needed.

References

Akalin, A. S., & Erişir, D. (2008). Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. Journal of Food Science, 73, M184-M188.

Amatayakul, T., Halmos, A. L., Sherkat, F., & Shah, N. P. (2006). Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. International Dairy Journal, 16, 40-51.

Anderson, G. J., & Connor, W. E. 1989. On the demonstration of ω-3 essential-fatty-acid deficiency. The American Journal of Clinical Nutrition, 49: 585-587.

Angers, P., Morales, M. R., & Simon, J. E. (1996). Fatty acid variation in seed oil among Ocimum species. Journal of the American Oil Chemists' Society, 73, 393-395.

Anton, M., Chapleau, N., Beaumal, V., Delepine, S., & de Lamballerie-Anton, M. (2001). Effect of high-pressure treatment on rheology of oil-in-water emulsions prepared with hen egg yolk. Innovative Food Science & Emerging Technologies, 2, 9-21.

AOAC. (1995). Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Arlington, VA. USA.

AOCS. (1980). Method Ce-2-66. Official and Tentative Methods of the

American Oil Chemists' Society, Champaign, IL, USA.

AOCS. (1990). In: Offial methods and recommended practices of the American Oil Chemists' Society (4th Eds). American Oil Chemists' Society, Champaign, IL, USA.

Augustin, M. A., & Sanguansri, L. (2008). Encapsulation of bioactives. In Food Materials Science. (pp. 577-601). Springer, New York.

Aymond, W. M., & Van Elswyk, M. E. (1995). Yolk thiobarbituric acid reactive substances and n-3 fatty acids in response to whole and ground flaxseed. Poultry Science, 74, 1388-1394.

Azorín-Ortuño, M., Urbán, C., Cerón, J. J., Tecles, F., Allende, A., Tomás-Barberán, F. A., & Espín, J. C. (2009). Effect of low inulin doses with different polymerisation degree on lipid metabolism, mineral absorption, and intestinal microbiota in rats with fat-supplemented diet. Food Chemistry, 113, 1058-1065.

Bancroft, W. D. (1913). The theory of emulsification, V. The Journal of Physical Chemistry, 17, 501-519.

Barnes, D. L., Harper, S. J., Bodyfelt, F. W., & McDaniel, M. R. (1991). Prediction of consumer acceptability of yogurt by sensory and analytical measures of sweetness and sourness. Journal of Dairy Science, 74, 3746-3754.

Bell, L.N. (2001). Stability testing of nutraceuticals and functional foods. In: R.E.C. Wildman (Eds), Handbook of Nutraceuticals and Functional Foods (pp. 501-506). CRC Press, New York.

Boersma, W. H., Laven, J., & Stein, H. N. (1990). Shear thickening (dilatancy) in concentrated dispersions. American Institute of Chemical Engineers Journal, 36, 321-332.

Bopp, M., Lovelady, C., Hunter, C., & Kinsella, T. (2005). Maternal diet and exercise: effects on long-chain polyunsaturated fatty acid concentrations in breast milk. Journal of the American Dietetic Association, 105, 1098-1103.

Bourre, J. M. (2005). Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: what is actually useful?. The Journal of Nutrition, Health & Aging, 10, 232-242.

Brazel, C.S. (1999). Microencapsulation: offering solutions for the food industry. Cereal Foods World 44, 388-393.

Burdge, G. C. (2006). Metabolism of α-linolenic acid in humans. Prostaglandins, Leukotrienes and Essential Fatty Acids, 75, 161-168.

Burdge, G. C., & Calder, P. C. (2005). Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults.

Reproduction Nutrition Development, 45, 581-598.

Burdge, G. C., & Wootton, S. A. (2002). Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. British Journal of Nutrition, 88, 411-420.

Burdge, G. C., Jones, A. E., & Wootton, S. A. (2002). Eicosapentaenoic and docosapentaenoic acids are the principal products of α-linolenic acid metabolism in young men. British Journal of Nutrition, 88, 355-363.

Cao, J., Schwichtenberg, K. A., Hanson, N. Q., & Tsai, M. Y. (2006). Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clinical Chemistry, 52, 2265-2272.

Caston, L. J., Squires, E. J., & Leeson, S. (1994). Hen performance, egg quality, and the sensory evaluation of eggs from SCWL hens fed dietary flax. Canadian Journal of Animal Science, 74, 347-353.

Champagne, C. P., & Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. Current Opinion in Biotechnology, 18, 184-190.

Chen, B., McClements, D. J., & Decker, E. A. (2010). Role of continuous phase anionic polysaccharides on the oxidative stability of menhaden oil-in-water emulsions. Journal of Agricultural and Food

Chemistry, 58, 3779-3784.

Chen, Y. C., & Chen, T. C. (2004). Mineral utilization in layers as influenced by dietary oligofructose and inulin. International Journal of Poultry Science, 3, 442-445.

Chen, Y. C., Nakthong, C., & Chen, T. C. (2005). Improvement of laying hen performance by dietary prebiotic chicory oligofructose and inulin. International Journal of Poultry Science, 4, 103-108.

Cho, Y., Shim, H., & Park, J. (2003). Encapsulation of fish oil by an enzymatic gelation process using transglutaminase cross-linked proteins. Journal of Food Science, 68, 2717-2723.

Choo, W. S., Birch, J., & Dufour, J. P. (2007). Physicochemical and quality characteristics of cold-pressed flaxseed oils. Journal of Food Composition and Analysis, 20, 202-211.

Crittenden, R. G., & Playne, M. J. (1996). Production, properties and applications of food-grade oligosaccharides. Trends in Food Science & Technology, 7, 353-361.

Das, U. N. (2006). Essential fatty acids-a review. Current Pharmaceutical Biotechnology, 7, 467-482.

Daun, J.K., Barthet, V. J., Chornick, T. L., & Duguid, S. (2003). Structure,

Composition, and Variety Development of Flaxseed. Flaxseed in Human Nutrition (2nd Eds), AOCS Press, USA.

Davidson, M. H., Burns, J. H., Subbaiah, P. V., Conn, M. E., & Drennan, K. B. (1991). Marine oil capsule therapy for the treatment of hyperlipidemia. Archives of Internal Medicine, 151, 1732-1740.

De Man, & John, M. 1999. Principles of food chemistry. 3rd ed. (pp. 54-65). Gaithersburg, MD: Aspen Publishers, Inc..

Delzenne, N. M., & Roberfroid, M. R. (1994). Physiological effects of non-digestible oligosaccharides. LWT-Food Science and Technology, 27, 1-6.

Dickinson, E., & Golding, M. (1997). Rheology of sodium caseinate stabilized oil-in-water emulsions. Journal of Colloid and Interface Science, 191, 166-176.

Djordjevic, D., McClements, D. J., & Decker, E. A. (2004). Oxidative stability of whey protein-stabilized oil-in-water emulsions at pH 3: Potential ω-3 fatty acid delivery systems (Part B). Journal of Food Science, 69, C356-C362.

Emken, E. (1994). Metabolism of dietary stearic acid relative to other fatty acids in human subjects. The American Journal of Clinical Nutrition, 60, 1023S-1028S.

Emken, E. A., Adlof, R. O., & Gulley, R. M. (1994). Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 1213, 277-288.

Esterbauer, H., Schaur, R. J., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biology and Medicine, 11, 81-128.

European Food Safety Authority. (2005). Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the Commission related to nutrition claims concerning omega-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat. European Food Safety Authority Journal, 253, 1-29.

Faber, N. K. M., Mojet, J., & Poelman, A. A. (2003). Simple improvement of consumer fit in external preference mapping. Food Quality and Preference, 14, 455-461.

Farrell, D. J. (1998). Enrichment of hen eggs with n-3 long-chain fatty acids and evaluation of enriched eggs in humans. The American Journal of Clinical Nutrition, 68, 538-544.

Farrell, D. J. (1998). Manipulation of the fatty acid composition of poultry meat and eggs to meet consumer demands. In Proceedings of the

6th Asian Pacific Poultry Congress. Nagoya, Japan (pp. 58-63).

Fenton, M., & Sim, J. S. (1991). Determination of egg yolk cholesterol content by on-column capillary gas chromatography. Journal of Chromatography A, 540, 323-329.

Fenwick, G. R., Curl, C. L., Pearson, A. W., & Butler, E. J. (1981). Production of egg taint by fish meal. Veterinary Record, 109, 292-292.

Ferrier, L. K., Caston, L. J., Leeson, S., Squires, J., Weaver, B. J., & Holub, B. J. (1995). alpha-Linolenic acid-and docosahexaenoic acid-enriched eggs from hens fed flaxseed: influence on blood lipids and platelet phospholipid fatty acids in humans. The American Journal of Clinical Nutrition, 62, 81-86.

Flamm, G., Glinsmann, W., Kritchevsky, D., Prosky, L., & Roberfroid, M. (2001). Inulin and oligofructose as dietary fiber: a review of the evidence. Critical Reviews in Food Science and Nutrition, 41, 353-362.

Flickinger, E. A., Schreijen, E. M. W. C., Patil, A. R., Hussein, H. S., Grieshop, C. M., Merchen, N. R., & Fahey, G. C. (2003). Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. Journal of Animal

Science, 81, 2008-2018.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226, 497-509.

Folkenberg, D. M., & Martens, M. (2003). Sensory properties of low fat yoghurts. Part A: Effect fat content, fermentation culture and addition of non-fat dry milk on the sensory properties of plain yoghurts. Milchwissenschaft, 58, 48-51.

Fox, P. F., & McSweeney, P. L. H. (1998). Dairy chemistry and biochemistry. Blackie Academic and Professional, London, UK.

Franck, A. (2005). Prebiotics stimulate calcium absorption: a review. Food Australia, 57, 530-532.

Frankel, E. N., Satué-Gracia, T., Meyer, A. S., & German, J. B. (2002). Oxidative stability of fish and algae oils containing long-chain polyunsaturated fatty acids in bulk and in oil-in-water emulsions. Journal of Agricultural and Food Chemistry, 50, 2094-2099.

Fuller, R. and G. Perdigón. 2003. The health benefits of probiotics and prebiotics, In G. R. Gibson, R. A. Rastall, and R. Fuller (Eds). Gut Flora, Nutrition, Immunity and Health. (pp. 52-53). Wiley-Blackwell.

Galobart, J., Barroeta, A. C., Baucells, M. D., Codony, R., & Ternes, W. (2001). Effect of dietary supplementation with rosemary extract and alpha-tocopheryl acetate on lipid oxidation in eggs enriched with omega3-fatty acids. Poultry Science, 80, 460-467.

Garg, M. L., Wood, L. G., Singh, H., & Moughan, P. J. (2006). Means of delivering recommended levels of long chain n-3 polyunsaturated fatty acids in human diets. Journal of Food Science, 71, R66-R71.

Gejl-Hansen, F., & Flink, J.M. (1977). Freeze-dried carbohydrate containing oil-in-water emulsions: microstructure and fat distribution. Journal of Food Science, 42, 1049-1055.

Gibbs, B.F., Kermasha, S., Alli, I., & Mulligan, C.N. (1999). Encapsulation in the food industry: a review. International Journal of Food Sciences and Nutrition, 50, 213-224.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. Journal of Nutrition, 125, 1401-1412.

Gonzalez-Esquerra, R., & Leeson, S. (2000). Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs. Poultry Science, 79, 1597-1602.

Gonzalez-Esquerra, R., & Leeson, S. (2001). Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids. Canadian Journal of Animal Science, 81, 295-305.

Grattard, N., Salaün, F., Champion, D., Roudaut, G., & LeMeste, M. (2002). Influence of physical state and molecular mobility of freeze-dried maltodextrin matrices on the oxidation rate of encapsulated lipids. Journal of Food Science, 67, 3002-3010.

Greenberg, A. S., & Obin, M. S. (2006). Obesity and the role of adipose tissue in inflammation and metabolism. The American Journal of Clinical Nutrition, 83, 461S-465S.

Griffin, W. S. T. (2006). Inflammation and neurodegenerative diseases. The American Journal of Clinical Nutrition, 83, 470S-474S.

Grummer, R. R. (1991). Effect of feed on the composition of milk fat. Journal of Dairy Science, 74, 3244-3257.

Hajati, H., & Rezaei, M. (2010). The application of prebiotics in poultry production. International Journal of Poultry Science, 9, 298-304.

Hamilton, R. M. G. (1982). Methods and factors that affect the measurement of egg shell quality. Poultry Science, 61, 2022-2039.

Haugh, R. R. (1937). The haugh unit for measuring egg quality. United

States Egg Poultry Magazine, 43, 552-573.

Heguy, J. M., Juchem, S. O., DePeters, E. J., Rosenberg, M., Santos, J. E. P., & Taylor, S. J. (2006). Whey protein gel composites of soybean and linseed oils as a dietary method to modify the unsaturated fatty acid composition of milk lipids. Animal Feed Science and Technology, 131, 370-388.

Herber-McNeill, S. M., & Van Elswyk, M. E. (1998). Dietary marine algae maintains egg consumer acceptability while enhancing yolk color. Poultry Science, 77, 493-496.

Hobson-Frohock, A., Land, D. G., Griffiths, N. M., & Curtis, R. F. (1973). Egg taints: association with trimethylamine. Nature, 243, 304-305.

Hall, C., Tulbek, M. C., & Yingying, X. (2006). Flaxseed. Advances in Food and Nutrition Research, 51, 1-97.

Holman, R. T., Johnson, S. B., & Hatch, T. F. (1982). A case of human linolenic acid deficiency involving neurological abnormalities. The American Journal of Clinical Nutrition, 35, 617-623.

Hough, G., & Sánchez, R. (1998). Descriptive analysis and external preference mapping of powdered chocolate milk. Food Quality and Preference, 9, 197-204.

http://www.fao.org/ag/aga/agap/frg/AFRIS/DATA/508.htm. (2014). Accessed on 31st May.

Hussein, N., Ah-Sing, E., Wilkinson, P., Leach, C., Griffin, B. A., & Millward, D. J. (2005). Long-chain conversion of [13C] linoleic acid and α-linolenic acid in response to marked changes in their dietary intake in men. Journal of Lipid Research, 46, 269-280.

Innis, S. M. (2000). Essential fatty acids in infant nutrition: lessons and limitations from animal studies in relation to studies on infant fatty acid requirements. The American Journal of Clinical Nutrition, 71, 238S-244S.

Jiang, Z., Ahn, D. U., & Sim, J. S. (1991). Effects of feeding flax and two types of sunflower seeds on fatty acid compositions of yolk lipid classes. Poultry Science, 70, 2467-2475.

Kim, R. S., & LaBella, F. S. (1987). Comparison of analytical methods for monitoring autoxidation profiles of authentic lipids. Journal of Lipid Research, 28, 1110-1117.

Koehler, H. H., & Bearse, G. E. (1975). Egg flavor quality as affected by fish meals or fish oils in laying rations. Poultry Science, 54, 881-889.

Kolanowski, W., & Laufenberg, G. (2006). Enrichment of food products with polyunsaturated fatty acids by fish oil addition. European Food

Research and Technology, 222, 472-477.

Kolanowski, W., & Weißbrodt, J. (2008). Possibilities of fisherman's friend type lozenges fortification with omega-3 LC PUFA by addition of microencapsulated fish oil. Journal of the American Oil Chemists' Society, 85, 339-345.

Koroleva, M. Y., & Yurtov, E. V. (2012). Nanoemulsions: the properties, methods of preparation and promising applications. Russian Chemical Reviews, 81, 21-43.

Kris-Etherton, P. M., Taylor, D. S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V., & Etherton, T. D. (2000). Polyunsaturated fatty acids in the food chain in the United States. The American Journal of Clinical Nutrition, 71, 179S-188S.

Krstonošić, V., Dokić, L., Dokić, P., & Dapčević, T. (2009). Effects of xanthan gum on physicochemical properties and stability of corn oil-in-water emulsions stabilized by polyoxyethylene (20) sorbitan monooleate. Food Hydrocolloids, 23, 2212-2218.

Labrousse, S., Roos, Y., & Karel, M. (1992). Collapse and crystallization in amorphous matrices with encapsulated compounds. Sciences des Aliments, 12, 757-769.

LaBrune, H. J., Reinhardt, C. D., Dikeman, M. E., & Drouillard, J. S. (2008).

Effects of grain processing and dietary lipid source on performance, carcass characteristics, plasma fatty acids, and sensory properties of steaks from finishing cattle. Journal of Animal Science, 86, 167-172.

Let, M. B., Jacobsen, C., Frankel, E. N., & Meyer, A. S. (2003). Oxidative flavour deterioration of fish oil enriched milk. European Journal of Lipid Science and Technology, 105, 518-528.

Let, M. B., Jacobsen, C., Sørensen, A. D. M., & Meyer, A. S. (2007). Homogenization conditions affect the oxidative stability of fish oil enriched milk emulsions: lipid oxidation. Journal of Agricultural and Food Chemistry, 55, 1773-1780.

Lewis, N. M., Seburg, S., & Flanagan, N. L. (2000). Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans. Poultry Science, 79, 971-974.

López-Ferrer, S., Baucells, M. D., Barroeta, A. C., & Grashorn, M. A. (2001). n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. Poultry Science, 80, 741-752.

Lubbers, S., Decourcelle, N., Vallet, N., & Guichard, E. (2004). Flavor release and rheology behavior of strawberry fat free stirred yogurt during storage. Journal of Agricultural and Food Chemistry, 52, 3077-

3082.

Maddock, T. D., Bauer, M. L., Koch, K. B., Anderson, V. L., Maddock, R. J., Barceló-Coblijn, G., & Lardy, G. P. (2006). Effect of processing flax in beef feedlot diets on performance, carcass characteristics, and trained sensory panel ratings. Journal of Animal Science, 84, 1544-1551.

Maddock, T. D., Anderson, V. L., & Lardy, G. P. (2005). Using flax in livestock diets. NDSU Extension Service.

Maga, J. A. (1982). Egg and egg product flavor. Journal of Agricultural and Food Chemistry, 30, 9-14.

Mahaffey, K. R. (2004). Fish and shellfish as dietary sources of methylmercury and the ω-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits. Environmental Research, 95, 414-428.

Mansbridge, R. J., & Blake, J. S. (1997). Nutritional factors affecting the fatty acid composition of bovine milk. British Journal of Nutrition, 78, S37-S47.

Mantzioris, E., Cleland, L. G., Gibson, R. A., Neumann, M. A., Demasi, M., & James, M. J. (2000). Biochemical effects of a diet containing foods enriched with n− 3 fatty acids. The American Journal of Clinical

Nutrition, 72, 42-48.

Marshall, R. T. (1992). Standard methods for the examination of dairy products. American Public Health Association, Washington, DC.

McClements, D. J. (2004). Protein-stabilized emulsions. Current Opinion in Colloid & Interface Science, 9, 305-313.

McClements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. Journal of Food Science, 65, 1270-1282.

Melanson, S. F., Lee Lewandrowski, E., Flood, J. G., & Lewandrowski, K. B. (2005). Measurement of organochlorines in commercial over-the-counter fish oil preparations: implications for dietary and therapeutic recommendations for omega-3 fatty acids and a review of the literature. Archives of Pathology & Laboratory Medicine, 129, 74-77.

Milinsk, M. C., Murakami, A. E., Gomes, S. T. M., Matsushita, M., & De Souza, N. E. (2003). Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids. Food Chemistry, 83, 287-292.

Ministry of Food and Drug Safety (MFDS). (2011). Health functional Foods Code (www.foodnara.go.kr/hfoodi/). The Food Safety Bureau. Republic of Korea. Accessed on 1st May.

Moghadasian, M. H. (2008). Advances in dietary enrichment with n-3 fatty acids. Critical Reviews in Food Science and Nutrition, 48, 402-410.

Morris, D.H. (2004). Flax: A Health and Nutrition Primer, Flax Council of Canada, Winnipeg, Manitoba, Canada.

Mussatto, S. I., & Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. Carbohydrate Polymers, 68, 587-597.

Nair, S. S., Leitch, J. W., Falconer, J., & Garg, M. L. (1997). Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action. The Journal of Nutrition, 127, 383-393.

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th ed. National Academy Press, Washington, DC.

Nitsan, Z., Mokady, S., & Sukenik, A. (1999). Enrichment of poultry products with ω3 fatty acids by dietary supplementation with the alga Nannochloropsis and mantur oil. Journal of Agricultural and Food Chemistry, 47, 5127-5132.

Nykter, M., Kymalainen, H. R., Gates, F., & Sjoberg, A. M. (2006). Quality characteristics of edible linseed oil. Agricultural and Food Science, 15, 402-413.

Oomah, B. D. (2001). Flaxseed as a functional food source. Journal of the

Science of Food and Agriculture, 81, 889-894.

Oomah, B.D. andMazza, G. (1998). Flaxseed products for disease prevention. In: Functional Foods: Biochemical and Processing Aspects, pp. 91-138. Mazza, G., Ed., Technomic Publishing Co, Inc., Lancaster, PA.

Packaged Facts. 2007. Omega-3 foods and the U.S. food and beverage market. Rockville, MD: Market Research.com, Inc.

Park, P.W., & Goins, R.E. (1994). In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. Journal of Food Science, 59, 1262-1266.

Park, S. O., & Park, B. S. (2012). Effect of feeding inulin oligosaccharides on cecum bacteria, egg quality and egg production in laying hens. African Journal of Biotechnology, 11, 9516-9521.

Parpinello, G. P., Meluzzi, A., Sirri, F., Tallarico, N., & Versari, A. (2006). Sensory evaluation of egg products and eggs laid from hens fed diets with different fatty acid composition and supplemented with antioxidants. Food Research International, 39, 47-52.

Pawlosky, R. J., Hibbeln, J. R., Novotny, J. A., & Salem, N. (2001). Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. Journal of Lipid Research, 42, 1257-1265.

Pearson, A. W., Greenwood, N. M., Butler, E. J., Curl, C. L., & Fenwick, G. R. (1983). Fish meal and egg taint. Journal of the Science of Food and Agriculture, 34, 277-285.

Peng, Y., Serra, M., Horne, D. S., & Lucey, J. A. (2009). Effect of fortification with various types of milk protein on the rheological properties and permeability of nonfat set yogurt. Journal of Food Science, 74, C666-C673.

Ponginebbi, L., Nawar, W. W., & Chinachoti, P. (1999). Oxidation of linoleic acid in emulsions: effect of substrate, emulsifier, and sugar concentration. Journal of the American Oil Chemists' Society, 76, 131-138.

Qiu, X. (2003). Biosynthesis of docosahexaenoic acid (DHA, 22: 6-4, 7, 10, 13, 16, 19): two distinct pathways. Prostaglandins, Leukotrienes and Essential Fatty Acids, 68, 181-186.

Rivero-Urgell, M., & Santamaria-Orleans, A. (2001). Oligosaccharides: Application in infant food. Early Human Development, 65, S43-S52.

Roberfroid, M., & Slavin, J. (2000). Nondigestible oligosaccharides. Critical Reviews in Food Science and Nutrition, 40, 461-480.

Roberts, D. D., Pollien, P., & Watzke, B. (2003). Experimental and modeling studies showing the effect of lipid type and level on flavor release

from milk-based liquid emulsions. Journal of Agricultural and Food Chemistry, 51, 189-195.

Rymer, C., & Givens, D. I. (2006). Effect of species and genotype on the efficiency of enrichment of poultry meat with n−3 polyunsaturated fatty acids. Lipids, 41, 445-451.

Sako, T., Matsumoto, K., & Tanaka, R. (1999). Recent progress on research and applications of non-digestible galacto-oligosaccharides. International Dairy Journal, 9, 69-80.

Salvador, A., & Fiszman, S. M. (2004). Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage. Journal of Dairy Science, 87, 4033-4041.

Sampath, H., & Ntambi, J. M. (2004). Polyunsaturated fatty acid regulation of gene expression. Nutrition Reviews, 62, 333-339.

Sattar, A., deMan, J. M., & Furia, T. E. (1975). Photooxidation of milk and milk products: A review. Critical Reviews in Food Science & Nutrition, 7, 13-37.

Scheideler, S. E., & Froning, G. W. (1996). The combined influence of dietary flaxseed variety, level, form, and storage conditions on egg production and composition among vitamin E-supplemented hens. Poultry Science, 75, 1221-1226.

Scheideler, S. E., Froning, G., & Cuppett, S. (1997). Studies of consumer acceptance of high omega-3 fatty acid-enriched eggs. The Journal of Applied Poultry Research, 6, 137-146.

Schmidl, M.K., and Labuza, T.P. (2000). Essentials of Functional Foods. Aspen Publishers, Gaithersburg, MD.; Hilliam, M. (2000). Functional food. The World of Ingredients. December, 50-52.

Schrooyen, P. M., Meer, R. V. D., & De Kruif, C. G. (2001). Microencapsulation: its application in nutrition. Proceedings of the Nutrition Society, 60, 475-479.

Shapira, N., Weill, P., & Loewenbach, R. (2008). Egg fortification with n-3 polyunsaturated fatty acids (PUFA): nutritional benefits versus high n-6 PUFA western diets, and consumer acceptance. The Israel Medical Association Journal: IMAJ, 10, 262-265.

Shimada, Y., Roos, Y., & Karel, M. (1991). Oxidation of methyl linoleate encapsulated in amorphous lactose-based food model. Journal of Agricultural and Food Chemistry, 39, 637-641.

Silva, M. H., Silva, M. T. C., Brandão, S. C., Gomes, J. C., Peternelli, L. A., & Franceschini, S. D. C. (2005). Fatty acid composition of mature breast milk in Brazilian women. Food Chemistry, 93, 297-303.

Singh, K. K., Mridula, D., Rehal, J., & Barnwal, P. (2011). Flaxseed: a

potential source of food, feed and fiber. Critical Reviews in Food Science and Nutrition, 51, 210-222.

Sørensen, A. D. M., Baron, C. P., Let, M. B., Brüggemann, D. A., Pedersen, L. R., & Jacobsen, C. (2007). Homogenization conditions affect the oxidative stability of fish oil enriched milk emulsions: Oxidation linked to changes in protein composition at the oil-water interface. Journal of Agricultural and Food Chemistry, 55, 1781-1789.

Souza, J. G., Costa, F. G. P., Queiroga, R. C. R. E., Silva, J. H. V., Schuler, A. R. P., & Goulart, C. C. (2008). Fatty acid profile of eggs of semi-heavy layers fed feeds containing linseed oil. Revista Brasileira de Ciência Avícola, 10(1), 37-44.

Stewart, C. S., K. Hillman, F. Maxwell, D. Kelly and T. P. King. (1993). Recent advances in probiosis in pigs: observations on the microbiology of the pig gut. In P. C. Garnsworthy and J. Wiseman (Eds), Recent Developments in Pig Nutrition (pp. 51-77). Nottingham: Nottingham University Press.

Sun, C., & Gunasekaran, S. (2009). Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. Food Hydrocolloids, 23, 165-174.

Sun, C., Gunasekaran, S., & Richards, M. P. (2007). Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. Food Hydrocolloids, 21, 555-564.

Tamime, A. Y., & Robinson, R. K. (1999). Yoghurt: science and technology. Woodhead Publishing.

Thompson, J. L., Lopetcharat, K., & Drake, M. A. (2007). Preferences for commercial strawberry drinkable yogurts among African American, Caucasian, and Hispanic consumers in the United States. Journal of Dairy Science, 90, 4974-4987.

Thybo, A. K., Kuhn, B. F., & Martens, H. (2004). Explaining Danish children's preferences for apples using instrumental, sensory and demographic/behavioural data. Food Quality and Preference, 15, 53-63.

Toborek, M., Lee, Y. W., Garrido, R., Kaiser, S., & Hennig, B. (2002). Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells. The American Journal of Clinical Nutrition, 75, 119-125.

Van Elswyk, M. E. (1997). Comparison of n–3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review. British Journal of Nutrition, 78, S61-S69.

Venkateshwarlu, G., Let, M. B., Meyer, A. S., & Jacobsen, C. (2004). Modeling the sensory impact of defined combinations of volatile lipid oxidation products on fishy and metallic off-flavors. Journal of Agricultural and Food Chemistry, 52, 1635-1641.

Verdonk, J. M. A. J., Shim, S. B., Van Leeuwen, P., & Verstegen, M. W. A. (2005). Application of inulin-type fructans in animal feed and pet food. British Journal of Nutrition, 93, S125-S138.

Voragen, A. G. J. (1998). Technological aspects of functional food-related carbohydrates. Trends in Food Science & Technology, 9, 328-335.

Watkins S. M., & German J. B. (1998). Omega fatty acids. In C. C. Akoh, & D. B. Min (Eds.), Food Lipids: Chemistry, Nutrition and Biotechnology (pp. 463-494). New York: Marcel Dekker.

Whelan, J., & Rust, C. (2006). Innovative dietary sources of n-3 fatty acids. Annual Review of Nutrition, 26, 75-103.

Wildman, R.E.C. (2001). Handbook of Nutraceuticals and Functional Foods. CRC Press, Boca Raton, FL.

Yildiz, G., Sacakli, P., & Gungor, T. (2006). The effect of dietary Jerusalem artichoke (*Helianthus tuberosus* L.) on performance, egg quality characteristics and egg cholesterol content in laying hens. Czech Journal of Animal Science, 51, 349-354.

Zare, F., Boye, J. I., Orsat, V., Champagne, C., & Simpson, B. K. (2011). Microbial, physical and sensory properties of yogurt supplemented with lentil flour. Food Research International, 44, 2482-2488.

Zentek, J. (2002). Egg taint–a problem of practical importance. Lohmann Information, 28, 3-6.

국문 초록

아마씨유와 올리고당의 유화물과 이를 이용한 식품의 물리화학적 특성

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최근 인간의 건강을 위한 식품의 역할에 대한 의식의 성장은 '기능성 식품'과 많은 전통식품의 기능적 특성에 대한 관심을 증가시기는 역할을 하였다. 기능성이 강화된 식품류의 개발이 활성화되고 있는 시점에 아마씨유는 심혈관계 질환 예방과 항염증에 잠재적 효과를 제공할 수 있는 오메가-3 지방산 급원으로 연구가 계속 진행되고 있다. 그러나, 이와 같은 장점에도 불구하고, 국내 아마씨유의 식품산업 적용도는 그리 높지 않

다. 아마씨유의 높은 ALA 함량은 산화에 취약하고, 이로 인하여 품질의 저하를 초래하기 때문이다. 따라서 본 연구의 목적은 ALA가 풍부한 아마씨유를 이용하여 산화적 또는 물리적으로 안정한 유화물을 개발하고 이를 식품에 적용하여 오메가-3 지방산이 강화된 식품을 개발하고자 한다.

첫번째 연구는 아마씨유(분산상)와 프락토올리고당(연속상)을 이용하여 실용적이고 간단한 방법으로 유화물을 만들고자 하였다. 이를 위해 유화제의 종류, 농도, 분산상과 연속상의 비율에 따른 유화물의 물리적 특성(유동학적 특성, 입자 분포도, 유화 안정성)을 조사하였다. 그 결과 HLB 값이 4.3, 8.6, 11.0, 14.5, 15.0인 유화제를 사용하여 아마씨유와 프락토올리고당의 유화물을 제조하였을 때 decaglycerol monolaurate(HLB 15)가 가장 안정적인 유화물을 형성하였다. 유화제(decaglycerol monolaurate)의 농도가 2% 이상일 때 안정적인 유화물이 형성되었다. 유화제의 농도가 증가할수록 점도가 증가하였으며, 입자의 크기가 감소하였고, pseudoplastic의 유동학적 특성을 보였다. 아마씨유와 프락토올리고당의

비율(3:7, 4:6, 5:5, 6:4, 7:3, 8:2)에 따른 유화물의 안정성을 측정한 결과, 아마씨유와 올리고당의 비율이 8:2인 것을 제외하고 모두 안정적인 유화물을 형성하여 아마씨유와 올리고당의 비율이 유동학적 특성에 영향을 크게 미치지 않았다. 아마씨유-올리고당 유화물은 아마씨유 자체보다는 산화가 억제되었으며, TBHQ를 처리한 아마씨유보다는 산화가 빠르게 진행되었다. 따라서 본 연구를 통하여 아마씨유 자체보다는 산화적으로 안정하고 물리적으로 안정한 유화물을 제조하였다. 이 새로운 형태의 유화물은 오메가-3 지방산(ALA) 강화를 목적으로 하는 식품에 쉽고 간편하게 첨가할 수 있을 것으로 판단한다.

두번째 연구는 총 6가지의 요거트((호상 요거트(Set-W), 아마씨유-올리고당 유화물을첨가한 호상 요거트(Set-WE), 아마씨유-올리고당 유화물을 첨가한 호상 탈지요거트(Set-NFE), 액상 요거트(Stir-W), 아마씨유-올리고당 유화물을 첨가한 액상 요거트(Stir-WE), 아마씨유-올리고당 유화물을 첨가한 액상 탈지요거트(Stir-NFE))를 제조하여 물리화학적 특성(pH, 산도,

고형분, 조단백, 조지방, 점도, firmness, 색도, 지방산 조성) 및 관능적 특성(정량적 묘사분석, 소비자 기호도)을 조사하였다. 아마씨유-올리고당 유화물의 첨가는 호상과 액상 요거트의 물리화학적 특성(pH, 산도, 고형분, 색도)에는 영향을 미치지 않았다($p>0.05$). 지방산 분석 결과, 아마씨유-올리고당 유화물의 첨가로 호상과 액상 요거트의 ALA의 함량이 크게 증가하였다($p<0.05$). 6가지 요거트를 정량적 묘사분석을 통해 평가한 결과, 총 14개의 묘사 용어(외관: 점도, 투명도; 향: 신향, 꼬린내, 비린향; 맛: 신맛, 치즈맛, 고소한맛, 비린맛; 입안의 느낌: 떫은맛, 끈적임, 코팅감; 후미: 신맛, 비린맛) 중에 13개의 용어(꼬린내 제외)에서 유의적인 차이가 나타났다($p<0.05$). 정량적 묘사 분석 결과, 아마씨유-올리고당 유화물을 첨가한 호상과 액상 요거트에서 이를 첨가하지 않은 요거트보다 비린 특성(향, 맛, 후미)과 관계된 묘사 특성이 더 강하였고, 탈지 요거트가 전지 요거트보다 비린 특성이 더 강하였다($p<0.05$). Set-W, Stir-W, Stir-WE 요거트는 다른 세가지 요거트보다 맛과 전반적인 기호도가 좋은 것으로 나타났다($p<0.05$). 따라서 본 연구 결과, 아마씨유-올리고당 유화물을 이용하여 n-

3 지방산이 강화된 요거트를 제조하고자 할 경우에는 호상 형태(발효 전 첨가)보다 액상 형태(발효 후 첨가)의 요거트에 사용하는 것이 관능적으로 바람직한 요거트를 제조하는 방법이라 판단한다.

마지막 연구는 n-3 지방산 강화 계란을 생산하기 위하여 아마씨유-올리고당 유화물과 어분 첨가 식이를 섭취한 산란계의 생산성(평균 난중, 산란율, 1일 산란양, 사료섭취량, 사료요구율)과 이로부터 얻은 계란의 지방산 조성과 계란 품질(난백높이, Haugh unit, 난각 두께, 난각 강도) 그리고 관능적 특성(소비자 기호도)에 미치는 영향을 알아보고자 아마씨유와 어분은 n-3 지방산의 급원으로, 프락토올리고당은 프리바이오틱스 급원으로 사용하였다. 산란계 식이에 대두유를 첨가한 것을 대조식이로 하고 아마씨유, 올리고당, 어분을 첨가한 6개의 군(1.5% 아마씨유, 1.5% 대두유+1.5% 올리고당, 1.5% 아마씨유+1.5% 올리고당, 1.5% 대두유+1.5% 어분, 1.5% 아마씨유+1.5% 어분, 1.5% 아마씨유+1.5% 어분+1.5% 올리고당) 식이를 산란계에 4주 동안 급여하였다. 프락토올리고당만을 섭취한 산란

계로부터 얻은 계란의 무게는 증가하였다($p<0.05$). 아마씨유 또는 아마씨유와 어분을 혼합한 식이를 섭취한 산란계로부터 얻은 계란은 대두유만을 섭취한 산란계가 낳은 계란에 비해 ALA, EPA, DHA가 증가하였다($p<0.05$). 아마씨유와 어분을 혼합한 식이를 섭취한 산란계가 낳은 계란은 대조군보다 이취, 비린내, 고소한맛의 기호도가 낮았으며, 전반적 기호도 또한 낮았다($p<0.05$). 아마씨유-올리고당 유화물과 어분을 첨가한 식이를 섭취한 산란계가 낳은 계란은 대조군과 비교하였을 때 모든 관능적 기호도에 있어서 유의적인 차이가 없었다($p>0.05$). 위 결과 아마씨유를 섭취한 산란계가 낳은 계란은 주요 n-3 지방산 중 ALA 함량이 증가하였고, 아마씨유와 어분을 혼합한 식이를 섭취한 산란계가 낳은 계란은 주요 n-3 지방산 중 EPA와 DHA가 증가하였다. 아마씨유와 어분의 혼합 식이에 프락토올리고당을 첨가할 경우 이를 섭취한 산란계가 낳은 계란은 n-3 지방산의 증가와 함께 관능적 기호도가 개선될 수 있다고 판단한다.

본 연구를 통하여 아마씨유를 이용한 n-3 지방산 강화 식품을 제조

하기 위한 방법으로 아마씨유와 프락토올리고당을 이용한 안정적인 수중 유적형 유화물을 제조하였고 이를 첨가한 식품들의 물리화학적, 관능적 특성을 확인하였다. 그 결과, 요거트 발효 전후에 아마씨유-올리고당 유화물의 첨가시 비린내, 비린맛, 비린 후미의 강도가 증가하였고, 발효전보다 발효후에 첨가하는 것이 모든 관능적 특성의 기도호가 더 좋았다. 또한 산란계 사료에 올리고당 급여로 인한 오메가-3 강화 계란의 관능적 품질 개선에 관한 자세한 메커니즘은 급여량과 기간에 따른 추가적인 연구가 필요할 것으로 생각한다. 결론적으로, 아마씨유-프락토올리고당 유화물은 다양한 n-3 지방산 강화 식품 제조에 활용할 수 있다고 판단한다.

주요어: 아마씨유, 프락토올리고당, 관능평가, 유화, 계란, 요거트

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