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Consumer Acceptability And Storage Stability Of Cupcake Supplemented With Fish Oil Emulsion

Olariyike A. Fasanya
North Carolina Agricultural and Technical State University

Consumer Acceptability and Storage Stability of Cupcake Supplemented with Fish Oil Emulsion

Olariyike A. Fasanya

North Carolina A&T State University

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Approved by:

Dr. Salam A. Ibrahim Major Professor	Dr. Mulumebet Worku Committee Member
Dr. Yi-Ling Pan Committee Member	Dr. Valerie Giddings Department Chairperson

Dr. Sanjiv Sarin
Dean, The Graduate School

Biographical Sketch

Olariyike A. Fasanya earned her Bachelor of Technology degree in Food Science from Ladoke Akintola University of Technology, Ogbomoso in Nigeria in 2004. In 2009 she received her post graduate degree diploma in Nutrition and Dietetics at the University of Agriculture Abeokuta Ogun state in Nigeria. She worked with Demoy's Nigeria Plc as a quality assurance manager in 2005. She worked with Nestle Nigeria Plc as a quality assurance technical service intern in 2003. She had also worked with Evans Nigeria Plc as a quality assurance technical service intern in 2002. In August 2011 she joined the Master's program at North Carolina Agricultural and Technical State University in food science. She is a member of the Family and Consumer Sciences Honor Society "Kappa Ominicro Nu." She is also a member of the Institute of Food Technology (IFT) and the American Chemical Society (ACS)

Olariyike Fasanya worked for Dr. Salam Ibrahim at North Carolina Agricultural & Technical State University in fall 2011 and summer 2012 as a graduate research assistant. In 2012 she had a poster presentation at Koury Convention Summer Conference on food safety practices. Her current research interest includes new product development, food safety, food processing, and food microbiology.

Dedication

This thesis is dedicated to God Almighty, the author and the finisher of my faith, the one that has given me life and breath and grace to successfully complete this study.

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Abstract

Omega-3 fatty acid (fish-oil) has gained attention as a functional food, and it is one of the best supplements used by several researchers to improve the nutritional value of different food products such as yogurt. The study was conducted to determine the effect of fish-oil emulsion on the physical attributes of shortened cupcake (control cake and five treatments) with different whey protein isolate concentrations. Emulsion concentration in each treatment included (1) 250mg Fish-oil + 0.5% Xanthan gum, (2) 250mg Fish-oil + 5% WPI, (3) 250mg Fish-oil +10% WPI, (4) 250mg Fish-oil + 0.5% Xanthan-gum + 5% WPI, and (5) 250mg Fish-oil + 0.5% Xanthan-gum + 10% WPI. Ninety untrained panelists at NC A&T participated in the study. A nine-point hedonic rating scale system was used for the evaluation. Sensory attributes evaluated included appearance, crust color, taste and texture, and consumer overall liking. Results of the study revealed the following: (a) no significant differences were found in consumer overall liking between the control and the treatment 2, and between the control and treatment 5; therefore, it can be concluded that cake is a good medium for omega-3 fish oil; (b) significant differences were found in consumer overall liking between the control and the remaining three other treatments; and (c) a significant difference was found between the storage days in the control cake; however, no significant differences were found between the storage days in treatments 2, 3, 4, and 5; therefore, it can be concluded that with the emulsion in the treatment cake storage life can be extended and still maintain quality. Overall, when considering the health benefits of omega-3 fish oil, this study revealed that cake supplemented with omega-3 fish oil maintains attributes desired by consumers and may provide a useful approach to improve nutritional intake.

CHAPTER 1

Introduction

1.1 Background

Diverse health benefits of omega-3 fatty acids have been documented (Ahmad, 1998). Omega-3 fatty acids are found in marine cold water fish and some vegetable oils. Fish oil is rich in docosahexaenoic acid (DHA) and eicosapentaenoic (EPA) while vegetable oils rich in omega-3 fatty acids provide Alpha Linolenic acid (ALA). These healthy fatty acids are found in high concentration in the cellular membranes of the body. Dietary DHA impacts the brain's structure and signaling systems and retina functions in infants. It also helps in reducing inflammation and oxidative stress while increasing membrane fluidity in the body, which contribute to reduced blood pressure and reduced risk of coronary heart disease (Ahmad, 1998). Intake of the plant-derived omega-3 fatty acid, alpha-linolenic acid, was associated with a reduction in the risk of Alzheimer's disease (Morris et al., 2003), whereby DHA supplementation not only improves memory in cases of Alzheimer's disease but has also been shown to improve age-related memory loss (Morris, Evans, Tangney, Bienias, & Wilson, 2005).

In other words, omega-3 fatty acid DHA is crucial for the healthy structure and function of the brain. An optimal intake of DHA is especially essential for pregnant and nursing mothers to ensure adequate brain development in their children and to prevent age-related memory decline. DHA helps promote nervous system development and optimal memory function. Its deficiency has been linked with many psychiatric disorders such as depression, suicidal behavior, anger, and hostility (Crawford, Hassam, & Stevens, 1981). Some studies showed that flaxseed oil has the highest content of linoleic acid in any food including flaxseeds, flaxseed meal, hempseed oil, hempseeds, walnuts, pumpkin seeds, Brazil nuts, sesame seeds, avocados, some dark leafy

green vegetables (kale, spinach, purslane, mustard greens, and collards), canola oil (cold-pressed and unrefined), soybean oil, and wheat germ oil. Other sources of omega-3 fatty acids include krill oils and algae (Park, 2006; Harper, Edwards, DeFilippis, & Jacobsen, 2006 Spences, Thornton, Muir, & Westcott, 2003). Omega-3 fatty acids are also available as dietary supplements which are taken to improve health and prevent various diseases

CHAPTER 2

Literature Review

2.1 Omega-3 Fatty Acid Polyunsaturated Fats (Omega 3 PUFA)

Omega 3 fatty acids have at least three double bonds starting from the third carbon atom at the methyl end of the fatty acid molecule (Pak, 2005). The essentiality of the structure is based on the chemical structure of these fatty acids and where the chemical bonds are located along the fatty acid structure. The location and amount of double bonds and length of the fatty acid chain in its chemical structure determines the biological importance of the essential fatty acid with regard to nutrition and health.

The first unsaturated carbon bond in omega-3 fatty acid occurs at the third carbon from the methyl end. The first unsaturated carbon bond in omega-6 and 9 fatty acids occurs at the sixth and ninth carbon atoms from the methyl end, respectively (Sun, Wang, Chen, & Chao, 2011). Omega-3 fatty acid polyunsaturated fats can be found in foods and are also available as dietary supplements.

In 2005, Scrimgeour defined omega-3 fatty acids as the natural product known as nonvitamin and nonmineral supplements, and most commonly used by adults in the United States. Fatty acids can be defined as compounds that are composed of long chains of carbon and hydrogen atoms (referred to as hydrocarbon molecules) containing a carboxylic acid at one end (COOH). Darlington and Stone (2001) categorized fatty acids into three major groups: saturated fatty acids contain no carbon-carbon double bonds and are covalently bonded, unsaturated fatty acids contain carbon-carbon double bonds, and polyunsaturated fatty acids (PUFAs) contain multiple sites of unsaturation. Polyunsaturated fatty acids are generally in liquid form at room temperature. Saturated fatty acids of less than eight carbon atoms are liquid at body temperature;

whereas those containing more than ten are solid at this temperature. The presence of carbon-carbon double bonds in fatty acids significantly lowers the melting point relative to that of a saturated fatty acid of the same number of carbon atoms. In the food industries, many animal- and plant-derived polyunsaturated fatty acids are chemically treated to introduce hydrogen atoms onto the carbon atoms that are double bonded to form a solid at room temperature.

2.1.1 Conversion of α- linolenic acid to EPA and DHA. In the western diet, about one third to one half of the fatty acids are saturated and the proportions of polyunsaturated fatty acids vary inversely with the saturated (Vessby, Gustafsson, Tengblad, Boberg, & Andersson, 2002). Whereas polyunsaturated fatty acids in the diet are linolenic acids (LA, 18:2n-6), this possesses a lower amount of fatty acids from the omega-3 series which are alpha linolenic acid (ALA, 18:3 n-3) and its two long chain metabolites eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). Fatty acids are used as sources of energy in the body, which can be metabolized by the desaturation and elongation to a longer chain and have more unsaturated fatty acids with specific properties (see Figure 1).

The desaturation steps are catalyzed by the enzyme desaturase, but with different affinities for the different fatty acids series. The preferred substrates for the 6 desaturase are 18:3 n-3 > 18:2n-6 > 18:1n-9 (Siguel & Maclure, 1987). Because of the product inhibition and competition between the substrate, the efficiency of the desaturation of a certain fatty acid is dependent not only on the amount in the diet but also on the content of others. It has been studied that, on the average, the body can only convert 5% of ALA to DHA and to EPA (Stark, 2008), and women can convert at rates approximately 2.5 times greater than men. This is made possible because of the developmental need to supply DHA to the fetus during pregnancy, as stated by Stark (2008). Therefore, the desaturation and elongation of ALA to long-chain polyunsaturated

omega-3 fatty acids (EPA and DHA) may be severely inhibited by the presence of a high proportion of LA in the diet; whereas a diet with a low proportion of omega-6 fatty acids and highly saturated fatty acids allows for an efficient processing of ALA (Vessby et al., 2002). In this process, ALA is first converted to EPA, and then to DHA by removing hydrogen molecules to form additional double bonds in a process known as desaturation, and addition of carbon atoms kown as elongation (Burdge, 2004).

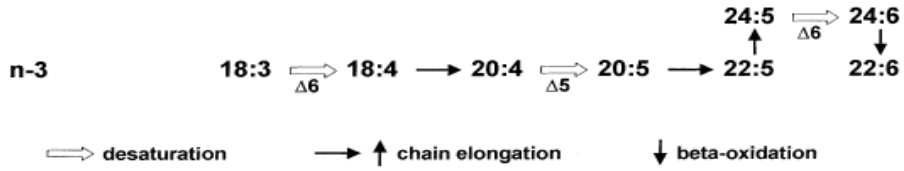


Figure 1. Long chain omega-3 fatty acid (Vessby et al., 2002).

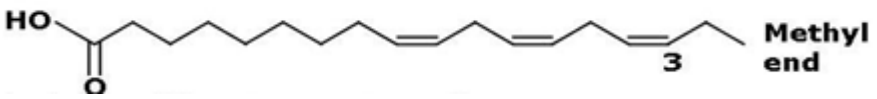
2.2 Chemical Structure of Omega-3 Fatty Acid

Unsaturated fatty acids consist of monounsaturated and polyunsaturated fatty acids. The two classes of PUFA are omega-3 and omega-6 fatty acids. Nutritionally-important omega-3 fatty acids in the body are α linolenic acid (ALA), eicosapentaenoic (EPA), and docosahexaenoic acid (DHA); their chemical structures are shown in Figure 2.

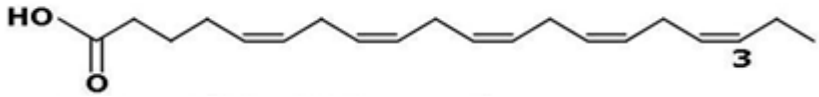
2.2.1 Eicosapentaenoic acid (EPA). EPA is a long-chain polyunsaturated fatty acid that reduces prostaglandins to help prevent clogged arteries. It acts as a precursor for thromboxane-3 to improve the dilation of arteries and supports the regulation of the immune responder leukotriene-5. EPA also supports optimal performance of the neurotransmitters needed for the nervous system to communicate effectively.

2.2.2 Docosahexaenoic acid (DHA). DHA is a long-chain, polyunsaturated fatty acid stored primarily in the brain and retina of the eye. The brain is composed mostly of fat (60%);

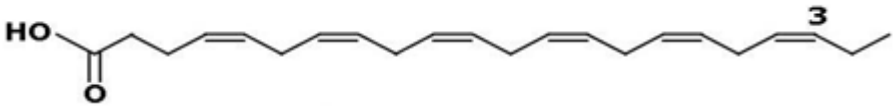
DHA makes up 30% of the fat, making it a critical component of the brain. DHA is crucial for brain development, maintaining brain function, and contributes to visual acuity.



(a) Alpha-linolenic acid (ALA, C18:3, omega)



(b) Eicosapentaenoic acid (EPA, C20:5, omega-3)



(c) Docosahexaenoic acid (DHA, C22:6, omega-3)

Figure 2. Chemical structure of omega-3 fatty acid **(Vessby et al., 2002)**

2.3 Chemical Structure of Omega-6 Fatty Acid

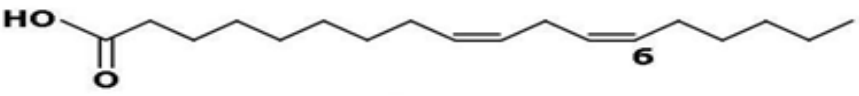
In our bodies the actions of omega-6 fatty acids are different and the biological effects of the omega-6 fatty acids are mediated by the conversion of *n*-6 eicosanoids that binds various types of receptors that are found in tissues of the body. The ratio of omega-6 to omega-3 fatty acids in the United States is estimated to be 10 to 1. Omega-6 and omega-3 fatty acids have different biological roles in the body and, in some cases, exert opposing effects but tend to maintain a balance in multiple systems, such as vascular health and immune functions.

2.3.1 Chemical structure of linoleic acid. Linoleic acid is an example of an omega-6 unsaturated fatty acid, and it is poorly soluble in aqueous media and susceptible to peroxidation. The body cannot synthesize linoleic acid from other food components. Fatty acids are precursors of other molecules such as prostaglandins, prostacyclins, thromboxanes, phospho-lipids,

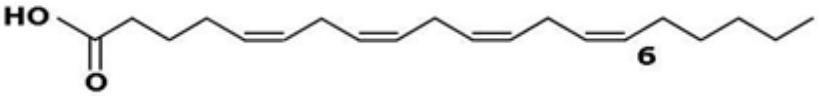
glycolipids, and vitamins. Fatty acids are esterified to a glycerol backbone to form a group of compounds known as mono-, di-, and tri-glycerides, which are neutral fats. Linoleic acid has been provided to cells in culture as a component of serum, albumin complex, or esterified to molecules such as cholesterol.

2.3.2 Chemical structure of arachidonic acid. Most biological actions of PUFA are derived from this long chain omega-6 (Arachidonic acid, AA). There are many different products (also known as metabolites) derived from long-chain PUFA that can be produced with varied actions depending upon the organ in the body with which they are associated and if the source of the metabolite was an omega-6.

In general, the actions of metabolites derived from AA are more pro-inflammatory while metabolites from EPA or DHA are considered less pro-inflammatory. Excessive products of AA in the circulatory system lead to changes in blood vessels that can limit blood flow to various organs and contribute to heart problems when dietary intakes of omega-3 PUFA are too low to counterbalance the omega-6 fatty acids.



(a) Linoleic acid (LA, C18:2, omega-6)



(b) Arachidonic acid (AA,C20:4,omega-6)

Figure 3. Chemical structure of omega-6 fatty acids. **(Vessby et al., 2002)**

2.4 Food Sources of Omega-3 PUFA and Omega-6 Fatty Acid

2.4.1 Omega-3 PUFA food sources. Fish oil is the main dietary source of long chain omega-3 polyunsaturated fatty acids. Omega-3 polyunsaturated fatty acid consists of α-linoleic acid and its longer chain metabolites which are eicosapentaenoic acid and docosahexaenoic acid. The richest dietary source of omega-3 LC PUFA is sea fish, especially fish oils. The major omega-3 LC PUFA is eicosapentaenoic and docosahexaenoic acids (EPA C20:5 and DHA C22:6, respectively). Pak (2005) revealed that the highest concentrations of the omega-3 PUFAs, EPA and DHA, are found in cold water fish such as salmon, mackerel, halibut, sardine, tuna, and herring. But ALA is found in flaxseed oil. Pak (2005) also revealed that flaxseed oil has the highest content of linoleic acid in any food, including flaxseeds, flaxseed meal, hempseed oil, hempseeds, walnuts, pumpkin seeds, Brazil nuts, sesame seeds, avocados, some dark leafy green vegetables (kale, spinach, purslane, mustard greens, and collards), and wheat germ oil.

2.4.2 Food sources of omega-6 fatty acid. Omega-6 fatty acids are found in a number of vegetable oils including corn oil, soybean oil, safflower oil, and sunflower oil. Processed and partially hydrogenated polyunsaturated fatty acids become trans fatty acids (Pak, 2005). Omega-6 fatty acids are also found in eggs and meats, margarine, and numerous baked or processed foods. The omega-6 fatty acids have their first double bond at the sixth carbon atom from the methyl end of the molecule. It was also concluded in Pak's (2005) study that omega-6 fatty acids are essential in the Western diet.

2.5 Potential Health Benefits of Omega-3 PUFA

Several studies performed on potential health benefits of enriched food consumption with omega-3 fatty acids showed that different amounts of DHA and EPA given in capsules or added to foods produce the same effects on blood lipid reduction (Wallace, McCabe, Robson, Keogh,

Murray, Kelly, and Strain, 2000). The ability of omega-3 polyunsaturated fatty acids to modulate tumor-cell growth was demonstrated for EPA by Chiu and Wan (1999). Several studies (Calder, 2004; Freeman et al., 2006; Psota, Gebauer and Kris-Etherton, 2006; Ruxton, Reed, Simpson, and Millington, 2004; Wang et al., 2006) revealed that omega-3 fatty acids can reduce cardiovascular disease. Likewise, additional studies (Wang et al. 2006; Harris, Miller, Tighe, Davidson and Schaefer, 2008) revealed that increasing the levels of intake of DHA and EPA, either by eating fish or taking fish oil supplements, lowers triglycerides, and slightly lowers blood pressure.

These studies also showed that DHA and EPA slow the progression of atherosclerosis (hardening of the arteries). The same authors concluded that omega-3 fatty acids reduce the risk of heart attack, stroke, and death among people with cardiovascular disease. Studies have found that fish oil may benefit people who have rheumatoid arthritis and high doses of fish oil have significantly reduced rheumatoid arthritis, morning stiffness, the number of swollen joints, and the need for corticosteroid drugs (Wang et al., 2006). Ward and Singh (2005) found that omega-3 fatty acids have potential applications in health promotion, including prevention of atherosclerosis, protection against arrhythmias, reduction of blood pressure, benefits for diabetic patients, the fight against manic-depressive illness, reduction of symptoms in asthma patients, protection against chronic obstructive pulmonary diseases, alleviation of symptoms of cystic fibrosis, the prevention of various cancers, improved bone health, and improved brain function in children. In addition, it has been revealed that omega-3 fatty acids play an important role in brain and nerve development in growing fetuses and infants (Honstra, 2001; Stephanie, 2001).

Omega-3 fatty acids can cure some forms of cancer and diseases with an immune-inflammatory component (Trebble et al., 2003). Morris et al. (2003) concluded in their study that

omega-3 fatty acids have some beneficial effects against neurological diseases such as depression and Alzheimer's disease. Additional supplementation with DHA and EPA has been shown to favorably influence serum lipid profiles in women. Laidlaw and Holub's (2003) findings showed that EPA arrested cell-cycle progression at G0/G1 phase, inducing necrosis in human leukemic HL-60 and K-562 cells in vitro. Likewise, EPA has induced apoptosis in HL-60 cells by down regulation of Bcl-2. Leaf and Kang (1997) and McLennan (1993) found that modest intake of omega-3 PUFA could reduce the risk of primary cardiac arrest by electrical stabilization of myocardial membranes and risk of sudden death.

2.5.1 Effect on cancer. PUFAs modify cell membrane, but phospholipids modify cellular functions which may reduce tumor motile/invasive potential that is directly toxic to tumor cells. Horia and Watkins (2005) also document that PUFAs and phospholipids are low in cytoxicity to normal cells. Many studies have shown that fish oil has important roles in the prevention of some types of cancer, including colon cancer (Jiang, Bryce, & Horrobin, 1998; Nano, 2003; Smyth & McGlynn, 2005), breast cancer (Horia & Watkins, 2005), renal cancer (Smyth & McGlynn, 2005), prostate cancer (Horrocks & Yeo, 1999), and pancreatic cell and liver cancer (Horia & Watkins, 2005; Jiang et al., 1998; Moyad, 2005).

2.5.2 Effect on cardiovascular disease. Health benefits of omega-3 PUFA on cardiovascular disease have been discovered over three decades (Abeywardena & Head, 2001). Omega-3 dietary supplements help to prevent heart disease through a variety of actions: (1) by the prevention of arrhythmias, which generate the prostanoids and leukotrienes with anti-inflammatory actions, and (2) by the inhibition of the synthesis of cytokines and the mitogens that augment the inflammation and promote plaque formulation (Uauy and Valenzuel, 2000).

2.5.3 Effects on human immune and inflammatory responses. The production of various eicosanoids explains the effects of long chain polyunsaturated fatty acid supplementation of various physiological functions (Uauy & Valenzuel, 2000). Eicosanoids are compounds that provide a link between the PUFAs' inflammation and immune functions, and they are synthesized from prostaglandins (PG), thromboxanes, leukotrienes (LT), lipoxins, and hydroperoxy-eicosatetraenoic acids (HETE). Prostaglandins, prostacyclins, throboxanes, and leukotrienes are derived from LCPUFA and they play a key role in modulating inflammation, cytokine release, immune response, platelet aggregation, vascular reactivity, thrombosis, and allergic phenomenon (Uauy & Valenzuel, 2000). The balance between Arachidonic acid (AA, n-6) and EPA (n-3) in biological membranes is regulated based on dietary supply. The n-6/n-3 ratio in phospholipids modulates the balance between prostanoids of the 2 and 3 series derived from AA and EPA, respectively (Uauy & Valenzuel, 2000).

2.5.4 Effect on plasma lipid. Marine oils have demonstrated effects on lowering the triglycerides in normal or hyperlipemic persons (Uauy and Valenzuel (2000). Studies on humans and animals have shown that fish oil concentrate inhibits hepatic triacylglycerol synthesis and the secretion of very-low-density lipoprotein (VLDL) from the liver. Omega-3 fatty acids do not affect post heparin enzymes activities. However, as it is revealed by Pak's (2005) study, omega-3 fatty acid possibly increases susceptibility of VLDL to lipoprotein lipase and hepatic lipase medicated lipolysis, thereby increasing the production of LDL from VLDL.

2.6 Fish Oil Recommended Dietary Intake

The long chain of omega-3 PUFAs has led to the commercial availability of purified fish oil supplement, which is available in drug and grocery stores (Horrocks & Yeo, 1999). An excessive intake of docosahexaenoic acid (DHA) can disturb membrane permeability and some

enzyme activities, but inadequate antioxidants can cause the accumulation of lipid peroxides (Pak, 2005). Recommendations for daily intake of omega-3 PUFAs have been published by several international scientific authorities (Kroes, Schaefer, Squire, & Williams, 2003). It is recommended that at least two or three fish meals a week be consumed, this could approximate to 200–400 mg of EPA and DHA a day. Nutrition recommendations published by Health and Welfare Canada provide a recommended daily intake of 1.0–1.8g omega-3 PUFAs. The World Health Organization (WHO) suggests servings of 200-500mg of DHA and EPA per week (WHO, 2003). The International Society for the study of Fatty Acids and Lipids (ISSFAL) recommended adequate intakes (AIs) of a minimum of 0.22 g daily of DHA and EPA combined, while the British Nutrition Foundation (BNF) has recommended a desirable population intake of 1.1 g (females) and 1.4 g (males) of DHA and EPA daily. In the United States, the Institute of Medicine (IOM) published a recommended AI of 0.5g omega-3 PUFAs (including DHA) daily for infants.

The American Heart Association recommends an average of 400-500mg EPA + DHA per day for people without heart disease and 1000mg for people with heart disease, and suggested that eating fish, especially fatty fish, at least 2-3 times in a week can average out to somewhere between 500 and 1,000mg EPA+DHA per day. The American Heart Association recommended that people who do not eat fish should consider getting EPA+ DHA from fish oil capsules (American Heart Association, 2010).

2.7 Types of Food Enriched with Omega-3 & 6 Fatty Acids

Presently, the terms ‘functional foods’ and ‘neutraceutical’ seem to be dominating the food market (Chingwaru, 2010). Because of the physiological functions of polyunsaturated fatty acids, omega-3 and omega-6 fatty acids are necessary for the growth and development of the

central nervous system, retina, and functioning of the cardiovascular systems (Nettleton, 1995; Simopoulos, 1991). Frequently, consumed foods are enriched with purified fish oil in order to increase the intake of omega-3 fatty acid in the diet. Fortification of food products such as bread, spreadable fats, dairy products, pasta, ice cream, milkshakes, or instant concentrate has been made possible by modern food technology, as stated by Kolanowski, Swiderski, and Berger (1999). They further stated in their studies that addition of fish oil introduces stability problems with regard to the taste and odor of the food products. Most USA products are enriched with omega-3 PUFA (Kolanowski & Laufenberg, 2006).

Table 1

Types of Food Products Enriched with Omega-3 PUFA

Milk and Dairy products	**Eggs and egg products**
Omega-3 milk (Parmalat, USA)	DHA Gold eggs (OmegaTech, USA)
YoBaby Plus fruit & Cereal with DHA (Stony field farm, USA)	Gold Circle Farms eggs (NutraSweet Kelco, USA
Wegmans Food You feel good about organic super Yogurt (Wegmans Grocery Rochester, NY, USA)	Eggs Plus (Pilgrim's Pride, USA)
Yo on the Go,(Whitney's Foods Inc., Jamaica, NY, US)	
Danino (Danone, Boucherville, Quebec, Canada, & Europe)	

(Kolanowski & Laufenberg, 2006)

Table 2

European Products Enriched with Omega-3 PUFA

Milk and Dairy products	Eggs and egg Products	Spreadable Fat	Juice and soft drink	Bread and Bakery Products	Meat and meat products
Plus omega-3-latte and omega-3-yogurt (Parmalat, Italy)	Eiplus eggs (Eifrisch, Germany)	Vitaquell omega-3 (Vitaquell, UK)	Supajus DHA rich orange drink (The Natural Fruit & Beverage Co., UK	Omega bread, sponge cakes, biscuits (Functional Nutrition, UK)	Omega-3 Jamon Cocido cooked ham breast (Carnicas Serrano, Spain)
Lauki omega+ skim milk (Candia, Spain)	Columbus eggs (Belovo, Netherlands)	Heartwatch omega reduced fat spread (Functional Nutrition, UK)	My way wellness drink (Designer Foods, Germany)	Nutribread (William Jackson Bakery, UK)	Terra y Mar turkey breast (Carnicas Serrano, Spain)
Lactel omega-3 milk (Lactalis Besnier Bridel, France)	Oro omega-3 eggs (Unione Cascine Valpadana, Italy)	Omega fat spread (Fjordland, Norway)	Timlic – orange nectar (Bauman-Fruits, Switzerland)	Nimble Heartbeat (British Bakeries, UK)	Omega cool burger (Pals, Norway)
Mleko omega-3 – UHT milk (SM Sudowa, Poland)	Ovo3 eggs (Maia Agrolimentare, Italy)	Gaio Spread (MD foods, Scandinavia)	Chikara Mizu soft drinks (Kirin Breweries, Japan)	Wellness Aktifit-Brot (Ruf Lebensmittelwerke, Germany)	Mega Off frozen chicken pieces enriched (Off Tene, Israel)
Omi-3 yoghurt (SM Siedlce, Poland)	Minicol omega pasteurized eggs (Wammala Food, Finland)	Vigor Omega Vitta (Vigor, Brazil)		F Plus fortified biscuits (Cuetara, Spain)	Strasburg sausage (Hans, Australia)
Omi-3 processed quark (OSM Ostrołka, Poland)		Dos Alamos Margarina (Grasco, Chile)		Diamant Vital omega-3 Kruste (Diamant M¨uhle, Germany)	
Especial omega-3 milk (Mimosa, Portugal)				Omega-3-bread and omega-3-rolls (VK Muhlen, Germany)	
ABC Infant Yogurt (Central Lechera, Australia, Spain)				Omega bread (Piekarnia Parkowa Wroclaw, Poland),	

2.7.1 Dairy products. Dairy products are food products which can be derived from animals such as cows, buffalo's milk, caribou, goat, sheep, yaks, and horses. Dairies come in many forms such as milk, butter, cheese, casein yogurt, clabber, gelato, and the favorite of all, ice cream. It can be classified into three large groups of dairy which are milk, cheese, and yogurt (Alexandriaruthk, 2012). All of these food products have been enriched with fish oil in one form or the other. Dairy products are rich in minerals and vitamins such as Calcium, Magnesium, Potassium, Riboflavin, Vitamin A & D, and proteins (Alexandriaruthk, 2012). Yogurt is a fermented food product and popular worldwide and has been consumed for the past thousand years.

Yogurt is a functional food made by a natural acidification of the milk by the action of lactic acid bacteria to make a gel-like form (Playne, Bennett, & Smithers, 2003). Yogurt is known as a functional food because of the live and active cultures that promote healthy digestion, boost the immune system, and provide other health benefits (National Yogurt Association, n.d.). Yogurt was prescribed by the ancient physician for curing disorders of the stomach, liver, intestines, and for appetite stimulation (Farnworth, 2003).

2.7.1.1 Importance of yogurt in health. Calcium in yogurt has beneficial effects such as reducing the risk of hypertension, cardiovascular disease, kidney stones, and colon cancer (Downey, 2005). Most importantly, yogurt provides healthy nutrition for children. Probiotics is a live culture present in some yogurt brands that helps in immunity boosting, keeping the gastrointestinal tract healthy, and preventing yeast infections. Balfour's (2009) findings revealed that consuming yogurt daily can help offset resulting stomachache issues; likewise, yogurt can be recommended for digestive problems such as bloating, gas, and irritable bowel syndrome.

In the United States, yogurt has been very popular during the past five years and it is showing no signs of slowing (Tamine & Robinson, 1999). Consuming yogurt has sustained a good growth pace as it captures a larger share of the cultured products market. From 2004 to 2008 yogurt was tracked in the snack food package market and showed a sales increase from 9.1 to 10.4% (SBI Market Profile, 1999). According to Datamonitor (2004), the yogurt global market sold the largest amount of yogurt to the United States.

In 2004, the functional food market also increased from 7.2% to $18.9 billion (Vierhile, 2006). Between 2006 & 2008, as documented by Packaged Facts (2009), omega-3 fatty acids was one of the fastest growing functional ingredients in the market as consumers in the United States had more knowledge of the benefits of omega-3 fatty acids.

2.7.1.2 Yogurt a model for omega-3 fatty acids. Incorporation of omega-3 into yogurt is an ideal medium due to its beneficial effects and the popularity of yogurt in the marketplace. Yogurt is a snack food that has been consumed by adults and kids, which makes it a major functional food in society. Fish oil-enriched food products have been developed and milk enriched with omega-3 has been marketed since 1998 (Mattila-Sandholm & Saarela, 2003). A study that included omega-3 fatty acid in yogurt described the oxidative stability of fish oil-enriched drinking yogurt together with the anti-oxidative effect of citric acid ester, vitamin-K, and sodium ethylenediaminetetaacetic acid (Nielsen, Debnath, & Jacobsen, 2007). It was also concluded in this study that there was no oxidation noticed in yogurt after being stored at $2^{o}C$ for up to 19 days and established that yogurt is stable toward oxidation. Diaz, Dunn, McClements, and Decker's (2003) study on the addition of fish oil to yogurt revealed the slow oxidation rate experienced could likely be due to the anti-oxidative properties of casein which is a milk protein and the strawberry flavor used to mask the fishy flavor. High oxidative susceptibility was

observed by Let, Jacobsen, and Meyer (2004a, 2004b) on fish oil by enriched milk, and they hypothesized that these may be due to the various factors in cow's milk. Fox and McSweeney (1998) suggested that both the transition metals and unsaturated phospholipids present in the milk fat globule membrane can promote milk oxidation. High levels of fat content in dairy products can be made possible by enriching with high levels of fish oil (Kolanowski & Weißbrodt, 2007). According to the study documented by Schram et al. (2007) that investigated the role of food matrix on bioavailability of omega-3 and the oxidative stress in plasma, incorporation of fish oil into food products such as yogurt drinks, fitness bars, bread and butter, and oil capsules, yogurt was the best matrix for providing fast absorption of lipids, including omega-3 fatty acids.

Studies have documented that different whey protein sources at a PH value of 3 can produce a stabilized emulsion system which can be arranged in the order of β- lacto globulin ≥ α lactalbumin ≥ whey protein isolate. Food ingredients such as whey protein have been widely used as an emulsifying agent in the food industry (Coupland and McClements, 1996).

2.7.2 Bakery products. Marine functional ingredients, such as fish oils, have found application in bakery, confectionary, and pasta products (Kadam & Prabhasankar, 2010). These are added in the form of fortifiers and nutritional enrichments. Fish oils which are rich in omega-3 are extensively used in bread and other products in the bakery industry (Kadam and Prabhasankar, 2010). These products have shown considerable improvement in EPA and DHA contents of breads and other bakery products leading to reduction of cardiovascular diseases, which is a major health concern of the twenty-first century (Nielsen et al., 2007). A wide range of omega-3 fatty acids in oil and powder forms are currently available for food fortification in the market in countries such as the UK, Korea, and Taiwan (Trautwein, 2001). Fortunately,

Europe and Japan have taken steps towards the enrichment of infant formulas with essential omega-6 and omega-3 fatty acids (Verardo et al., 2009). Oxidation of omega-3 and omega-6 fatty acids can deteriorate the taste, odor, and shelf life of enriched foods; thus, adequate enrichment levels, shelf life, and the addition of antioxidants need to be considered to ensure good quality (Dalton et al., 2006; Jacoben, 2008; Let, Jacobsen, Sorensen, & Meyer, 2007).

Omega-3 oils are incorporated in bakery products, pastas, and dairy products such as milk, yogurt, and juice, as well as nutrition bars. DHA supplementation in humans increased serum, DHA as non-esterifies fatty acid at levels that were potentially antithrombotic (Conquer & Holub, 1998). According to Liu, Wallin, and Saldeen's (2001) study, there was a significant increase in plasma and HDL-cholesterol in 36 hyperlipidemia patients fed with bread containing fish oil. Recent research has shown that seaweed can be used as a rich source of carotenoids such as taxanthin, fucoxanthin, and dietary fiber, and can be incorporated in pasta products without much intervention in sensory quality (Kadam & Prabhasankar, 2010). Incorporation of Wakame seaweed in pasta (Pinnatifida, 2009) has shown great potential to improve fucoxanthin content. Kadam & Prabhasankar (2010) documented that consumers also accepted surimi, a processed fish product, at moderate concentrations in pasta.

An excellent product in which the incorporation of 'neutraceutical' or 'pharmafoods' is attempted is bread. One of the latest enrichments has been the addition of omega-3 PUFA to improve essential fatty acid intake (Kadam and Prabhasankar, 2010). A study conducted in Europe by Kadam (2010) revealed that the consumption of bread enriched with omega-3 PUFA steadily increased because Europeans recognize the healthy component of omega-3 PUFA products. The encapsulation method that could be used to enhance the stability of omega-3 lipids was patented by Yan (2003). The author used a multi-core approach by first encapsulating the

omega-3 lipids and then applying a second shell over the agglomerated encapsulated lipids. This approach provides a product that has multi-layers of protection. Kadam and Prabhasankar (2010) documented in their report that Ocean Nutrition Canada started to use the encapsulated method developed by Yan in 2005 under the Powder-loc trademark to produce stable omega-3 encapsulated powders. The powder that resulted from this process delivered 500–800 mg oil per gram of dry powder. In contrast, 200–350 mg oil per gram of dried powder is typically delivered for conventional spray-dried encapsulated powders. The Wright Group (Crowley) uses a proprietary technology called Super Micro Atomization Retention Technology (SMART) to produce Super Coat omega-3 products. The application of specific fatty acids to bread was a new approach to incorporate more omega-3 into the diet.

Bread enriched with Microencapsulated Tuna Oil (MTO) increases DHA and determined the acute and chronic effects of low doses of long chain on plasma (Yep, Li, Mann, Bode, & Sinclair, 2002). The purpose of Yep's et al (2002) study was to determine whether the consumption of bread enriched with a low dose of MTO would improve LC n−3 PUFA status in healthy individuals, as measured by plasma levels of PUFA. The author used 36 subjects with hyperlipidemia and it was stated in the report that subjects were randomly divided into three groups: (a) stable fish oil with oat fiber, (b) control with oat fiber, and (c) control with wheat fiber. Their results showed that triglycerides were decreased and HDL cholesterol increased after intake of the bread containing stable fish oil. The study further showed a significant increase in omega-3 fatty acids among subjects with hyperlipidemia intake of bread containing a small amount of fish oil. Saldeen, Wallin, and Marklinder's (1998) study on bread intakes revealed that bread is a reliable and significant source of higher n−3 PUFA (with 20 and 22 C atoms).

Functional foods are capable of reducing risk factors for coronary heart disease (Harrison et al., 2004). It was hypothesized in Harrison et al.'s study that bread, cracker biscuits, and snack bars fortified with DHA (long-chain omega-3) would have a positive impact on cholesterol level and blood pressure. Evaluations of instant foods were suitable for fortification with fish oil at limited levels and obtained levels of fortification were much lower than in the case of spreadable fats and bread or dairy products (except liquid milk; Roche & Gibney, 1994). It was also concluded in the study conducted by Roche and Gibney (1994) that fortification of instant foods at different levels might increase omega-3 LC PUFA levels in the average diet, especially when combined with other fish oil-fortified foods.

In the microencapsulated study conducted by Koletzko (1996), the highest fortification level was reached in the case of potato mash, as well as banana flavored milk-wheat and banana flavored milk-rice, both are children's cereals. Neilson (1992) investigated the effectiveness of promoting white bread enriched with omega-3 fatty acids using gelatin-coated fish oil. White bread was eventually marketed in Denmark as 'Omega Bread.' Neilson (1992)

2.8 Mechanism of Autoxidation of Lipids

There are three processes involved in autoxidation of a free radical, and it can be divided into 3 stages: initiation, propagation, and termination. During the process of the initiation some event causes free radicals to be formed such as benzoylperoxide, which can be formed purposefully by the decomposition of a radical initiator. Destructive autoxidation stages can be initiated by pollution and when free radicals are formed, materials are converted to hydroperoxide due to a reaction in the chain of the free radical and the chain is ended by termination reactions whereby the free radicals collide and join their odd electrons to form a new bond, as shown in the structure in Figure 4.

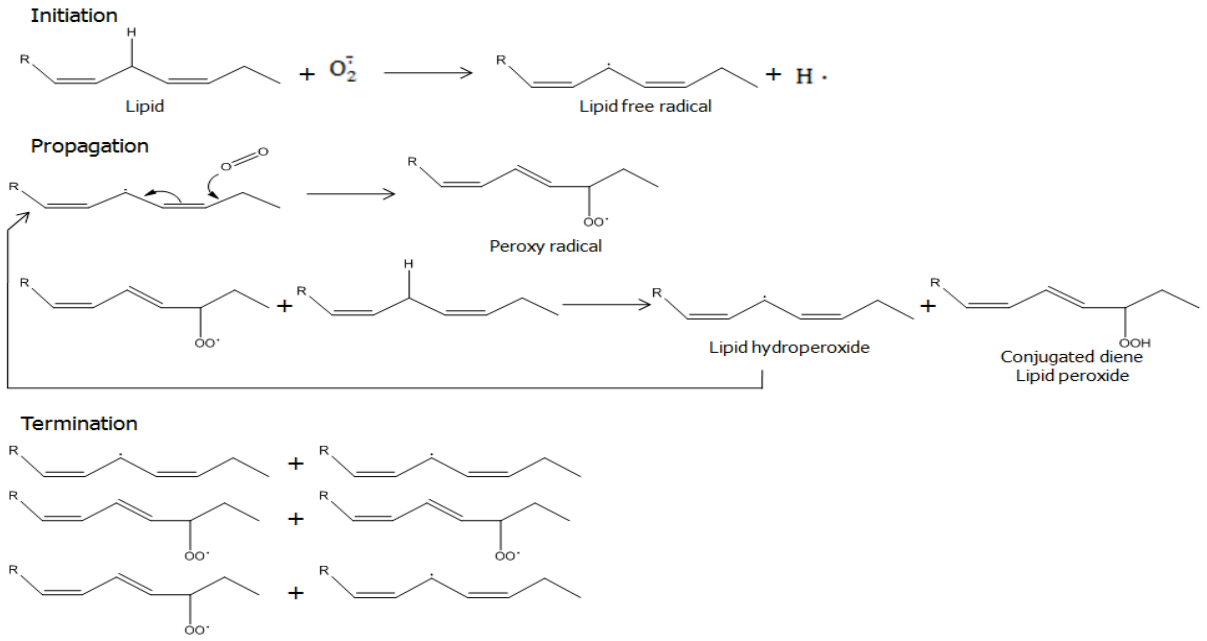


Figure 4. Lipid Auto oxidation (Rognlien, 2010)

2.8.1 Oxidation of fish oil. Fish oil rich in long chain omega-3 polyunsaturated fatty acids have been studied a great deal and recognized for their nutritional importance (Carroll, 1986; Simopoulos, 1991). However, the utilization of these oils mostly as a nutritional supplement in fortified foods and food processing is limited by the oxidation of lipid, and these oxidative problems may be attributed to the composition of triglycerides in fish oil and the wide variations in fatty acids (Frankel, Satué-Gracia, Meyer, & German, 2002; Moffat, 1995). According to a study conducted by Yu and Sinnhuber (1967), in many studies on the oxidative stability of lipid PUFA such as gas chromatography, oxygen absorption is based on an unspecific method and unrealistic method for the measurement of lipid oxidation and stability. The results of studies conducted by Han, Yi, and Shin (1991) stated that the effectiveness of antioxidants in oil protection of long chain PUFA have been particularly difficult to interpret. Antioxidant effects in mayonnaise enriched with 16% fish oil was documented by Jacobsen et al. (2001). Results of their study revealed that EDTA inhibited the formation of free radicals, volatiles, the

fishy flavors, the rancidity off flavors, and the lipid hydrogen peroxides. As reported by Jacobsen et al., the oxidative effect of EDTA was attributed to the ability to chelate free metal ions along with the egg yolk ion located at the oil water interface. The authors concluded that free radicals can be decreased in level by the addition of Gallic acid and oxidative flavor in mayonnaise deterioration. In the same study, Jacobsen et al. stated that additional emulsification did not have an impact on the oxidative flavor or the formation of free radicals but it did affect the volatiles profile in mayonnaise.

Ascorbic acid as an antioxidant in mayonnaise reduces the value of peroxides and promotes volatile oxidation formation at a low PH of 3.8- 4.2 (Djordjevic, McClements, & Decker, 2004). Whey protein isolate can be used in the stability of algae oil in water emulsion, and at a PH value of 3 yields high physical and oxidative stability (Djordjevic et al., 2004). It was also determined by (Djordjevic et al., 2004) that Whey protein stabilizes algal oil in water emulsions stored at a refrigerated temperature.

Antioxidant such as Ascorbyl palmitate completely stopped oxidation in the fish oil-enriched milk emulsion as reported in the Let, Jacobsen, Pham, and Meyer (2005) study. Enrichment of milk with highly unsaturated fish oil is possible without the formation of fish off-flavor, provided that the oxidative quality of the fish oil is most favorable when the peroxide value approached zero (Let, Jacobsen, Frankel, & Meyer, 2003). The effect of oxidation was studied in three different products, namely yogurt, milk, and salad dressing by either the addition of the fish oil in water emulsion or a neat fish oil to these products directly. Milk turns out to have a higher level of fish off flavor and oxidized faster irrespective of the mode of addition than fish oil-enriched yogurt and salad dressing; therefore yogurt turns out to be a good vehicle for omega-3 polyunsaturated fatty acid. Yogurt and salad dressing enriched in neat fish oil were

stable compared to the water emulsion fish oil, and it can be concluded that application of the neat fish oil is a good preservative for products to ensure the final quality of the products (Let & Meyer, 2007). An energy bar was supplemented with 5% fish oil in N. S. Neilsen and Jacobsen's (2009) study, and it was documented that heating of bars during baking did not increase oxidation but addition of the neat fish oil makes the bar oxidatively unstable.

2.9 Ingredients Used in Emulsion System of Fish Oil

Omega-3 oils are much more popular and extensively used than any other ingredient of marine source. Chitin and chitosan polysaccharides have gained attention and the quality of the ingredients used to manufacture food emulsions can have a pronounced impact on oxidative stability (McClements & Decker, 2000). Transition metals in an emulsion in reasonable amounts can accelerate lipid oxidation through their ability to promote the breakdown of hydroperoxide (McClements & Decker, 2000). Algae and seaweed have been found to be a good source of dietary fiber, antioxidants, and carotenoids; on other hand, fish bone and shark cartilage are extensively used as source of calcium (Schrooyen, van der Meer, & De Kruif, 2001). Seafood is characteristically tender, easily digested, and a good source of many important minerals (Kadam & Prabhasankar, 2010).

An antioxidant can be defined as a substance that inhibits the rate of the reaction with oxygen (Markley 1961; Stansby, 1967). The antioxidant phenomenon is particularly important with fish oil, the fatty acids which are generally unsaturated and generally susceptible to attack by oxygen in the air (Stansby, 1967). Natural antioxidants are used in the food industry to protect lipid-bearing foods against oxidation. In a reviewed report by Jacobsen, et al., (2008), the results of the variety of antioxidants used in various food matrices determined the different effects in different food matrices. Impacts of food ingredients on the oxidative stability of food products

was studied by Jacobsen et al. (2001). Results of their study revealed that ingredients such as NaCl, sugar, and vinegar in the production of mayonnaise may impact the oxidative stability. Meanwhile, the addition of tragon vinegar to mayonnaise decreases the Ph to less than 4.2 in order to ensure microbial stability. According to Jacobsen et al. (2008), menhaden oil in water emulsion containing EDTA could be used as an ingredient system delivery to include omega-3 fatty acids into foods.

2.9.1 Storage stability and sensory properties of fish oil enriched food products. The most important cause of deterioration in the quality of fish oil, from a flavor and odor standpoint, is oxidation (Stansby, 1967). The sites of attack by oxygen are the unsaturated portions of the fatty acid moieties of triglycerides. Boran, et al., (2006) expanded on oxidative spoilage of fish oil. Fish oils are highly susceptible to oxidative spoilage and the rate of fish oil oxidation is significantly different from that of other oils. However, purification technologies have emerged that remove most of the unpleasant fishy flavor. According to Trautwein (2001), PUFA and highly unsaturated EPA and DHA are very susceptible to oxidation. A study conducted by Kolanowski et al. (1999) revealed that since oxidation can cause rancidity and negative impacts on sensory properties such as taste and odor, fish oils are usually stabilized with vitamin E and other natural antioxidants. Therefore, for food enrichment only unhydrogenated fish oil should be used. The hydrogenation process may improve stability and shelf life of fish oil but saturation of double bounds eliminates the desirable health properties of omega-3 PUFA (Muller et al., 1998). To prevent oxidation of fortified foods, El-Sayed, Abd El-Gawad, Murad, and Salah (2002) recommended the use of vacuum or inert gases packaging in one-portion units and its consumption shortly after package opening. When fish oil-enriched food exerts an access to oxygen, it promotes oxidation and deterioration of EPA and DHA. This helps to increase

unacceptability of fishy flavor, which is the most negative influence on shelf-life and sensory quality of fish oil-enriched foods (Kolanowski et al., 1999). Spreadable fats are a good example of food products suitable for enrichment with EPA and DHA by fish oil addition (Kolanowski, Swiderski, Jaworska, and Berger, 2004). Kolanowski et al. (2004) revealed that the level of fish oil addition to the recommended consumption used did not have a significant effect on the textural attributes of the spread and significantly improved the fatty acid profile. These authors also reported that a daily intake of 30g of enriched spread could provide 0.25 g of EPA and DHA. Thus, a regular consumption of such a spread would increase the amount of long-chain omega-3 PUFAs in the diet.

Omega-3 long chain PUFA has various challenges ahead for use of marine functional ingredients in the daily diet. These include low consumer awareness among people about potential health benefits, pollution of seafood with various hazardous components such as industrial waste, metals etc.; sensory changes in the product with the incorporation of marine food; and changes in physicochemical properties of food (Katsuda et al., 2008). The number of various food products enriched with omega-3 PUFA available on the market is increasing substantially, but the bioavailability and health effects derived from regular consumption of those products still remain to be tested (Katsuda et al., 2008). Bioavailability and physiological effects of omega-3 fatty acids added to foods may differ depending on the type and quantity of other nutrients present in the food product (Kolanowski & Laufenberg, 2006).

2.10 Xanthan Gum

Xanthan gum is a polysaccharide, which is manufactured using natural microbial fermentation processes. This process converts corn syrup to Xanthan gum using the microorganism Xanthomonas campestris, a bacterium that is naturally found on the cabbage

plant, which produces slimy and gummy colonies. The microorganism produces these slimy and gummy colonies, which are called extracellular polysaccharides. These polysaccharides are released from the bacterium cell because no covalent bonds are formed to the cell wall (Sadar, 2004). The extracellular polysaccharide released from Xanthomonas campestris is called xanthan gum. Xanthan gum is produced by the process of submerged aerobic fermentation using glucose as the primary carbohydrate source. The xanthan gum is recovered, purified, dried, and milled into a white powder. Due to a microorganism which is commonly found in green leafy vegetables, cabbage produces xanthan gum as a protective coating which is precipitated and ground into a powder wall (Sadar, 2004). Various mesh sizes xanthan gum are used in the food industry for suspension, addition of viscosity and also to stabilize the emulsion system wall (Sadar, 2004). As documented in El-Sayed et al's (2002) report, Xanthan gum has the ability to produce a large viscosity by adding very small quantity of gum.

Xanthan gum solutions are pseudo plastic and can lose viscosity immediately when a high shear is applied (Chanamai & McClements, 2001). Xanthan gum inhibits syneresis and prevents fillings from being absorbed in pastries. Xanthan gum also helps to stabilize frozen products through the freeze and thaw cycles by controlling ice crystal growth. Xanthan which is blended with Guar, locust bean gum, are both effective stabilizers for ice cream, ice milk, sherbet, and water ices (Chanamai & McClements, 2001). According to Morris, Rees, Young, Walkinshaw, and Darke's (1977) study, the synergistic interaction between xanthan gum and galactomanans in solutions results in enhanced viscosity or gelation. Xanthan gum is a non-adsorbing polysaccharide and does not bind to whey protein and stabilized in a droplet surface (Sun et al., 2007).

Xanthan gum can be defined as an anionic linear hydrocolloid with a (1, 4) linked β-D-glucose backbone, as in the cellulose. Cellulose has a large side unit on every other glucose unit at location C-3. The unit side, a trisaccharide, contains a glucuronic acid residue linked (1,4) to a terminal mannose unit and (1,2) to a second mannose which connects to the glucose backbone (Sworn, 2000). The mannose unit connected to the backbone usually contains an acetyl group. Approximately 50% of the terminal mannose molecules carry a pyruvic acid residue (Kovac, 1977; Sworn, 2000; Zirnsak, Boger, & Tirtaatmadja, 1999). The primary structure is shown in Figure 5.

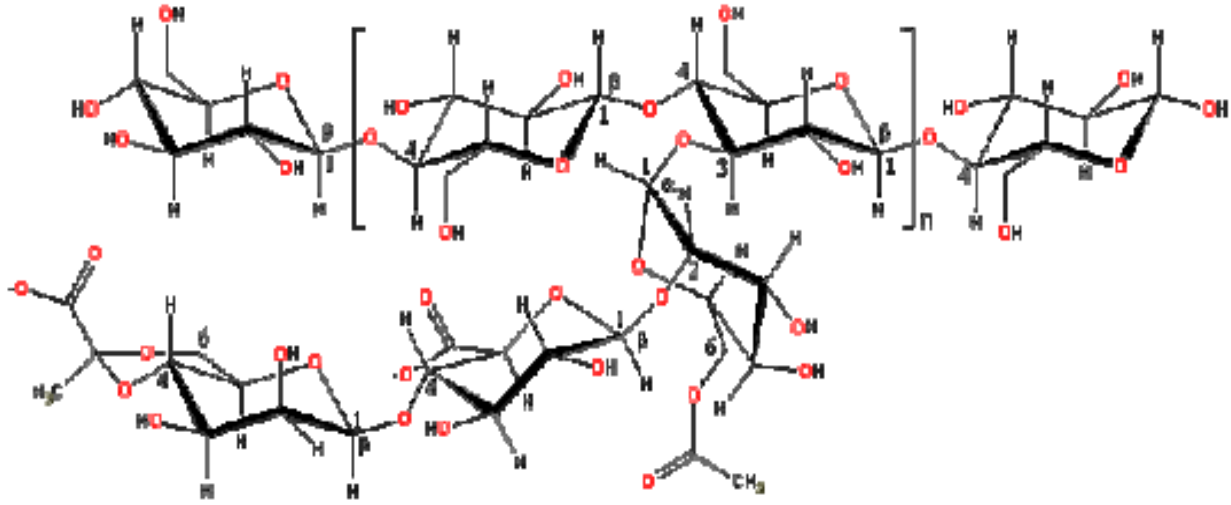


Figure 5. Molecular structure of xanthan gum (Sadar, 2004).

2.10.1 Physical properties of food products using xanthan gum. Xanthan gum is an excellent thickening agent which exhibits pseudo plastic rheological characteristics. Once the shear is removed, the starting viscosity is recovered. The reason this occurs with xanthan gum is the ability of the xanthan molecules to form aggregates through hydrogen bonds and polymer entanglement (Sworn, 2000). At low shear rates, xanthan solutions are highly ordered, entangled, stiff molecules. As shear is increased, the aggregates are interrupted and individual polymer

molecules align in the direction of the shear force, which results in the condition known as pseudo plastic (Deis, 2001). According to Sworn's (2000) report on xanthan gum when 1.0% xanthan gum is used, an almost gel-like consistency will be observed at rest, but when shear is applied, it exhibits the same rheological properties. Xanthan gum is stable over the pH range 2 to 12; at pH below 2 and above 12, viscosity tends to decrease slightly (Dziesak 1991; Sworn, 2000). However, it was concluded in Sworn's (2000) study, that as the concentration of xanthan gum is reduced, a decrease in viscosity of the xanthan gum was observed.

Xanthan gum, unlike many other food gums, is stable at a range of temperatures. The viscosity will not change significantly between ambient temperature and a definitely "melting temperature," which is usually around 60ºC according to research by Sworn (2000). If viscosity is lost due to an increase in temperature, it is reversible and as the solution cools, the initial viscosity will return. Depending on the concentration of xanthan gum, salts may either decrease or increase viscosity of xanthan gum (Sworn, 2000). At 0.25% xanthan gum concentration or below, monovalent salts may cause a slight decrease in viscosity. Unlike most hydrocolloids, xanthan gum has not been found degraded frequently by enzymes unlike how proteases, pectinases, cellulases and amylases are found in many food systems. It is believed the arrangement of the trisaccharide side unit is responsible for this enzyme resistance (Sworn, 2000). The side unit prevents enzymes from attacking the β-(1, 4) linkages located on the backbone.

Therefore, xanthan gum can be used in food products containing active enzymes. Although xanthan gum is not a gelling agent, it can form elastic, thermo reversible gels when combined with locust bean gum. High viscosities are achieved when combined with galactomanans such a locust bean gum and guar gum (Dziesak 1991).

2.10.2 Emulsion system of food products using xanthan gum. Xanthan gum is approved for food use as a stabilizer, emulsifier, thickener, suspending agent, and foam enhancer (Sanderson, 1996). It has been used in many food products including baked goods, pie fillings, and bread mixes. Xanthan gum contributes to smoothness, air incorporation, and retention for batters of cakes, muffins, and biscuits since its introduction to the marketplace in the 1960s. Xanthan gum adds volume and moisture, which leads to higher crumb strength with less crumbling. The use of xanthan gum in microwave cake mix applications facilitates better moisture retention, and better stabilization and structure formation which helps produce a more tender and moist cake (Anon, 1989).

Cold or hot process bakery and fruit pie fillings can be improved in texture and flavor release by the addition of xanthan gum. Shelf life can also be extended, as well as syneresis control and stability during freeze-thaw cycles. Xanthan gum is used in wet prepared batters and batter coatings. In prepared batters, xanthan gum acts as a suspension agent and improves gas retention (Sadar, 2004). Shrimp, chicken, fish, and onion rings can use xanthan gum in their batter formulations for consistent adhesion and stabilization (Sadar, 2004). Frequently, xanthan gum is used in salad dressing. The pseudo plasticity of xanthan gum causes it to flow easily when poured and once the flow stops it recovers its viscosity, resulting in cling or adhesion. Small particles of xanthan gum can be added to dry mixes for milk shakes, sauces, gravies, dips, soups, and beverages (Anon, 1989).

Xanthan gum provides stability, syneresis control, and consistent viscosity when exposed to freeze-thaw cycles (Sadar, 2004; Sanderson, 1996). Xanthan has various uses which can also be added to frozen products like whipped toppings, batters, soufflés, gravies, entrees, and retorted products due to the stability over a wide range of temperatures. Although the viscosity of

xanthan gum would be low at high retort temperatures, upon cooling the original viscosity would return. In addition to this reason, the xanthan can improve the filling process and reduce splashing (Sworn, 2000). Syrups and toppings also have a use for xanthan gum. Xanthan gum allows these products to have excellent pouring and clinging properties as well as good stability and uniform suspension of ingredients. In general terms, xanthan thins under shear in the mouth facilitating flavor release (Chinachoti, 1995). The table below shows some food applications of Xanthan Gum.

Table 3

Food Applications of Xanthan Gum

Food Products	Uses	References
Baking goods	Enhance or Pumping, Stabilizer	Garcia-Ochoa et al., 2000
Dairy Products (Ice cream)	Enhance viscosity, Stabilizer	Urlachaer & Noble, 1997
Microwave cakes	Helps in moisture retention, stabilizer	Anon, 1989
Frozen food	Improve in freeze thaw stability	Garcia-Ochoa et al., 2000
Juice drinks	Use as a suspending agent	Garcia-Ochoa et al., 2000 Becker et al., 1998
Salad dressings	Use as a stabilizer, suspending agent and a emulsifier	Garcia-Ochoa et al., 2000
Cheese	Inhibitor	Garcia-Ochoa et al., 2000 Becker et al., 1998
Sausage casings	Formation of film	Urlachaer & Noble, 1997
Gravies and sauces	Thickener, Flavor release heat resistant	Urlachaer & Noble, 1997
Fruit gels	Prevent sticking	Dziezak, 1991
Icing and glazing	Use as adhesive	Becker et al., 1998
Dry mixes	Makes dispersion in cold or hot water easy	Garcia-Ochoa et al., 2000
Syrups	Use a thickener	Garcia-Ochoa et al., 2000
Beverages	Use as stabilizer	Giese, 1995

2.10.3 Using xanthan gum as an ingredient to improve food stability. The polysaccharide finds important uses, especially in the food industry, mainly as an emulsion stabilizer and thickener, but also as a gelling agent in combination with other polysaccharides (Parker, Gunning, & Robins, 1995). The ability of xanthan to thicken and stabilize emulsion systems such as salad dressings is attributed to a weak gel-like structure in solution formed by the gum molecules in the emulsion's continuous phase which prevents the oil droplets from creaming since the gravitational lift on the droplets is less than the yield stress of the xanthan weak gel (Parker, Gunning, & Robins, 1995).

2.11 Whey Protein Isolate

Whey protein isolates are mixtures of globular proteins isolated from whey. They are excellent emulsifiers; it is one of the two major groups of proteins found in milk and a highly digestible source of protein (Agboola, Singh, Munro, Dalgleish, & Singh, 1998). Whey proteins are used in a variety of foods including ice cream, bread, and infant formula. Whey protein has been used in fat replacers for low-fat ice cream and as an ingredient in milk replacement products. Whey protein isolates sometimes hydrolyze to produce small fragments and free amino acids, which are more easily digested (Agboola et al., 1998). Hydrolyzed whey proteins have been used extensively in infant and specialized adult nutritional formulations (Mahmoud, 1994). Stable oil in water emulsions could be formed with whey protein as the sole emulsifier when using appropriate WPH concentration and homogenization conditions (Agboola et al., 1998). The emulsions made with various concentrations of whey protein isolate are most stable at a pH of 7 and less stable at a pH of 5.5 (Hunt, 1994). Hunt also reports that whey protein is a popular dietary supplement for improving muscle strength and body composition and for the prevention of heart disease, diabetes, and age-related bone loss.

Whey protein may aid in the prevention of some hereditary conditions, such as the tendency to develop allergies. It may act as an appetite suppressant and aid in the control of blood sugar. The best known effects of whey protein are its ability to help promote weight loss, increase lean muscle mass, and boost the immune system (Agboola et al., 1998). Whey protein contains high levels of essential and branched-chain amino acid which is known to help people maintain or build muscle tissue. This can be important for athletes, people trying to lose body fat and older adults concerned about maintaining their muscle mass. Whey protein may also help with weight loss by increasing feelings of fullness and maintaining blood glucose at constant levels. It boosts the immune system by helping the body produce an antioxidant called glutathione. Glutathione protects against free radical damage, pollution, toxins, infection, and sunlight exposure. Adding whey protein to the diet may help protect health in people of all ages.

The oil in water emulsion is the basis of many food products and their properties define food quality to a great extent. The most important emulsion properties are emulsion stability, rheological behavior, interfacial properties, interactions, texture, and flavor (Coupland & McClements, 1996). It is documented that the factors affecting the oxidative stability of whey emulsion are the chemical structure of lipids, interfacial characteristics, droplet characteristics, the concentration, size, and the interactions with aqueous phase component of the salt, sugar, and proteins. Lipid oxidation has been extensively studied in bulk fats and oils, as well as in emulsified lipids. According to Hung et al. (1997), various molecules in an emulsion distribute themselves between three different regions which are the interior of a droplet, the continuous phase and the interfacial region and according to their polarity and surface activity. The process of lipid oxidation depends on the type of emulsifier and emulsified oil (Frankel, 1993; Mei et al.,

1999). Varieties of different types of oils have traditionally been used in food emulsions including soybeans, corn, canola, olive, safflower, and sunflower oils (Hui, 1992).

The oxygen-barrier properties of whey protein coatings have the potential to increase the shelf life of foods such as roasted peanuts by reducing lipid oxidation rate (Coupland & McClements, 1996). The high pressure homogenization process of emulsions can produce emulsions with the smallest droplet size, increasing the interaction between the interface (oil in water) and the protein used as emulsifier. However, such a process can unexpectedly produce unstable emulsions. This fact can be attributed to the coalescence of the dispersed phase because of the interaction of the small droplets as a consequence of high pressure leading to aggregation of unfolded proteins. Also, findings show that homogenization at high pressures (500 to 2000 bar) of emulsions (10% w/w soybean oil and 0.5% w/w whey proteins) result in partial desaturation of protein adsorbed at the (oil in water) interface with the exposure of its hydrophobic groups in a similar way to that observed in heating processes. Thus, the improved adsorption of proteins at the interface, caused by high pressure homogenization, contributed to the stability of the system. The Food and Drug Administration (FDA) announced the availability of a qualified health claim for reduced risk of coronary heart disease (CHD) on conventional foods that contain eicosapentaenoic acid (EPA) and DHA omega-3 fatty acids (FDA, 2004).

Different studies on the effects of fish oil emulsion on food products have revealed different results. Rognelien (2010) revealed that untrained panelists were unable to differentiate 0.5% wt/wt fish oil and butter oil in unflavored yogurt (unflavored yogurt was the control). However, they were able to detect oxidized fish oil compared to butter oil or fish oil under the same test conditions. Rognelien's (2010) study was conducted to differentiate among butter, fish and oxidized fish oil at 0.5% (wt/wt) levels in unflavored yogurts. In 1999 Kolanowski,

Swiderski, and Berger conducted a study on the possibilities of fish oil application for food products enriched with omega-3 PUFA. The results of the study revealed that yogurts enriched with fish oil up to 0.3% (0.1% DHA/EPA) were acceptable to a sensory panel. Likewise, Kolanowski et al (1999) compared different food products enriched with some percentage of fish oil (e.g. Fish oil added to milk (0.15%) and flavored yogurt (0.3%) was compared to soy bean oil (1.5%), fat spreads (1.5%), an orange drink (0.3%) and apple-beetroot juice (0.15%)). Results showed that food products supplemented with stronger flavor and sweetener were better at masking the fishy flavor and aroma. Higuchi, Shirai, Suzuki, Kawashima, Tamura (2008) studied the effects of yogurt supplemented with fish oil on plasma lipid and glucose concentrations, and liver lipid contents in mice. Results suggest that plasma triacylglycerol and glucose concentrations are effectively decreased by supplementation of yogurt with fish oil.

Serna-Saldivar, Zorrilla, De La Parra, Stagnitti, Abril (2006) revealed that after 6 days the control bread had higher acceptability compared with the rest of the breads enriched with high levels of DHA or omega-3 oils. The high-enriched fish oil bread was well accepted during the first days of storage but had the least preferred acceptability after 13 days. In 2001, Liu, et al studied the effect of bread containing stable fish oil on plasma phospholipid fatty acids, triglycerides, HDL-cholesterol, and malondialdehyde in subjects with hyperlipedemia. Results of the study showed that in subjects with hyperlipedemia intake of bread containing a small amount of fish oil revealed a significant increase in omega-3 fatty acids, an increase in HDL-cholesterol, and a decrease in triglycerides and MDA, which may reduce the risk of ischemic heart disease.

Furthermore, results of Ramcharitar, Badrie, Mattfeldt-Beman, Matsuo, and Ridley's study, (2005) on muffins revealed that the majority of consumers used in the sensory evaluation rated the control muffin (0 % flaxseed) higher than the flax muffin for appearance, color, flavor,

texture, overall acceptability, and food acceptance. Flaxseed muffin was “neither liked nor disliked” to “liked slightly” in overall acceptability. The purpose of this study is to determine the effect of fish oil emulsion on the physical attributes of shortened cupcake with different whey protein isolate concentrations.

2.12 Hypotheses

Three hypotheses were formulated:

Hypothesis I

a. There will be no significant difference between the control cake and the cake sample supplemented with 250 mg fish oil + 5 % whey protein isolate wt/wt% .
b. There will be no significant difference between the control cake (0% fish oil) and the cake sample supplemented with 250mg fish oil + 10% whey protein isolate wt/wt%.
c. There will be no significant difference between the control cake (0% fish oil) and the cake sample supplemented with 250mg fish oil + 0.5 % xanthan gum + 10 % whey protein isolate wt/wt%.

Hypothesis 2

There will be no significant difference among cupcake characteristics supplemented with different emulsions [i.e. Different treatments: a) 250 mg fish oil plus 0.5% Xanthan gum (wt/Wt) %; b) 250 mg fish oil, plus 5% Whey-protein isolate (wt/Wt) %; c) 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) %; d) 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein (wt/Wt) %; and e) 250 mg fish oil, plus 0.5 % Xanthan gum, plus 10 % Whey-protein (wt/Wt) % see Table 5].

Hypothesis 3

There will be no effect of baked cake refrigeration length of 0, 2 & 5 days on sensory evaluation.

2.13 Objectives

1. To develop an emulsion system of fish oil for a cupcake food product.
2. To evaluate the sensory characteristics and consumer acceptability of cupcake supplemented with fish oil emulsion.
3. To study the storage stability of cupcake supplemented with fish oil during refrigerated storage.

CHAPTER 3

Methodology

3.1 Method and Participants

Ninety untrained panelists participated in this study. Participants were recruited through personal acquaintances from the Department of Family & Consumer Sciences at North Carolina A&T State University. Included in the study were students and employees. The population used in this study was determined using Cochran's (1963) sample size determination formula shown in equation (1) at significant level of 0.05 with the assumption that the population is large. Therefore, assuming $p = 0.5$ (maximum variability), the sample error is calculated to be 11%.

$$n = \frac{Z^2 pq}{e^2} \tag{1}$$

3.2 Apparatus/Materials

Apparatuses used in this study were publicly available. A digital kitchen scale, oven, hand mixer, cimarec mixer, and homogenizer were used in the study.

A digital scale was used to measure the weight or mass of items ranging from kitchen ingredients to laboratory substances. Modern digital scales also provide accurate readings to one milligram and accurately measure products in chemical, physics, and medical research laboratories. A mainstay's digital kitchen scale model no MS19-041-400-33 was used in this study to measure all the recipes and was calibrated to zero before weighing the recipes. The electric oven is a thermally insulated chamber used for the heating, baking, or drying of a substance. A Hotpoint electric oven model no RB532 GOJ2AD (10.6 kilowatt) was used. A kitchen hand mixer was used in recipe mixing. A Black & Decker product, model no. MX217

and 120 volts of 250 Watt was used in this study to ensure mixture of the batter and the emulsion.

A stirrer is an instrument used to stir substances together. Barnstead/Thermolyne Cimarec stirrers, model no. SP131635 and120 volts were used to stir fish oil emulsion recipes before homogenization. Homogenization is an intensive blending of mutually-related substances. Fisher scientific 1800 si power gen with 115 volts homogenizer was used in the study to ensure proper mixture and uniformity of the treated recipe samples. For storage purposes a refrigerator was used to store the cake for five days. A refrigerator is a common household appliance that consists of a thermally insulated compartment and a heat pump. A refrigerator is used to slow down bacteria process or growth of harmful bacteria including pathogens so as to increase the shelf life of food. A Hot Point refrigerator was used to store the baked cake. These cakes were stored for 0, 2, and 5 days maximum before sensory evaluations were conducted.

3.3 Procedure

The procedure for the baking of the shortened cupcake was divided into three different steps which are described in the following sections.

3.3.1 Cupcake basic formulation. Ingredients used in all cupcake formulations included pure granulated sugar, eggs, margarine, milk, fish oil, and flour. All ingredients were purchased from local grocery stores in Greensboro, NC. Ingredients such as xanthan gum and whey protein isolate WPI were obtained from TIC Gum, Belcamp, MD and Main Street Ingredients, La Crosse, WI, respectively. Cake batters were made according to the formula and methods described in www. Joy of baking.com online manual. Cakes ingredients were measured in a bowl as mentioned in Table 4. All ingredients were mixed with a hand mixer, measured sugar and margarine were beaten together until light and fluffy; eggs were added one at a time and

beaten well after each addition. Flour and eggs were alternately added in three additions, at the beginning and at the end with the flour. The mixture of the basic ingredients was divided into six equal portions and was placed separately and labeled from sample one to sample six.

Table 4

Formulations (% wt/w of Ingredients) for Five Cupcake Treatment with 250mg of Fish Oil

Ingredients	Free Oil	Fish oil +0.5% Xanthan	Fish oil +5% WPI	Fish oil+10% WPI	Fish oil +0.5% Xanthan +5% WPI	Fish oil +0.5% Xanthan +10% WPI
Flour	29.31	29.16	27.91	26.64	27.78	26.51
Margarine	16.98	16.89	16.17	15.44	16.095	15.36
Sugar	19.05	19.44	18.61	17.76	18.52	17.67
Eggs	22.54	22.43	21.47	20.495	21.37	20.39
Milk	9.299	9.253	8.855	8.454	8.81	8.411
Xanthan gum	-	0.494	-	-	0.470	0.449
Fish oil	-	2.288	2.288	2.288	2.288	2.288
Salt	0.030	0.029	0.029	0.027	0.029	0.027
Whey protein Isolate	-	-	4.766	9.086	4.757	9.09

3.3.2 Fish oil emulsion delivery system. Ingredients used in formulation of the fish oil emulsion delivery system included whey protein isolate, fish oil, milk, and xanthan gum. All ingredients were measured as shown in Table 3.1.

Whey protein isolate was measured and dissolved in 2% reduced fat liquid milk to create a solution in a bowl. It was then slightly mixed with a cimarec stirrer. Fish oil was then added to the solution with continuous stirring followed by the addition of xanthan gum gradually, until the solution was properly mixed. The emulsion was then mixed using a homogenizer to ensure

proper mixture and uniformity. Each formulation was mixed as described in Table 3.1 from free oil to the fifth treatment and then separately placed on a counter and labeled from sample one through sample six.

3.3.3 Formulation of cupcake batter treatment. The basic ingredients and the fish oil emulsion formulations were matched equally according to their label and mixed together uniformly using a hand mixer. Twelve cupcakes from each treatment were made by evenly filling the batter into circular cake pans. Cakes were baked in a general electric oven at 350^{o} F for 20 minutes. After baking, cakes were cooled to ambient temperature. Once cakes were fully cooled they were bagged in a polyethylene film bag, until testing.

3.4 Sensory Evaluation Procedure

Twelve cupcakes were baked for each treatment and each cake sample for each treatment was divided into eight smaller pieces. All together there were 96 pieces of cake in a treatment and 576 pieces for all cake treatments. Thirty pieces from each cake treatment were evaluated one hour after baking in day zero. Sixty pieces of cake samples were divided into two (30 pieces each), bagged separately and stored in a refrigerator at 37^{o}F for an hour after baking. One bag containing 30 cake samples was taken out of the refrigerator after two days of storage and was allowed to sit for one hour at room temperature before the sensory evaluation. The second bag was allowed to be in the refrigerator at the same temperature for five days. After five days of storage, cake samples were brought out of the refrigerator and were allowed to sit at room temperature for an hour before being used for the sensory evaluation. Thirty panelists participated in each day of the evaluation and each panelist was given six cake samples to evaluate, one sample from each treatment. Each sample was placed in a plastic sample cup, which was then covered and labeled. Thereafter, a plastic sample cup from each treatment was

placed in a zip lock bag and was taken to the evaluation room. Cakes were coded using unique randomized numbers: 456 represented control sample 1; 797 represented treatment 1 sample 2; 214 represented treatment 2, sample 3; 265 represented treatment 3, sample 4; 699 represented treatment 4, sample 5; and 566 represented treatment 5, sample 6. The consumer acceptability study was submitted to North Carolina A & T State University Institutional Review Board (IRB) and was approved as an exemption.

Prior to sensory evaluation processes, as panelists entered into the testing room, they were welcomed, and briefed with the procedures for the sensory evaluation. Any questions asked were answered and the participants who agreed to continue with the evaluation processes were given the sensory evaluation form. Those who declined were thanked for their time and allowed to leave. The researcher gave the panelists instructions on how the sensory evaluation would be conducted. Instructions included number of cake samples panelists have to evaluate at a time (three samples), how to use the water presented in a paper cup to cleanse their palate after each taste of the cake sample and the process of recording their observation of the cake characteristics on the evaluation form. The panelists' task was to the evaluate cake sample characteristics and score the cake based on the 9-point hedonic scale rating on the sensory evaluation form presented at the beginning of the experiment (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely"). The panelist response evaluation form is shown in Appendix B. Panelists' ratings on each cake characteristics (appearance, crust color, taste, tenderness, texture, smell and overall liking) were recorded and represented the data used for the analysis.

3.5 Data Collection

From the sensory evaluation form completed by each panelist, the ratings for each cake characteristic were recorded and tabulated on a spreadsheet using Microsoft Excel ® version 2010. The recorded data were used for the data analyses. IBM SPSS software 7.5 versions 2009 was used for the data analysis to verify the hypotheses stated for each cake characteristics. Scheffé multiple comparisons tests were conducted to confirm the significant differences among cake samples.

CHAPTER 4

Results

4.1 Results

The formulations for each cake sample used in the study are ranged from cake sample 1 to cake sample 6. The mean and standard deviation of panelist rating of cupcake sample (Table 5) are summarized in Appendix A.

Table 5

Cake Sample Formulations

SAMPLES	FORMULATION (wt/wt %)
Sample 1 (SAP1-456)	Control (0% fish oil)
Sample 2 (SAP2-797)	contained 250 mg fish oil plus 0.5% Xanthan gum
Sample 3 (SAP3-214)	contained 250 mg fish oil, plus 5% Whey-protein isolate
Sample 4 (SAP4-265)	contained 250 mg fish oil, plus 10 % Whey-protein isolate
Sample 5 (SAP5-699)	contained 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein
Sample 6 (SAP6-566)	contained 250 mg fish oil, plus 0.5 % Xanthan gum, plus 10 % Whey-protein

Descriptive statistics for appearance, crust color, tenderness, texture, smell, taste, color, and overall liking across the six samples for the three different days showed that on average,

sample 1 (SAP1-456) was rated highest in day zero of testing followed by sample 2 (SAP2-797) and sample 4 (SAP4-265) rated lowest in appearance. Sample 1 (SAP1-456) maintained the lead rating throughout the time tested for appearance (i.e. day zero, day two and day five). However, in the fifth day, sample 2 (SAP2-797) was rated the lowest in appearance. Panelists rated Sample 1 (SAP1-456) the highest in crust color, followed by sample 2 (SAP2-797) and sample 5 was rated the lowest in average in day 0 for crust color of the sensory evaluation. On average sample 2 (SAP2-797) was rated highest in day two followed by sample 1 (SAP1-456) and sample 3 (SAP3-214) was rated the lowest in crust color. On average tenderness in day zero was rated highest for sample 2 (SAP2-797), followed by sample 1 (SAP1-456) and sample 4 (SAP4-256). However, sample 6 (SAP6-566) was rated the lowest in day 2 for tenderness.

Results further showed that sample 2 (SAP2-797) was rated the highest in texture, followed by sample 1, and the least rated cake sample for texture was sample 6 (SAP6-566) in day 0. However, in day two and day five of sensory evaluation, sample 1 was rated the highest for texture. Panelists' ratings across cake samples on the smell revealed that sample 1 was rated the highest for each of the days followed by sample 2. The results of the panelists rating indicated that panelists like cake sample 2 textures more than all other cake samples evaluated in day zero, they liked cake sample 1 for texture in day two and day five of the sensory evaluation. Similar results were also observed on panelists' ratings for the taste. Overall, cake sample 4 (SAP4-265) was rated the lowest in overall liking. Figure 6 illustrates graphically the relationship between panelists' ratings across cake characteristics on day zero.

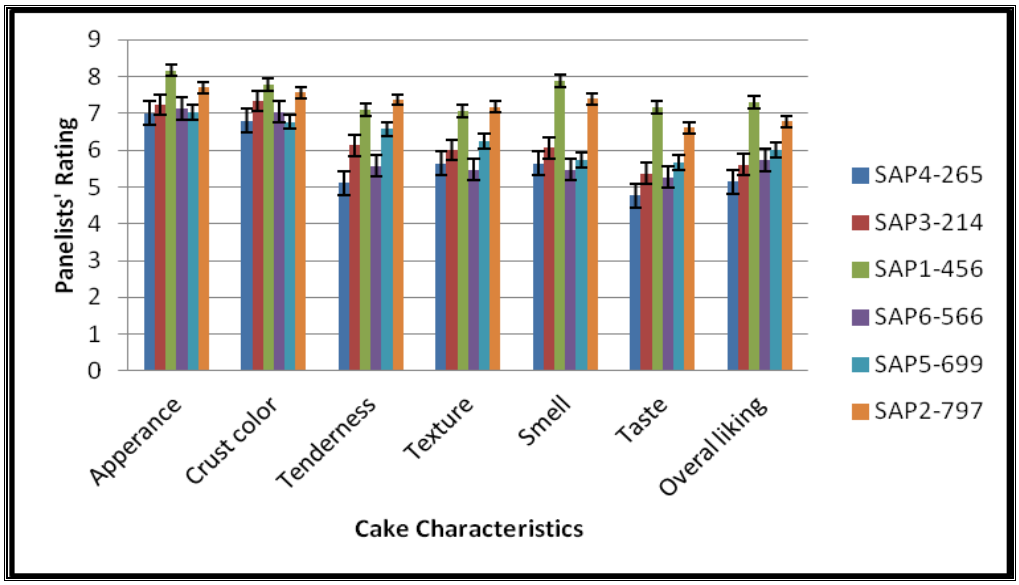


Figure 6. Panelists' ratings in cup cake physical characteristics on day zero.

Figure 7 illustrates the differences in panelists' ratings among cake samples on day 2. Panelists' highest ratings were found for sample 1 and sample 2. This means that sometimes panelists' ratings for sample 2 were the highest and sometimes their ratings for sample 1 were found to be the highest.

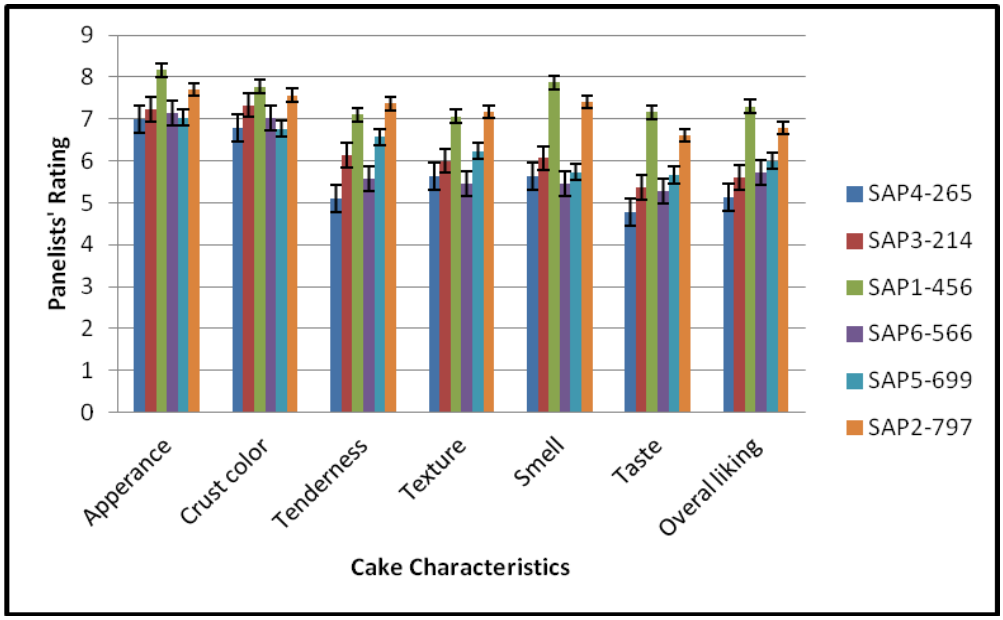


Figure 7. Panelists' ratings in cup cake physical characteristics on day two.

Figure 8 illustrates the differences among cake samples in day five according to panelists' ratings. It is shown in this figure that panelists rated sample 1 the highest among all samples tested across the cake characteristics. However, the differences in the ratings were very close among samples; therefore, inferential statistics were conducted to check for significance of the differences across cake characteristics.

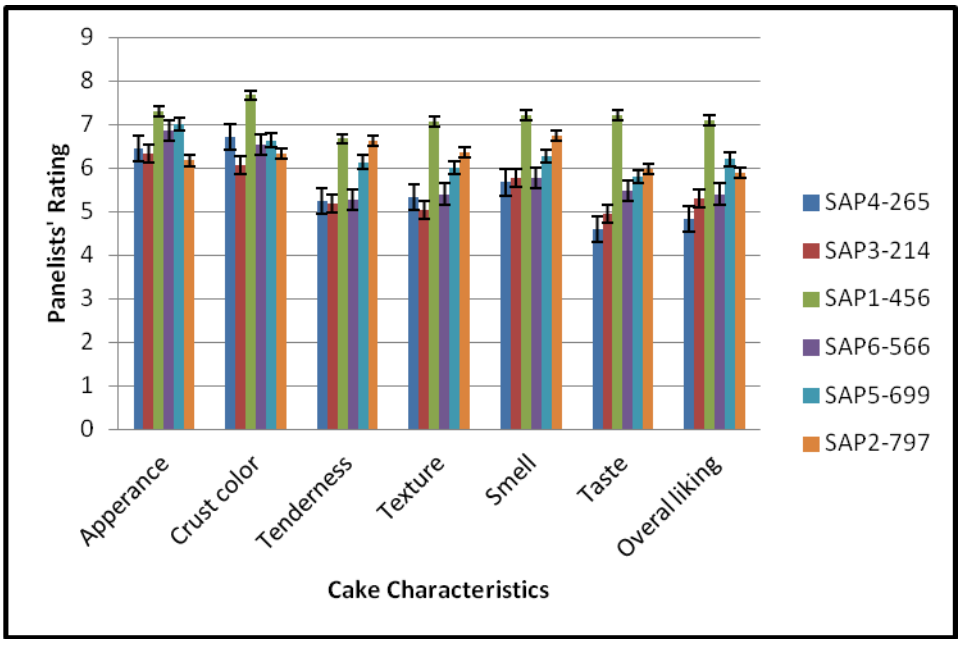


Figure 8. Panelists' ratings in cup cake physical characteristics on day five.

Hypothesis I investigated the differences between the control (0% fish oil) and all treated cakes in overall liking. Results revealed that panelists found significant differences in the comparison between the control (0% fish oil) and cake samples containing 250mg fish oil+5% whey protein isolate wt/wt% ($p < 0.0001$); differences were also found between the control (0% fish oil) and cake samples that contained 250mg fish oil + 10% whey protein isolate wt/wt% ($p < 0.0001$). Differences were also found between the control (0% fish oil) and cake samples that contained 250mg fish oil +0.5% xanthan gum +10% whey protein isolate ($p < 0.0001$). However,

no significant differences were found between the control (0% fish oil) and all other cake treatments.

Hypothesis II stated that there will be no significant difference among cupcake characteristics supplemented with different emulsions. In the analysis of this hypothesis, days of evaluation were not considered as a factor, therefore the following results were found in the analysis:

1. For appearance, results revealed a significant difference in panelists rating between the control (0% fish oil) and cake samples that contained 250mg fish oil+ 5% whey protein isolate wt/wt % ($p = 0.0140$) and between the control (0% fish oil) and cake samples that contained 250mg fish oil + 10% whey protein isolate wt/wt % ($p = 0.0160$). Results revealed no significant difference between any other two cake samples compared. The results indicated that panelists showed more interest in crust color for the control cake sample than for both cake samples that contained 5% and 10% whey protein isolate for appearance.
2. For crust color, results revealed a significant difference in panelists rating between the control (0% fish oil) and cake samples that contained 250mg fish oil +5% whey protein isolate wt/wt % ($p = 0.003$); and between the control (0% fish oil) and cake samples that contain 250mg fish oil + 10% whey protein isolate ($p = 0.040$). Results from the panelist ratings revealed no statistical significant difference between any other two cake samples compared. The results indicated that panelists showed more interest in crust color for cake sample control (0% fish oil) than they did for both cakes that contained 5% and 10% whey protein isolate for crust color.

3. For tenderness, results revealed differences in panelists rating between cake samples that contained 250mg fish oil + 5% whey protein isolate wt/wt% and cake samples that contained 250mg fish oil+ 0.5% xanthan gum ($p < 0.0001$). Differences were also found between cake samples that contained 250mg fish oil+ 5% whey protein isolate and the control ($p = 0.001$). Results also revealed differences in panelists rating between cake samples that contained 250mg fish oil+ 10% whey protein isolate wt/wt% and the control (0% fish oil) ($p < 0.0001$).

   Differences were also found between cake samples that contained 250mg fish oil +10% whey protein isolate and cake sample that contained 250mg fish oil +0.5% xanthan gum +5% whey protein isolate ($p = 0.012$); likewise, cake samples that contained 250mg fish oil + 10% whey protein isolate was found to be different from cake samples that contained 250mg fish oil +0.5% xanthan gum ($p < 0.0001$). The results also revealed a difference between sample control (0% fish oil) and cake sample that contained 250mg fish oil + 0.5% xanthan gum + 10% whey protein isolate ($p < 0.0001$) and also between samples that contained 250mg fish oil + 0.5% xanthan gum + 10% whey protein isolate and samples that contained 250mg fish oil +0.5%xanthan gum +5% whey protein isolate ($p = 0.045$). Results further revealed a significant difference between cake samples that contained 250mg fish oil +0.5% xanthan gum + 10% whey protein isolate and cake samples that contained 250mg fish oil +0.5% xanthan gum ($p < 0.0001$). However, results of the analyses further revealed no statistical significant difference between any other two cake samples compared. The results of this analysis suggested that panelists liked one cake sample's tenderness more than the other when compared at the same time.

4. For texture, results from the panelists ratings revealed a significant difference in the comparison between cake samples that contained 20mg fish oil +5% whey protein isolate and the control (0% fish oil) ($p < 0.0001$); differences were found between cake samples that contained 250mg fish oil +10% whey protein isolate wt/wt% and the control ($p < 0.0001$); differences were also found between cake samples that contained 250mg fish oil +10% whey protein isolate wt/wt% and cake samples that contained 250mg fish oil +0.5% xanthan gum ($p < 0.0001$). Results further revealed a significant differences between cake samples that contained 250mg fish oil + 5% whey protein isolate wt/wt% and cake samples that contained 250mg fish oil + 0.5% xanthan gum ($p = 0.004$); significant differences were also found between the control and the cake samples that contained 250mg fish oil + 0.5% xanthan gum +10% whey protein isolate wt/wt% ($p < 0.0001$). Likewise, a significant difference between cake samples that contained 250mg fish oil + 0.5% xanthan gum+ 10% whey protein isolate wt/w% and cake samples that contained 250mg fish oil +0.5% xanthan gum wt/wt % was revealed from the results ($p < 0.0001$). No statistical significant difference in panelist rating between any other two cake samples compared was found. This indicated that panelists liked one cake sample's texture compared to the other cake samples evaluated at the same time.
5. For smell, results revealed significant differences in the panelist rating between cake samples that contained 250mg fish oil+ 5% whey protein isolate wt/wt% and the control (0% fish oil) ($p < 0.0001$); differences were also revealed between cake samples that contained 250mg fish oil + 5% whey protein isolate wt/wt% and cake samples that contained 250mg fish oil +0.5% xanthan gum wt/wt% ($p = 0.004$).

Results also revealed a significant difference in panelists rating between cake samples that contained 250mg fish oil +10% whey protein isolate wt/wt% and the control (0% fish oil) ($p < 0.0001$); differences were found between cake samples that contained 250mg fish oil +10% whey protein isolate wt/wt% and cake samples that contained 250mg fish oil+0.5% xanthan gum wt/wt % ($p < 0.0001$). Significant differences were also found in panelist rating between the control (0% fish oil) and the cake samples that contained 250mg fish oil +0.5% xanthan gum +10% whey protein isolate wt/wt % ($p < 0.0001$). Additionally, results of the analysis of the panelists rating revealed a significant difference between the control and cake samples that contained 250mg fish oil +0.5% xanthan gum +5% whey protein isolate wt/wt % ($p = 0.004$).

A significant difference was also revealed in panelist rating between cake samples that contained 250mg fish oil +0.5% xanthan gum wt/wt% and cake sample that contained 250mg fish oil +0.5% xanthan gum+10% whey protein isolate wt/wt% ($p < 0.0001$). No statistical significance difference in panelist rating between any other two cake samples was found. The results of this analysis suggest that panelists like one cake sample's smell compared to the other tested at the same time.

6. For taste, the results revealed significant differences in the panelists rating between cake samples that contained 250mg fish oil +5% whey protein isolate wt/wt% and the control (0% fish oil) ($p < 0.0001$); differences were also found in panelists rating between cake sample that contained 250mg fish oil + 5% whey protein isolate wt/wt and cake sample that contained 250mg fish oil +0.5% xanthan gum ($p = 0.029$). The data analysis also revealed differences between cake samples that contained 250mg fish oil +10% whey protein isolate wt/wt % and the control (0% fish oil) ($p < 0.0001$); differences were also

found between cake samples that contained 250mg fish oil + 10% whey protein isolate wt/wt% and cake samples that contained 250mg fish oil +0.5% xanthan gum+ 5% whey protein isolate wt/wt% ($p = 0.042$).

Differences were also found between cake samples that contained 250mg fish oil + 10% whey protein isolate wt/wt% and cake samples that contained 250mg fish oil + 0.5% xanthan gum ($p < 0.0001$); differences were further found between the control (0% fish oil) and cake samples that contained 250mg fish oil +0.5% xanthan gum + 10% whey protein isolate wt /wt % ($p < 0.0001$). Additionally, significant differences were found between the control and cake samples that contained 250mg fish oil +0.5% xanthan gum + 5% whey protein isolate wt/wt % ($p = 0.002$).

Finally, differences were found between cake samples that contained 250mg fish oil +0.5% xanthan gum +10% whey protein isolate wt/wt% and cake sample that contained 250mg fish oil+ 0.5% xanthan gum wt/wt % ($p = 0.038$). Results revealed no statistical significance differences between any other two cake samples compared.

Hypothesis III stated that storage days would not significantly affect cake characteristics. ANOVA results on the control (0% fish oil) revealed a statistical significance effect of storage time ($p = 0.036$) on each cake sample characteristics (on all the treatments and the control (0% fish oil). This means that the number of storage days affected panelists rating of the characteristics of the control sample (0% fish oil). However, no statistical significant effects of storage days on panelists rating of the treated cake samples were revealed. This means that panelists found no difference in the treated cake samples between 2 days of storage and 5 days; between 0 days of storage and 2 days and between 0 days of storage and 5 days.

CHAPTER 5

Discussion, Future Research and Conclusion

5.1 Discussion

5.1.1 Differences between average cake treatments and the control. It was hypothesized in the study that there would be no significant difference between each cupcake enriched with different percentages of fish oil and the control (0% fish oil) in panelist overall liking. Results of the analyses revealed that the control (0% fish oil) was statistically significantly different from the cupcake enriched with 250 mg fish oil, plus 5% Whey-protein isolate (wt/Wt) % (cake sample 3), cupcake enriched with 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) % (cake sample 4), and cupcake enriched with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 10 % Whey-protein (wt/Wt) % (cake sample 5). This means that at alpha level of ($\alpha = 0.05$) panelists preferred control (0% fish oil) compared to cupcake sample 3, 4, and 5. The results of this study aligned with the findings of Ramcharitar, et al (2005), who found in their study that the majority of consumers rated the control muffin (0 % flaxseed) higher than the flax muffin for overall acceptability. However, no statistically significant differences were found between the control (0% fish oil) and the cupcake enriched with 250 mg fish oil plus 0.5% Xanthan gum (wt/Wt) % (cake sample 2) and the cupcake enriched with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein (wt/Wt) % (cake sample 5). These results indicated that at the alpha level ($\alpha = 0.05$), panelists found no significant difference between the control (0% fish oil) and the cake samples supplemented with 250 mg fish oil plus 0.5% Xanthan gum and the cake samples supplemented with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein. However, the health benefits of omega-3 could be taken as the advantages cake samples 2 and 5 had over the control.

5.1.2 Differences between cake samples supplemented with fish oil emulsion. The second hypothesis in this study stated that there would be no significant difference in characteristics among cupcakes supplemented with different emulsions. Results revealed no statistically significant difference among all cake treatments in appearance. Likewise, no statistically significance difference was found in crust color among all cake treated with emulsion. For tenderness, significant differences were found between cake samples supplemented with 250 mg fish oil, plus 5% Whey-protein isolate (wt/Wt) % and cake samples supplemented with 250 mg fish oil plus 0.5% xanthan gum; cake samples supplemented with 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) % and cake samples supplemented with 250 mg fish oil, plus 0.5 % xanthan gum, plus 5 % Whey-protein; cake samples supplemented with 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) % and cake samples supplemented with 250 mg fish oil plus 0.5% xanthan gum; cake samples supplemented with 250 mg fish oil, plus 0.5 % xanthan gum, plus 10 % Whey-protein and cake samples supplemented with 250 mg fish oil, plus 0.5 % xanthan gum, plus 5 % Whey-protein and between cake samples supplemented with 250 mg fish oil, plus 0.5 % xanthan gum, plus 10 % Whey-protein and cake samples supplemented with 250 mg fish oil, plus 0.5 % xanthan gum, plus 5 % Whey-protein. This means that panelists found differences in cake tenderness of the two cakes compared. Cake sample enriched with 250 mg fish oil plus 0.5% xanthan gum was found to be more tender. Meanwhile, no significant differences were found in all other comparisons. For texture, only three comparisons showed statistically significant differences, and no significant differences were found in the other eight comparisons. The combinations that panelists found to be more tender significant differences were cake samples supplemented with 250 mg fish oil, plus 5% Whey-protein isolate (wt/Wt) % and cake samples supplemented with 250 mg fish oil plus 0.5%

Xanthan gum; cake samples supplemented with 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) % and cake samples supplemented with 250 mg fish oil plus 0.5% Xanthan gum; and cake samples supplemented with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 10 % Whey-protein and cake samples supplemented with 250 mg fish oil plus 0.5% Xanthan gum. For smell, significant differences were also found in the comparisons between cake samples supplemented with 250 mg fish oil, plus 5% Whey-protein isolate (wt/Wt) % and (cake samples supplemented with 250 mg fish oil plus 0.5% Xanthan gum); cake samples supplemented with 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) % and cake samples supplemented with contained 250 mg fish oil plus 0.5% Xanthan gum; and cake samples supplemented with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 10 % Whey-protein and cake samples supplemented with contained 250 mg fish oil plus 0.5% Xanthan gum. No significant differences were found in all other combination comparisons. For taste, statistically significant differences were found between cake samples supplemented 250 mg fish oil, plus 5% Whey-protein isolate (wt/Wt) % and cake samples supplemented with contained 250 mg fish oil plus 0.5% Xanthan gum; cake samples supplemented with 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) % and cake samples supplemented with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein, cake samples supplemented with 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) % and cake samples supplemented with contained 250 mg fish oil plus 0.5% Xanthan gum, and between cake samples supplemented with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 10 % Whey-protein and cake samples supplemented with contained 250 mg fish oil plus 0.5% Xanthan gum. Overall, cake sample enriched with 0% fish oil was found best in taste. No statistically significant differences were found between all other combination comparisons. In general, these results indicated that different emulsions had different effects on the cake samples. For each

characteristic cake samples supplemented with 250 mg fish oil plus 0.5% Xanthan gum (sample 2) was found to be significantly different from all other cake samples; this means that cake sample 2 was more preferable for the panelists.

5.1.3 Effects of storage days on cake samples. Hypotheses 3 stated that there would be no statistically significant effects of storage days on sensory evaluation of cupcake supplemented with fish oil. Results of the analysis revealed a statistically significant difference in the control cake between storage day 0 and day 2 of the evaluation ($p = 0.036$). This means that panelists found differences in the control cake (0% fish oil) characteristics between cupcakes stored in day 0 and in day 5. No significant difference was found in the control (0% fish oil) between day 2 and day 5 ($p = 0.1972$). The results of this study aligned with the findings of Serna-Saldivar et al (2006), who found that control breads and breads enriched with high levels of DHA or omega-3 oils acceptability were not significantly different until after 6 days of storage. For cake samples that contained 250 mg of fish oil plus 0.5% xanthan gum, the results revealed no significant differences between storage days in the panelists' evaluations. Also there were no significant differences between storage day 0 and day 2 ($p = 0.0782$), and between day 2 and day 5 ($p = 0.9289$). For cake samples that contained 250 mg fish oil, plus 5% Whey-protein isolate (wt/Wt) %, the results revealed no statistically significant differences between storage day 0 and day 2 ($p = 0.3283$), and between storage day two and day five ($p = 0.69189$). For cake samples that contained 250 mg fish oil, plus 10 % Whey-protein isolate (wt/Wt) %, no statistically significant differences were found between storage day 0 and day 2 ($p = 0.4679$), and between storage day 2 and day 5 ($p = 0.9716$). For cake samples that contained 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein, no statistically significant differences were found between storage day 0 and day 2 ($p = 0.2879$), and between storage day 2 and day 5 ($p = 0.7435$). Finally, for

cake samples that contained 250 mg fish oil, plus 0.5 % Xanthan gum, plus 10 % Whey-protein, a statistically significance difference was found between storage day 0 and day 2 ($p = 0.035$). However, results revealed no statistically significant difference between cake samples stored in day 2 and in day 5 ($p = 0.2808$).

5.2 Future Research

A limited number of people participated in the sensory evaluation processes and those who participated were not trained panelists. Therefore, for future research trained panelists should be used and the number of panelists should also be increased. This might result in different conclusions.

For future research, the cake samples should be stored for additional days so as to determine the day consumer liking would be significantly different from the day it was freshly baked. This may help to determine when the expiration day should be.

For future research, similar emulsion should be used to evaluate different food products so as to determine which food product would maintain its stability at different days of storage.

5.3 Conclusion

The following conclusions could be reached:

1. No significant differences were found in consumer overall liking between the control (0% fish oil) and the treatment 2, and between the control (0% fish oil) and treatment 5. Cake treatment 2 is the cake samples supplemented with 250 mg fish oil plus 0.5% Xanthan gum and cake treatment 5 is the cake samples supplemented with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein. Therefore, it can be concluded that 250 mg fish oil, plus 0.5 % Xanthan gum is a good emulsion for cake baking. For this sample the data analysis revealed no significant differences between the control (0% fish

oil) and the cupcake enriched with 250 mg fish oil plus 0.5% Xanthan gum (wt/Wt) % and cupcake enriched with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein (wt/Wt) %. However, the addition of the fish oil and Xanthan gum stabilized the treated cake samples which made cake samples 2 and 5 the best among all cakes tested in this study. According to Sadar (2004) xanthan gum is used in the food industry for suspension, addition of viscosity and also to stabilize the emulsion system wall. This might be the reason why panelists found no significant differences between the control (0% fish oil) and cupcake enriched with 250 mg fish oil plus 0.5% Xanthan gum (wt/Wt) % and cupcake enriched with 250 mg fish oil, plus 0.5 % Xanthan gum, plus 5 % Whey-protein (wt/Wt) %.

2. Significant differences were found in consumer overall liking between the control (0% fish oil) and the other three remaining treatments. The panelists liked the cupcake supplemented with fish oil.
3. A significant difference was found between the storage days in the control (0% fish oil); however, no significant differences were found between the storage days in treatment 2, 3, 4, and 5. Therefore, it can be concluded that the emulsion in the treated cakes did not have any significant effects on the quality of the cakes' characteristics over five days.

The results showed that the addition of fish oil emulsion systems in cake products maintained consumer acceptability for about 5 days without any changes noticed in the tastes and all other cake characteristics by the panelists. The results of this study aligned properly with the results of Serna-Saldivar et al (2006). It was revealed in Serna-Saldivar et al study that the addition of omega-3 oil to bread did not significantly affect consumer overall acceptability for the first 6 days of storage. As it is documented in Rognlien (2010) report, untrained panelists were unable

to differentiate 0.5% wt/wt fish oil and butter oil in unflavored yogurt (unflavored yogurt was the control). Omega-3 has been added to different food products; however, the application of omega-3 fish oil to cupcake was a new approach to incorporate more of omega-3 fish oil in the diet.

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Appendix A

Descriptive Statistics of Samples across Cake Characteristics

		SAP1 (456)			SAP2 (797)			SAP3 (214)		
		S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
SC1	AVE	8.17	7.30	7.30	7.70	6.97	6.17	7.23	6.17	6.33
	SD	0.87	1.58	1.64	1.18	1.90	2.49	1.55	1.97	2.28
SC2	AVE	7.77	6.97	7.67	7.57	7.00	6.33	7.33	5.73	6.07
	SD	1.07	1.45	1.37	1.17	1.49	2.20	1.40	2.41	1.78
SC3	AVE	7.10	6.57	6.67	7.37	6.50	6.63	6.13	5.13	5.17
	SD	1.45	1.59	1.75	1.33	1.70	1.96	1.89	2.15	2.32
SC4	AVE	7.07	6.47	7.07	7.17	6.20	6.37	6.00	5.20	5.03
	SD	1.29	1.70	1.66	1.29	1.75	2.19	1.72	2.09	2.09
SC5	AVE	7.87	6.43	7.20	7.40	6.43	6.73	6.07	5.20	5.77
	SD	0.97	1.83	1.83	1.07	1.78	1.57	2.03	2.28	2.22
SC6	AVE	7.17	6.37	7.20	6.60	5.77	5.97	5.37	4.80	4.93
	SD	1.37	1.90	1.65	1.71	2.13	2.20	2.13	2.09	2.50
SC7	AVE	7.30	6.37	7.08	6.78	5.93	5.88	5.60	5.05	5.30
	SD	1.26	2.30	1.61	1.78	2.13	2.28	1.96	2.38	2.09
		SAP4 (265)			**SAP5 (699)**			**SAP6 (566)**		
		S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
SC1	AVE	7.00	6.33	6.43	7.03	6.80	7.00	7.13	6.87	6.87
	SD	1.49	1.69	2.16	1.63	1.88	1.78	1.63	1.59	1.76
SC2	AVE	6.80	6.30	6.70	6.77	6.97	6.63	7.03	6.67	6.53
	SD	1.67	1.78	1.93	1.65	1.54	1.65	1.22	1.69	1.93
SC3	AVE	5.10	4.67	5.23	6.57	5.53	6.13	5.57	4.57	5.27
	SD	1.92	2.09	2.01	1.52	1.72	2.06	1.76	1.98	1.96
SC4	AVE	5.63	4.53	5.33	6.23	5.63	6.00	5.47	4.90	5.40
	SD	1.97	2.03	2.11	1.41	1.65	2.15	1.93	2.19	1.99
SC5	AVE	5.63	4.83	5.67	5.73	6.00	6.27	5.47	5.33	5.77
	SD	1.83	2.29	1.69	1.91	1.64	2.08	2.10	1.60	2.18
SC6	AVE	4.77	4.30	4.60	5.67	5.30	5.80	5.27	4.47	5.47
	SD	1.74	2.12	2.04	2.06	2.02	2.22	2.27	1.93	2.22
SC7	AVE	5.13	4.82	4.83	6.00	5.57	6.20	5.73	4.83	5.40
	SD	1.96	2.09	1.84	1.98	1.79	2.16	1.98	1.88	2.18

Appendix B

Sensory Analysis and Evaluation Performance Checklist

Sensory Analysis and Evaluation Performance checklist

Instructions: Prior to performing this sensory, kindly examine the food product provided in the plate. Conduct the following analysis and score in the corresponding sections. When you have completed the study, please discard product, leave pencils and score sheet on the/ counter. Thank you for your participation

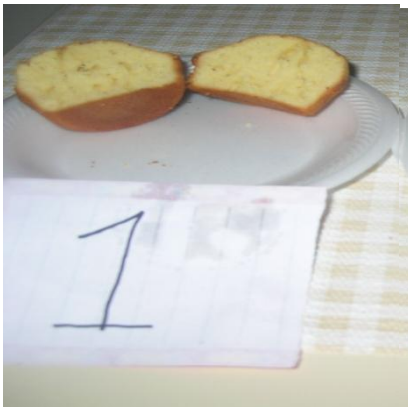
Date----------

Characteristics		Samples				
	456	797	214	265	699	566
Appearance						
Crust color						
Tenderness						
Texture						
Smell						
Taste						
Overall liking						

Scale: 9 = Like extremely
8 = Like very much
7 = Like moderately
6 = Like slightly
5 = Neither like nor dislike
4 = Dislike slightly
3 = Dislike moderately
2 = Dislike very much
1 = Dislike extremely

Appendix C

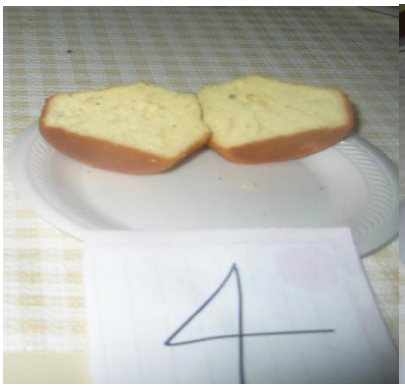
Cup-Cake samples and the Emulsions



Cake Sample 1 (SAP1-456)

Cake Sample 2 (SAP2-797)

Cake Sample 3 (SAP3-214)



Cake Sample 4 (SAP4-265)

Cake Sample 5 (SAP5-699)

Cake Sample 6 (SAP6-566)

All Cake Samples

Batter Ingredients

Batter Ingredients

Cake batter+ emulsion before baking for sample 1, 2, & 3

Cake batter+ emulsion before baking for sample 4, 5, & 6