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Effect Of Bay (Laurus Nobilis) Essential Oil On Physicochemical Properties Of Alaska Pollock (Theragra Chalgoramma) Surimi Nutrified With Salmon And Flaxseed Oils Under Refrigerated Storage

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Biographical Sketch

Ugochukwu C. Anyanwu was born on October 21, 1986 in Owerri, Nigeria to Prof. and Dr. (Mrs.) C.U. Anyanwu. He obtained his bachelor's degree in Food Science and Technology from Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State in 2008. Ugochukwu enrolled at North Carolina Agricultural and Technical State University for his graduate studies (Masters) in Food and Nutritional Sciences program to further his education. While pursuing his masters, he focused his research on product development/formulation of functional food under the advisement of Dr. Reza Tahergorabi. His research was funded by the department of Family and Consumer Sciences at North Carolina Agricultural and Technical State University. While enrolled as a graduate student, Ugochukwu served as a Research Assistant in the Family and Consumer Sciences Department. Upon graduation, he plans to pursue a doctorate degree in food science and subsequently find a placement in the food industry.

Dedication

This research work is dedicated to my parents, Prof. and Dr. (Mrs.) C.U Anyanwu and siblings for their support and inspiration towards achieving a higher education.

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Lastly, and most importantly, I am grateful to my beloved family for their encouragement and patience throughout my studies.

Table of Contents

List of Figures

List of Tables

List of Symbols and Abbreviations

α	Alpha
ALA	Alpha-Linolenic Acid
BEO	Bay Essential Oil
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
ºC	Celsius
DPPH	1, 1-diphenyl-2-picrylhydrazyl
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
EO	Essential oil
FA	Fatty Acids
FAP	Fatty Acid Profile
LA	Linoleic Acid
MDA	Malondialdehyde
ω	Omega
PUFAs	Polyunsaturated Fatty Acids
TBARS	Thiobarbituric Reactive Substances
TBHQ	Tertiary Butyl Hydroquinone
TPA	Texture Profile Analysis
a_w	Water Activity

Abstract

Surimi seafood products are widely accepted and consumed in the U.S. and other parts of the world. In recent time, there has been increased demand for surimi seafood products nutrified with ω-3 polyunsaturated fatty acids (PUFAs) as a result of their reported health benefits, but this may have a negative effect on the storage time of the product due to lipid oxidation of fatty acids (FA). Bay (*Laurus nobilis*) essential oil (BEO) which has demonstrated antioxidant properties was incorporated to the surimi seafood to reduce lipid oxidation thereby extending storage time. The objectives of this study were to (1) evaluate the physicochemical properties (proximate composition, pH, water activity, texture and color) and (2) determine the fatty acid profile (FAP) and oxidation rate of surimi seafood nutrified with ω-3 PUFA-rich oils from flaxseed and salmon and stabilized with BEO during storage time. Alaska pollock surimi gels were formulated at 78% moisture by ice addition. ω-3 oils were added at 5% by replacing ice at 1:1 along with 0 (control), 0.5, 1% BEO, followed by cooking (90^0C for 30min) in hotdog casings, vacuum packed and stored at 4^0C for 6 days. Texture properties of surimi gels were determined by Kramer shear and texture profile analysis. Color values were measured with L*a*b*. FAP was determined with gas chromatography, lipid oxidation with TBARS. Analysis of variances was performed using two-way ANOVA (SAS, version 16.0). Proximate analyses (ash, moisture, protein, and total fat) showed differences ($P<0.05$) in moisture and fat between the treatments containing ω-3 rich oils stabilized with BEO and the control. Whiteness of surimi gels increased significantly with the addition of BEO between treatments and storage time. Lipid oxidation significantly decreased over storage time for treatments with 1% BEO. Addition of BEO and ω-3 rich oils had no detrimental effect on the texture of surimi gels. Significant difference ($P<0.05$) was observed between the FA content of surimi gels treated with flaxseed, and salmon oils and the control. These results suggest that the incorporation of BEO may allow food manufacturers

to nutrify surimi seafood with beneficial ω-3 rich oils without affecting product quality while extending storage time.

CHAPTER 1

Introduction

Surimi is a Japanese term for mechanically deboned fish flesh, washed with water and blended with cryoprotectants. It is an intermediate product used in the production of foods ranging from the traditional kamaboko products of Japan to other surimi seafood products. Alaska pollock (*Theragra chalcogramma*), the first large-scale source of fish in the United States is regarded as the premium fish species for production of surimi seafood products (Tina, Nurul, & Ruzita, 2010). It is the largest white fish biomass in the world, harvested from various habitats in the North Pacific Ocean, covering from Hokkaido (Japan), Kamchatka (Russia), Alaska (United States), to Vancouver Island (Canada).

Surimi has been consumed in North America as "imitation crabmeat" (Park & Lin, 2005) and is a much enjoyed food product in most countries and available in many shapes and textures (Hall, 2011; Park & Lin, 2005). Surimi popularity remains high and continues to grow due to the low-cholesterol, low-fat, and high nutrient content of surimi seafood (Campo & Tovar, 2008).

According to the American Heart Association, cardiovascular disease (CVD) has had an unquestioned status of the number one cause of death in the U.S. since 1921 (American Heath Association, 2009). Omega-3 polyunsaturated essential fatty acids (ω-3 PUFAs), particularly alpha linolenic acid (ALA, 18:3ω3), eicosapentaenoic (EPA, 20:5 ω-3) and docosahexaenoic (DHA, 22:6 ω-3) FAs are associated with multifaceted health benefits, including lowering blood triglycerides and improving cardiovascular health in humans. DHA and EPA are primarily found in fatty fish such as salmon, char and mackerel; whereas ALA is found in plants and seeds.

Current Western diets include high amounts of omega-6 polyunsaturated fatty acids (ω-6 PUFAs) and low amounts of ω-3 PUFAs, with a ω-6/ω-3 ratio at 15-20/1 (Simopoulos, 2002).

Recommended ω-6/ω-3 ratios range from 1:1 – 1:5 to promote the suppression of chronic inflammatory diseases like CVD.

Therefore, in an effort to improve cardiovascular health, health and professional organizations recommend increased consumption of foods rich in ω-3 PUFAs. Although consumers are becoming familiar with the benefits of ω-3 PUFAs, surimi seafood is not fortified with these essential nutrients. Due to its low fat content, nutritional quality and high functional proteins, surimi is a logical vehicle for functional additives such as ω-3 PUFAs. The food products nutrified with ω-3 PUFAs provide a means to achieve desired biochemical effects of these nutrients without the ingestion of dietary supplements, medications or a major change in dietary habits (Tahergorabi, Matak, & Jaczynski, 2014). However, a potential consequence of the ω-3 PUFAs fortification is increased lipid oxidation, which may lead to rancidity, texture and color deterioration, as well as loss of nutrients, especially the ω-3 PUFAs (i.e., ALA, EPA, and DHA).

Various synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been widely used to help meat and seafood preservation. However, over the past few years, increasing consumer demand for more natural “preservative-free” products has led the food industry to consider the incorporation of natural antioxidants in a range of products. The use of natural antioxidants has the advantage of being more acceptable by the consumers as these are considered as ‘‘non chemical”. This antioxidant effect of plant EOs and extracts are mainly due to the presence of hydroxyl groups in their chemical structure (Shahidi, 2000; Shahidi, Janitha, & Wanasundara, 1992).

In the last years, many researchers have evaluated the antioxidant properties of extracts from different plants and vegetables (Chen, Wong, Leung, He, & Huang, 2002; Ibanez et al., 2003). Fresh or dried bay leaves (*Laurus nobilis*) are used in cooking for their distinctive flavor and fragrance. The leaves are often used to flavor soups, stews, braises and *pâtés* in Mediterranean cuisine. The antioxidant activity of BEO is mainly related to 1, 8-cineole, eugenol and methyl eugenol which are considered three effective free-radical scavengers (Politeo, Juki, & Milo, 2007). The antioxidant activity of these molecules has been compared to that from other recognized antioxidant substances. Politeo et al. (2007) indicated that the antioxidant potential of these compounds was comparable to that of BHT and BHA. Nevertheless, the protective activity of BEO against lipid oxidation of nutrified surimi with ω-3 rich oils remains unknown.

The overall objective of this study was to improve oxidative stability of surimi seafood containing salmon and flaxseed oils by incorporation of BEO during refrigerated storage. The specific objectives of our study were to:

1. Evaluate the physicochemical properties of the studied surimi seafood.
2. Determine the fatty acid composition and oxidation rate of the studied surimi seafood.

CHAPTER 2

Literature Review

2.1 Surimi

Surimi is referred to as a refined fish proteins produced through various step-by-step processes including heading, gutting, filleting, deboning, washing, dewatering, refining, mixing with cryoprotectants, and freezing. Minced and deboned fish is washed to remove sarcoplasmic proteins, pigments, lipids, enzymes, and heme compounds resulting in whiter color values, which is an indicator of a higher quality surimi product (Hastings, Keay, & Young, 1990). This production process further increases the concentration of actin and myosin in the final product resulting in a product with better gelation qualities (Hall, & Ahmad, 1997). Excess water is pressed out of the surimi and cryoprotectants are added to prevent protein denaturation during freezing (Hall, & Ahmad, 1997). After production, the surimi can be stored at very low temperatures for later use (Park, & Lin, 2005).

Over the past few decades, production of the processed fish-based product, surimi, has constantly increased. In Europe, France is the first consumer of surimi-based products and approximately 500,000 tons were produced worldwide in 2008 (Guenneugues, & Morrissey, 2005). The history of surimi production started with the Japanese fish processing industry and has expanded into the United States, Korea, and Southeast Asia. Guenneugues, & Morrissey (2005) reported that the surimi production in recent years is estimated at 600,000 metric tons a year, and for the last 25 years consumption of U.S. surimi seafood has increased to 200 million pounds. World demand for surimi, a raw food ingredient, continues to grow due to its unique textural properties, storage properties and high nutritional value (Park, & Morrissey, 2000; Bourtoom, Chinnan, Jantawat, & Sanguandeekul, 2009).

Alaska pollock is mostly used for surimi production and is shaped, textured and flavored to imitate some other fish products (ex. surimi sticks/shredded, crab, lobster, scallops). Mansfield (2003) reported that Alaska pollock (*Theragra charcogramma*) is the third most abundantly harvested species in the world and the main source for surimi production. This is mainly as a result of its high whiteness values and lack of protease activity which contribute to a higher quality product. Its production is divided into two major sections: raw materials are first transformed into the "surimi base" then additives such as starch, egg white, aromas and colorants are added. The product is then cooked and packaged (Lee, 1999). In general, Alaska pollock is favored in the production of surimi because it has fewer color pigments and fewer lipids (Hall, 2011).

Figure 1. A Block of Frozen Surimi (Usually formed in 10kg blocks)

Below (Figure *2*) is a flowchart for surimi manufacturing process. It begins with whole fish, sorting by size and ends with frozen storage.

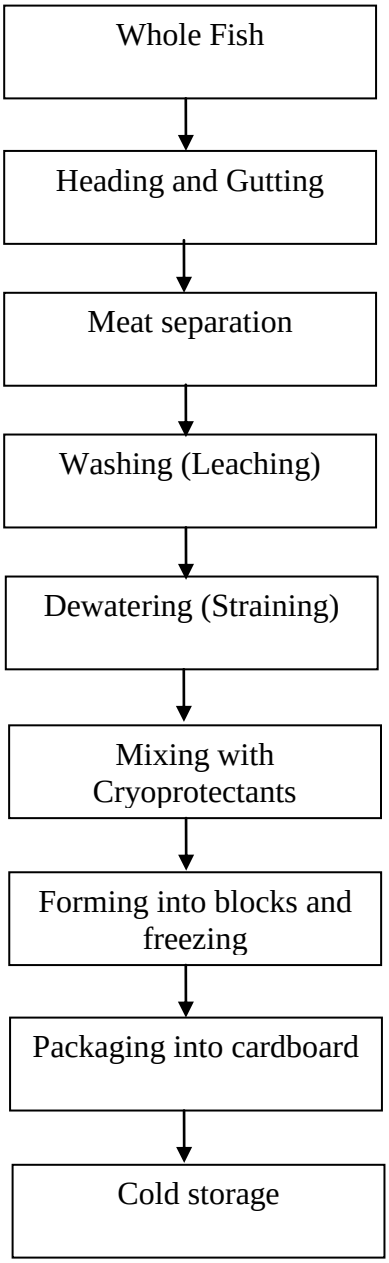


Figure 2. Flow Diagram for Surimi Processing (Park, 2013)

2.2 Omega-3 Polyunsaturated Fatty Acids (PUFAs)

Fat, as reported by (Burr, & Burr, 1929), is an essential component of the diet and the fatty acids have different roles in the human body. Bjerregaard, & Dyerberg (1988) reported that Danish researchers in the early 1970s discovered that Greenland Inuit who consumed large amounts of marine lipids had lower cardiovascular mortality when compared to Danes who consumed lower amount of these lipids. This discovery hence triggered new research into the role of long-chain polyunsaturated fatty acids in the development of cardiovascular disease and its beneficial effect when incorporated into foods. This long chain polyunsaturated fatty acids (PUFAs) can be divided into three groups: omega-3 (ω-3) PUFAs, omega-6 (ω-6) PUFAs and omega-9 (ω-9) PUFAs. The intake of ω-3 PUFA in industrialized countries is about 4-10 percent; hence, several bodies have issued PUFA guidelines to encourage more consumption of ω-3 fatty acids to optimize its health benefits. Alpha linolenic acid (ALA, 18:3ω3) is the essential FA precursor for docosahexaenoic acid (DHA, 22:6 ω3) and eicosapentaenoic acid (EPA, 20:5n3). Humans are unable to synthesize ALA *de novo* as a result of the lack of delta (Δ) 15 desaturase enzymes, which insert a double bond at carbon 15 from the delta end (Gebauer, Psota, Harris, & Kris-Etherton, 2006). Thus, ALA is considered an essential FA and must be obtained through the diet.

According to the National Research Council (1993), flaxseed oil contains the highest concentration of ALA among plant-derived oils, and fish as well as other marine animals are the best sources of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) (Anselmino, & Hornstra, 2000). Oomah, & Maza (1998) have shown that flaxseed oil reduces hypertension, cholesterol, inflammatory markers, blood pressure, triglyceride level and cardiovascular disease. Weber, & Raederstorf (2003) reported that several mechanisms have been suggested to explain

the preventive effect of ω-3 PUFA on cardiovascular diseases. Furthermore, they stated that ω-3 PUFA can reduce triglyceride levels by lowering hepatic triglyceride synthesis and by decreasing the release of triglyceride-rich very low-density lipoproteins (VLDLs) into the blood. Hypertension is another important cardiovascular risk factor. High doses of ω-3 PUFA have been shown to reduce hypertension by influencing membrane fluidity and the balance of the prostanoids that control the constriction and dilation of the small arteries and arterioles. Other studies have shown that ω-3 LC PUFA have anti-aggregant activity (Hirai et al., 1989). Dietary sources of EPA and DHA are primarily marine sources (i.e., algae and fatty fish). Juturu (2008) went further to state that ω-3 PUFAs also improve endothelial functions, reduces vasoconstriction, platelet aggregation and the risk of sudden cardiac death. Hence, there is an increasing interest in the fortification of food products with ω-3 PUFAs.

The Food and Drug Administration (FDA) in 2004 approved a health claim for reduced risk of cardiovascular diseases for foods containing ω-3 PUFAs, mainly EPA and DHA. This provided a marketing leverage for functional foods fortified with ω-3 PUFAs. Lanier, Martin, & Bimbo (1988) reported that since surimi seafood comprises formulated food products associated with marine sources of wide acceptance, it is therefore a suitable vehicle for increasing the consumption of ω-3 PUFAs without the need for dietary supplements in a pill or capsule form. Apart from the nutritional value of these oils, they also help to improve the texture and increase the whiteness of surimi seafood and prevent sponge-like texture development during extended frozen storage and reduce brittleness. They have the ability to replace water by 1:1 up to 6% without changing the shear stress and shear strain values.

2.3 Lipid Oxidation

Lipid oxidation serves as a mediator of important processes in living biological systems. It is one of the major causes of deterioration in cold stored fish muscle and negatively affects color, odor and flavor, protein functionality and conformation, and overall nutritional content of fish muscle (Pearson, Gray, Wolzak, & Horenstein, 1983). This deterioration in quality is due to the high content of PUFAs contained within fish muscle along with highly active pro-oxidants (Hultin, 1994). Notable reactants in lipid oxidation are oxygen and unsaturated fatty acids. Oxidized lipids in lipid-protein systems are known to induce polymerization of proteins resulting in decreased solubility and formation of colored complexes (Desai, & Tappel, 1963).

Triacylglycerides (fats and oils) are largely removed during surimi manufacture by flotation, aided by mechanical action and possible melting/softening. Most of the depot fat is removed when fish are headed, gutted, and skinned because fish generally deposit most of their fat in these regions. There are, however, a small percentage of membrane phospholipids in fish muscle that is difficult to remove by washing. These phospholipids are highly unsaturated and often in contact with muscle heme iron and are therefore very sensitive to spoilage by oxidation. Such oxidation causes off-flavors and may hasten denaturation of the myofibrillar proteins (Lanier, 2000). Lipids in surimi are even more unstable if pro-oxidants, such as iron (e.g., from water pipes, machinery, or residual heme proteins), are present. Washing and mincing procedures generally incorporate a large amount of oxygen into the surimi, making lipid oxidation even more likely.

With the addition of ω-3 PUFAs to surimi seafood, there is an increased risk of oxidation which may be due to the fact that PUFAs are unstable and more prone to oxidation. The production of volatiles is influenced by the degree of unsaturation of the oil (Pérez-Mateos,

Boyd, & Lanier, 2004). Lipid oxidation induces formation of an array of products, thereby directly or indirectly decreasing the sensory quality of fish and fish products (Jacobsen, 1999). Saeed, & Howell (2002) reported that oxidized lipids also interact with proteins inducing modification of textural properties. Also, Murakawa, Benjakul, Visessanguan, & Tanaka (2003) reported that oxidized lipids can interact with proteins causing denaturation, changes in functional properties, polymerization, and brings about an adverse effect on the quality of surimi products.

2-Thiobarbituric Acid Reactive Substances (TBARS), as reported by Botsoglou et al. (1994), are naturally present in biological specimens and composed of lipid hydroperoxides and aldehydes which increase in concentration as a response to oxidative stress. The sensitivity of measuring TBARS has made this assay the method of choice for screening and monitoring lipid peroxidation which is a major indicator of oxidative stress. TBARS assay values are usually reported in malonaldehyde (MDA) equivalents, which is a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides. This assay is well-recognized and an established method for quantifying lipid peroxides.

2.4 Bay Essential Oil

Over the past years, and even recently, there has been great public concern about the safety of synthetic antioxidants (e.g. BHT, BHA and TBHQ) in food preservation besides health implications. These synthetic antioxidants are known to have toxic and carcinogenic effects on human and food systems (Siddhuraju, & Manian, 2006). Synthetic antioxidants may cause liver swelling and influence liver system activities and cerebro-vascular diseases (Choi, Jeong, & Lee, 2007). As a result, there is a strong need for effective and safer antioxidants based on natural sources, as alternatives, to prevent the deterioration of foods. Literatures show many reports of

extracts from natural sources that have demonstrated strong antioxidant activity (Descalzo, & Sancho, 2008). Descalzo, & Sancho (2008) reported that essential oils (EO) and extracts from botanical materials are known to have varying degrees of antioxidant activities. Some of these EOs and extracts have been reported to be more effective than synthetic antioxidants (Mimica-Dukic, Bozin, Sokovic, & Simin, 2004).

Bay (*Laurus nobilis*) is an evergreen tree of Luraceae family which is from the Mediterranean region. Its leaves have seen application in culinary and food industry as a spice and flavoring agent. It is also popular in the field of herbal medicine for its antifungal, antibacterial, anti-diabetes and ant-inflammatory properties (Fang et al. 2005). Because of the strong, spicy aroma of the essential oil of bay leaves as well as its dried form, they are therefore widely used as flavor enhancers for foods such as meats, soups, sauces, and confectionery (Marion, Audrin, Maignial, & Brevard, 1994). The chemical composition of bay leaf EOs from different origins has been studied by different researchers and in all cases, 1, 8-cineole was found to be the major component with percentages ranging between 31.4% and 56% (Pino, Borges, & Roncal, 1992); followed by R-terpinyl acetate, linalool, and several monoterpene hydrocarbons such as *â*-pinene and sabinene. Benzene compounds (eugenol, methyl eugenol, and elemicin), present in percentages ranging between 1% and 12%, are responsible for the spicy aroma of bay leaves and are important factors determining the sensory quality of bay leaves (Borges, Pino, & Sa´nchez, 1992).

Bay, in addition to its antioxidant properties, also interests researchers as a result of its possible antimicrobial quality. Dadalioglu, & Evrendilek (2004) studied the antibacterial properties of bay EO using four different foodborne pathogens (*Staphylococcus aureus, S.* Typhimurium, *Listeria monocytogenes* and *E. coli*.O157:H7), they discovered strong

antibacterial activity in all the cases. Cherrat et al. (2014) introduced hurdle technology where EO extracted from bay were combined with physical methods (mild heat treatment, pulsed electric fields and high hydrostatic pressure) to study its antimicrobial activities. The antimicrobial activity was measured using agar disc diffusion method. This combined method showed strong antimicrobial activity compared to previous individual studies. Studies conducted by Da Silveira et al. (2014) using Tuscan sausages, which were reported to be affected by foodborne pathogens, were subjected to tests on antimicrobial activities using bay EO. The application of this EO reduced the amount of pathogens which resulted in an increase of shelf life by two days.

2.5 pH and Water Activity

The growth of microorganisms in foods can be affected by many factors, mainly temperature, nutrients, oxygen content, pH value, and water activity (a_w) (Olsen, MacKinon, Goulding, Bean, & Slutsker, 2000). Therefore, controlling growth of microorganisms and production of toxins in foods is the main approach to preserving quality and ensuring safety of products. The presence of competing microorganisms as well as naturally occurring anti-microbial substances are other factors which may also influence growth of microorganisms in foods.

Myofibrillar proteins of various fish species are most stable at neutral pH. It is observed that the pH of minced fish and laboratory-prepared surimi samples increased during the storage and this is possible due to the production of amines and other basic products. Fukuda, Yamaguchi, Sakukida, & Kawamura (1981) in his work used ATPase activity to measure denaturation rate with respect to pH of mackerel actomyosin during frozen storage. Results showed that denaturation rate increased rapidly when pH was lower than 6.5. He further stated

that gel forming ability also decreases with reduction in pH. Lanier (1992) reported that pH of surimi relates to the water-holding and gel-forming properties of cooked gels. Changes in pH during frozen storage seem to be species dependent. Freshness of the fish when processed can also affect the pH of the surimi (Huss, 1988). Wicker, Lanier, Hamann, & Akahane (1986) stated that pH has a significant effect on the texture properties of various protein gels. They further went to state that alkaline pH was found to improve the texture properties of fish protein gels in terms of increasing gel strength and/or elasticity.

Water activity is one of the factors that determine the growth of microorganisms in surimi. Water activity is a measure of water in food that is available for microbial growth and chemical reactions (Aberoumand, 2010). Reducing the water activity in surimi seafood generally results in retardation or inhibition of microbial growth. Most bacteria, including many foodborne pathogens, require a minimum water activity of 0.93 to grow. However, some bacteria, including *Staphylococcus aureus*, are salt tolerant and can grow at water activity as low as 0.86. Certain processes, including dehydration, refrigeration, freezing, and addition of preservatives, can be used to inhibit growth of microorganisms. These processes can either be used alone or combined together to increase the shelf life of products. Surimi seafood can be formulated to contain low water activity (<0.85), high salt content (>20%), or low pH (<4.6) for extended storage (Jay, 2000).

2.6 Texture

Texture remains one of the ultimate criteria of product acceptability by the consumer. Food industry efforts are to develop the proper texture for food products (Rohm, 1990; Sundaram, & Ak, 2003). The International Organization for Standardization (1992) defines the texture of food as the rheological and structural attributes of a food product which is perceived

by human senses. There are different methods that can be used to measure texture, each of which provides slightly different information: Texture Profile Analysis (TPA), Kramer shear.

Texture is one of the most important factors governing the quality of surimi as it is with other foods. It is a major component when measuring the functional characteristics of raw surimi materials, as well as the properties of the finished surimi seafood product and effects of manufacturing conditions. The gel-forming properties of surimi make it a valuable texture-building agent in formulated muscle foods. This however, goes a long way to determine consumers' preference and acceptance of processed foods as they always go for soft textured foods. There are different methods which can be used to measure texture, each of which provides different information: Texture Profile Analysis (TPA), and Kramer shear, which are considered empirical tests that characterize results so that they can be directly related to overall acceptance or hedonic ratings (Kim, Park, & Yoon, 2005).

2.6.1 Texture Profile Analysis

The texture profile analysis quantifies specific characteristics that are directly related to the overall acceptance of surimi seafood and has been widely used for the empirical determination of a number of textural attributes of muscle foods and surimi gels. These specific attributes according to Mallikarjunan (2006), include: cohesiveness, springiness, chewiness, resilience, gumminess and hardness. Hardness is measured as the maximum force (N) detected during first compression. Cohesiveness is the ratio of the positive force during the second compression to the positive force during the first compression, and it determines the deformation of a material before it breaks. Gumminess is the product of hardness and cohesiveness while springiness is determined as the ratio of the distance from the second area to the second probe reversal over the distance and shows the ability of a material to recover its original shape after

the removal of applied force. Chewiness is a measure of the energy required to chew a solid food to the point adequate for swallowing and is the product of gumminess and springiness. Resilience shows the degree of how well a sample recovers from deformation in relation to speed and force applied (Alvarez, Canet, & Lopez, 2002). Apart from hardness, it is worthy to note that cohesiveness, gumminess, springiness, resilience and chewiness have no units.

TPA test involves repeatedly compressing a bite-size piece of food, a cube approximately 2cm, to 25% of its original height, between two parallel surfaces, recording force versus displacement. This process imitates the action of the human jaw. From the resulting force–time curve, the aforementioned textural attributes that correlate well with the sensory evaluations of those parameters are determined (Szczesniak, 1986).

2.6.2 Kramer Shear Force

The Kramer shear is a typical system that has 6 shear blades which are 3.2 mm thick and separated by a distance equal to its thickness. To carry out this test, the sample holder is filled with the food of 8cm in length, and then shear blades are forced through the material until they pass through the bars in the bottom of the sample container. Force on the ram holding the blades is measured over time and correlated to the firmness of the product. Parameters usually measured include maximum force at a given sample weight, slope, and energy of the force-deformation curve. Some food products do not display a linear relationship between maximum force and sample weight. Therefore, it has been advised to use a constant weight of sample in the test cell unless a linear relationship is demonstrated between sample weight and maximum force for that food (Bourne, 2002).

2.7 Color

According to Froning (1995), color is an important meat quality which greatly affects consumers' preference. The heme pigment – myoglobin and hemoglobin are responsible for the color of meat, with myoglobin as the determining factor for meat color because the hemoglobin (blood pigment) is mainly removed after slaughter. Color is seen as another important feature of surimi seafood. The three color hue values commonly measured in the surimi seafood industry are L*, a*, and b* with color quality determined by whiteness (Park, 1995). In this regard, the moisture content of the surimi sample is an important factor influencing the color measurement. Hence, if the sample has more water content, because of greater light reflection, it will have higher lightness value.

The L* indicates lightness; a* and b* are the color coordinates. While a* is the red/green axis ("+" being toward the red and "–" being toward the green), b* is the yellow/blue axis ("+" being toward the yellow and "–" being toward the blue). Park (1995) further reported that the a* values of pollock and whiting gels were consistent regardless of moisture contents, cooking/setting conditions, sample size, or frozen storage. Wang, Martinez, & Olsen (2009) stated that about 40-50% of the total fish harvested around the world is made up of dark-muscle species. They went further to say that many of these could be utilized as raw material for surimi based products if the flesh color could be lightened. Lightness in fish flesh can be estimated by the "L value," which will be higher for white-fleshed fish than for dark-muscle species (Wang et al., 2009). Lanier (2000) associated lower L-values with a high concentration of heme proteins in blood (hemoglobin). Lanier also noted that for complete removal of the heme containing substances from the muscle during refining, the heme proteins are required to be maintained in a nearly "native or undenatured" state. If the protein is denatured, the heme component will bind to

myofibrillar proteins and leave the surimi darkened. Vegetable fat-based substances and hydrophilic colloids regarded as whitening agents have been tested and known to whiten fish flesh and the resultant surimi. Lanier (2000) also claimed that the addition of sodium tri-polyphosphate to a bleaching solution had a synergistic effect on both color and texture; provided that the temperature was kept below 15^0C.

CHAPTER 3

Methodology

3.1 Objective 1

3.1.1 Surimi

Frozen blocks of Alaska pollock (*Theragra chalcogramma*) surimi containing cryoprotectants (4% sugar, 5% sorbitol, 0.15 g/100 g of sodium tripolyphosphate and 0.15 g/100 g of tetrasodium pyrophosphate) were purchased from Trident Seafoods Corps. (Seattle, WA.). The frozen blocks were shipped overnight in large coolers surrounded with ice to Food and Nutritional Sciences Laboratory at North Carolina Agricultural and Technical State University. On arrival, the frozen surimi was cut into approximately 1000g blocks, individually vacuum packed and stored frozen at -80^0C until used in surimi paste preparation.

3.1.2 Surimi Paste Preparation

Surimi paste was prepared using a procedure described by (Jaczynski, & Park, 2004) with slight modifications. Frozen surimi blocks (~1000 g) were partially thawed at refrigeration temperature (4°C) overnight. Blocks were then cut into cubes (2-3 cm), weighed (~833.33g) and chopped in a universal food processor (UM 5 universal, Stephan Machinery Corp, Columbus, OH, USA) equipped with cooling jacket. Surimi was chopped at low speed (1,800 rpm) for 1 min. A surimi paste was obtained by adding 2 g/100g of NaCl and chopping continued at low speed (1,800 rpm) for 30sec. Moisture content of surimi was adjusted to 78% by adding ice before continuing to chop at low speed (1,800 rpm) for 1 min. The ω-3 PUFA-rich oils (flaxseed and salmon oils) were obtained from Jedwards International Inc. (Quincy, MA) and were added at 5 g/100g by replacing ice (1:1) added to the surimi paste. Bay essential oil (New Directions Aromatics, Cheektowaga, NY) was also added at 0.5 and 1% to each treatment except the control

samples. The blend treatment was prepared as (flaxseed: salmon, 4:1).The chopping continued under vacuum (0.5 bar) and at high speed (3,600 rpm) for 3mins. The paste temperature was controlled between 1–4 °C during chopping. Surimi pastes were prepared in 1 kg batches. The last chopping step resulted in the formation of the final surimi paste.

3.1.3 Surimi Gel Preparation

The prepared paste was packed into a polyethylene bag and vacuum sealed using a vacuum machine (Vacu-Fresh TC-420-F-G-C; CA, USA) to remove air pockets. Then surimi paste was placed into a sausage stuffer (The Sausage Maker, Buffalo, NY) and force applied to stuff the surimi paste into plastic hotdog casings (Lem Products Direct; West Chester, OH) (diameter:2.6 cm, length: 17.5 cm), then tied with knots. The stuffed "hotdog-like" surimi paste were then cooked in water bath at 90 °C for 30mins and subsequently cooled in an ice bath for 20mins. After cooling, the gel was extruded from the hotdog casing, vacuum sealed and stored for 6 days at 4 $^{\circ}$C for laboratory analysis. Prepared gel was used to determine proximate composition, pH, water activity, texture properties, color, lipid oxidation and fatty acid profile.

3.1.4 Proximate Composition of Surimi Gels

Moisture content, crude protein content, total fat, and ash content were determined for surimi gels. Each parameter was determined using about 2g of surimi sample. For moisture determination, sample was placed on an aluminum dish (Fisher Scientific Co., Fairlawn, NJ) and oven dried at 105°C for 24 h (Association of Official Analytical Chemists, 1995). Crude protein was determined by Kjeldahl assay and expressed as g/100 g (wet weight basis) (AOAC, 1995). Ash content was performed by incinerating samples in a muffle furnace at 550°C for 24h and expressed as g/100 g (wet weight basis) (AOAC, 1995). Total fat content was determined

according to the Soxhlet extraction method and expressed as g/100 g (wet weight basis) (AOAC, 1995). All proximate analyses are reported as mean values of at least three replicates.

3.1.5 Change in pH Value

The pH values were measured using a hand-held pH meter (Oakton, Illinois, U.S.A) and the probe was calibrated before each measurement (Xu, Xia, Yang, & Nie, 2010).

3.1.6 Water Activity (a_w) Measurement

The water activity of the surimi gel was measured at 25°C (±0.2°C) using an electronic dew-point water activity meter (Aqualab Series 4 model TE, Decagon Devices, Pullman, WA). The a_w meter was equipped with a temperature control to maintain a constant temperature. The measurements were performed in triplicate and mean values ± standard deviation are reported.

3.1.7 Texture Properties of Surimi Gels

Two methods for determination of textural properties were employed in this study: texture profile analysis (TPA) and Kramer shear test, each characterizing different textural parameters. These methods provide a comprehensive understanding of textural properties of surimi gel, hence their use in the present study. Surimi gels cut into cylindrical shapes (length = 8.0 cm, diameter = 2.6 cm) per treatment were subjected to Kramer shear test using a texture analyzer (Model TA-XT2, Texture Analyzer, Texture Technologies Corp., Scarsdale, NY) with a Kramer cell attachment consisting of five 3.0-mm thick and 70-mm wide shear blades passing through a cell with a corresponding number of slots. The weight of each individual gel sample was recorded and gel placed under the blades in the Kramer cell. Shear force was measured at a 120mm/min crosshead speed and expressed as maximum peak force (N peak force/g of gel sample). The texture profile analysis was performed according to Bourne (2002) with some

modifications. At least six cylindrical gels (height = 2.50 cm, diameter = 2.6 cm) per treatment were subjected to two-cycle compression (50% compression) using the texture analyzer equipped with a round 70-mm diameter TPA plate attachment moving at a speed of 90mm/min. from the resulting force-time curves, hardness, cohesiveness, springiness, gumminess, chewiness, and resilience were determined as defined by Bourne (2002).

3.1.8 Color Properties of Surimi Gels

The surimi gel samples were cut to 2.5cm in length and used to determine the color of the samples. The color properties of surimi gels were determined using a Minolta Chroma Meter CR-400/410 colorimeter (Konica Minolta Co. Ltd., Osaka, Japan) calibrated with white calibration plate (L^* = 97.57, a^* = -1.08 and b^* =1.25) supplied by the manufacturer which was placed under the orifice of the instrument (Chen, & Jaczynski, 2007a; 2007b). Using CIE (Commission Internationale d'Eclairage of France) color system, the L* (lightness) a* (red to green) and b* (yellow to blue) tristimulus color values were determined (Lanier, 1992). However, at intervals of measuring the color, the surface of the colorimeter was lightly patted dry with kimtech wipes. This is to minimize the 'shininess' effect, caused by surface moisture, which would artificially increase the lightness and whiteness value recorded. At least 3 replicates were performed per treatment and readings were taken on day 0, 3, and 6 at room temperature. Whiteness of gels was calculated by the following equation (National Fisheries Institute, 1991; Taskaya, Chen, & Jaczynski, 2010):

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

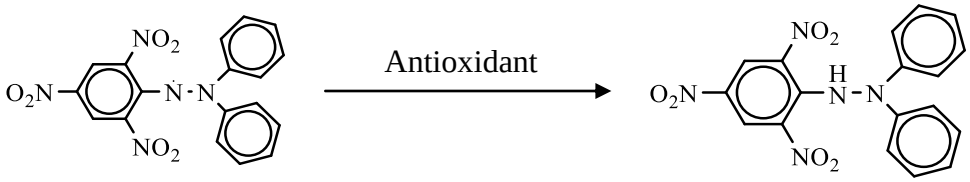
3.2 Objective 2

3.2.1 Lipid Oxidation of Surimi Gels

Oxidative rancidity of surimi gel was measured by 2-thiobarbituric acid reactive substance (TBARS) assay of malonaldehyde (MDA) as described (Chen Nguyen, Semmens, Beamer, & Jaczynski, 2008; Jaczynski, & Park, 2003). MDA is a major compound generated from the oxidation of fatty acids. The absorbance was measured at 535 nm using a Genesys spectrophotometer (model 10S UV-Vis; Thermo Fisher Scientific Inc., Shanghai, China). The TBARS values were calculated using molar absorptivity of MDA (156 000 M^{-1} cm^{-1}) and results reported as mg MDA/kg of sample. The TBARS values are reported as mean values ± standard deviation of at least three replicates for days 0, 3 and 6.

3.2.2 DPPH Assay of Bay Essential Oil

1, 1-diphenyl-2 picrylhydrazyl (DPPH•) scavenging activity is an assay to test the capacity of a substance to neutralize free radical either by donating electron or hydrogen. This is based on the reduction of DPPH radical to non-radical form by an antioxidant. DPPH• is a stable free radical because the unpaired electron spreads out over the whole molecule. The solution of this radical is characterized by purple color with maximum absorption at 517nm. When DPPH• radical is reduced by accepting hydrogen or electron from an antioxidant, the color of the solution becomes yellow which is determined by spectrophotometer (Molyneux, 2004).



1, 1-diphenyl-2 picrylhydrazyl (free radical) 1, 1-diphenyl-2 picrylhydrazine (nonradical)

Figure 3. Reduction of DPPH radical to non-radical form by an antioxidant

DPPH assay was performed using a method described by Yu et al. (2002) with some modifications. Different concentrations (%, v/v) of BEO were prepared. BEO was prepared in 0.00625, 0.0125, 0.025, 0.05, 0.1, 0.5 and 1 (%, v/v). BEO concentrations were dissolved in methanol. 100 µL of essential oil and 900 µL of 70 µM DPPH• solution were mixed in a tube. Methanol was used as blank. After mixing this solution, they were kept in dark for 30 min at room temperature. The reaction was measured by using a Genesys spectrophotometer (model 10S UV-Vis; Thermo Fisher Scientific Inc., Shanghai, China) at 517nm. The experiment was performed in triplicate. Data collected was calculated as % radical scavenging activity using the following equation:

% scavenging activity = $[1 – (Abs_{sample}/Abs_{control})] \times 100$

$Abs_{control}$ = Absorbance of DPPH solution

Abs_{sample} = Absorbance of essential oil

3.2.3 Fatty Acid Profile (FAP) of Surimi Gels

Fatty acid profile (FAP) as described by Chen et al. (2008) was determined for surimi gels by extracting lipids with acid hydrolysis into ether followed by methylation to fatty acid methyl esters (FAMEs). The FAMEs were measured quantitatively using a capillary gas–liquid chromatograph (GLC) (Model 7890A equipped with a 7683B series injector, Agilent Technologies, Santa Clara, CA) against an internal standard (C19:1). Helium was used as carrier gas at 0.75 ml/min flow rate and a 200:1 as split ratio. The initial temperature of 100^0C was held for 4 min and then increased to the final temperature of 240^0C at a heating ramp of 3^0C /min. The final temperature was held for 15 min. The injector and detector temperatures were 225^0C and

285^0C, respectively. The data are reported as mean values (±standard deviation) of at least three replicates and the mean values are expressed as percent of a fatty acid in total fatty acids.

3.3 Statistical Analysis

The experiments were independently triplicated (n=3). Results were reported as mean values ± standard deviation (SD). Analysis of variances was performed using two-way ANOVA (SAS, version 16.0) (SAS Institute Cary, North Carolina). Differences among the mean values of the various treatments and storage periods were determined using Duncan's test, and significant difference was defined as $p < 0.05$.

CHAPTER 4

Results

4.1 Objective 1

4.1.1 Physicochemical properties of surimi

The physicochemical properties (proximate composition, pH, water activity, texture, and color) of surimi seafood nutrified with ω-3 PUFA-rich oils from flaxseed, salmon, and blend (flaxseed: salmon 4:1) and stabilized with BEO during storage time (6 days) at 4^0C were assessed. The explanation of different treatments is represented in Table 1 along with the experimental codes representing each treatment on the tables and graphs.

4.1.1.1 Proximate composition of heat-set surimi gels

Proximate composition of surimi gels was determined and shown in Table 2. Reported proximate composition of surimi (wet weight basis) according to the United States Department of Agriculture (2010) is 76.3, 15.2, 0.9, and 0.6, g/100 g for moisture, crude protein, total fat and ash respectively. In the present study, surimi gels without the addition of oil had a proximate composition of 77.15, 15.97, 1.67 and 2.23 g/100 g for moisture, crude protein, total fat and ash, respectively (wet weight basis). Expectedly, the proximate composition for total fat, ash and moisture (wet weight basis) content for surimi with the addition of oil was significantly different ($P < 0.05$) from the control (no oil) due to the development and formulation of surimi paste. There was no significant difference ($P > 0.05$) in the protein content of the control sample as compared with the other treatments. Moisture content was observed to be higher in the control sample with treatment 8 (flaxseed: salmon: bay; 4:1:1) having the least moisture content. There was interaction in moisture content between the other samples. Statistical difference exists between the samples in terms of fat content with the control having the least because of no added

oil. The ash content values ranged from 2.23-2.11 g/100g with treatment 8 having the least ash content although statistical interaction was observed between the treatments.

4.1.1.2 pH value and water activity of heat-set surimi gel

The pH and water activity of heat-set surimi gel was determined over a storage time of 6-days and results obtained are shown in Table 3. There was significant differences in pH values between the control sample (no added oil) and the other treatments in terms of storage time ($P<0.05$). Same difference was seen between treatments. The average pH of surimi gel is seen to be slightly acidic ranging from 6.69-6.97. The optimum pH for gelation is reported to be within the range of 6.5-7.5 and the pH for this study was found to lie between this ranges. Between treatments, the pH value was found to increase for control and treatment 1 and decreases for all other treatments with increase in storage time.

The water activity which is an indicator for level of microbial activity was also determined. Table 2 shows the results obtained for a 6-day storage time. The water activity of heat-set surimi was observed to be between 0.96-0.98 which is within the expected range for meat and fish products (Rodel, Scheuer, & Wang, 1990). Significant difference was observed between and within treatment in relation to storage time. The addition of flaxseed and salmon oils and BEO had no significant effect on the mean value for water activity of the samples.

Table 1

Description of Experimental Treatments and Treatment Codes

Experimental Codes	Flaxseed Oil (g/1000g)	Salmon Oil (g/1000g)	Bay Essential Oil (g/1000g)	Surimi (g/1000g)	Salt (g/1000g)	Ice (g/1000g)	Batch Weight (g)
1	0	0	0	833.3	20	146.7	1000
2	50	0	0	833.3	20	96.7	1000
3	0	50	0	833.3	20	96.7	1000
4	50	0	5	833.3	20	91.7	1000
5	50	0	10	833.3	20	86.7	1000
6	0	50	5	833.3	20	91.7	1000
7	0	50	10	833.3	20	86.7	1000
8	40	10	10	833.3	20	86.7	1000

Table 2

Proximate Composition[a] of Heat-set Surimi Gels Developed with the Addition of Flaxseed Oil, Salmon Oil, and Bay Essential Oil (BEO), and Blend (blend was flaxseed: salmon: BEO, 4:1:1)

	Experimental Treatment Codes[b]							
	1	2	3	4	5	6	7	8
Moisture (g/100g)	77.15±0.07 [a]	71.79±0.10[cb]	71.85±0.03[b]	71.01±0.28[ed]	71.50±0.60[cbd]	71.07±0.38[ed]	71.27±0.35[ced]	70.77±0.29[e]
Protein (g/100g)	15.97±1.27[a]	15.87±1.19[a]	15.43±0.61[a]	16.90±0.28[a]	15.35±0.78[a]	15.15±0.92[a]	15.30±1.67[a]	15.60±2.12[a]
Fat (g/100g)	1.67±0.30[b]	3.15±0.58[a]	2.98±0.68[a]	2.50±0.61[ba]	2.30±0.58[ba]	3.01±0.46[a]	2.71±0.92[ba]	2.26±0.69[ba]
Ash (g/100g)	2.23±0.53 [bac]	1.99±0.03[c]	2.49±0.06[ba]	2.54±0.13[a]	2.23±0.12 [bac]	2.33±0.08[bac]	2.16±0.06[bc]	2.11±0.12 [c]

[a]Data are given as mean values ± standard deviation (n = 3). Different letters within the same row and column indicate significant differences (Duncan Test, $P<0.05$) between mean values. [b]Experimental Treatment codes are shown in Table 1.

Table 3

pH[a] and Water Activity[a] of Heat-set Surimi Gels Developed with the Addition of Flaxseed Oil, Salmon Oil, and Bay Essential Oil (BEO), and Blend (blend was flaxseed: salmon: BEO, 4:1:1)

Experimental Treatment Codes[b]								
Storage Time (Days)	1	2	3	4	5	6	7	8
pH								
1	6.69±0.26^{h}	6.77±0.01^{hgf}	6.76±0.03^{hgf}	6.96±0.01^{ba}	6.86±0.01^{ebdacf}	6.83±0.03^{ebdacf}	6.91±0.01^{ebdac}	6.94±0.01^{bac}
3	6.97±0.01^{a}	6.78±0.01^{hgf}	6.71±0.01^{hg}	6.77±0.02^{hgf}	6.92±0.01^{ebdac}	6.81±0.01^{edgf}	6.93±0.01^{bdac}	6.79±0.01^{ehgf}
6	6.94±0.01^{bdac}	6.92±0.01^{ebdac}	6.83±0.01^{edgcf}	6.74±0.01^{hgf}	6.87±0.02^{ebdacf}	6.84±0.0^{1ebdacf}	6.76±0.01^{hgf}	6.84±0.01^{ebdacf}
Water Activity								
1	0.97±0.00^{c}	0.97±0.00^{bc}	0.98±0.00^{ba}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.00^{dc}
3	0.98±0.00^{a}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.01^{dc}
6	0.98±0.00^{a}	0.97±0.00^{bc}	0.97±0.00^{bc}	0.97±0.00^{dc}	0.96±0.00^{d}	0.97±0.01^{bc}	0.97±0.00^{bc}	0.97±0.01^{dc}

[a]Data are given as mean values ± standard deviation (n = 3). Different letters within the same row and column indicate significant differences (Duncan Test, $P<0.05$) between mean values. [b]Experimental Treatment codes are shown in Table 1.

4.1.1.3 Texture profile analysis of heat-set surimi gel

The texture profile analysis (TPA) of surimi gels with and without added oil was measured and obtained results are shown in Table 4 and Figure 3 for TPA and hardness, respectively. These results show that addition of oil resulted in treatments with the greatest ($P<0.05$) chewiness, and gumminess as compared with the control in which no oil was added. In terms of springiness, resilience and cohesiveness, no difference ($P<0.05$) was detected among the treatments and storage time although there was interaction between the control and other treatments for control day 1(cohesiveness) and treatment 1, day 1 (resilience). This signifies that addition of oil does not affect these parameters within quantity tested in this study. There was a decrease in gumminess among treatments and increase in gumminess between days for all treatments. This may be as a result of type of oil used and amount added to each treatment. However, the blend had significantly greater gumminess and chewiness than other treatments except treatment 1 (having only flaxseed oil). For TPA parameter tested, it can be seen from the table that each parameter especially chewiness and gumminess tends to increase over storage time for each treatment and tends to vary between treatments.

The hardness of surimi gels (Figure 3) was determined and results showed that the treatment with only flaxseed oil (treatment 1) had the greatest hardness between treatment and storage time, followed by treatment 6 and varied among all other treatments and days with the control (no oil) day 1 having the least hardness. In general hardness increased with addition of oils. These results indicate that addition of flaxseed oil (50g) will produce gels with greater hardness and gumminess.

4.1.1.4 Kramer shear test of heat-set surimi gel

The Kramer shear test is another measure of gel strength (Figure 4). Treatment with 50g salmon oil demonstrated the highest Kramer force ($P<0.05$), followed by the treatment with 50g flaxseed oil. The Kramer force tends to increase within treatments over storage time with the exception of treatment 1 and the blend (Treatment 8) which decreased with storage time. The Kramer force correlates with TPA results in that both tends to increase with increase in storage time. This is an indication of gel strength of heat-set surimi gels.

4.1.1.5 Color determination of heat-set surimi gel

Table 5 and Figure 5 shows the tristimulus color values (L* a* b*) and a plot of the whiteness values respectively. L* is a scale, 0-100, of blackness and whiteness with 0 being more black and 100 being more white. The value a* correlates with redness (positive values) and greenness (negative values), and b* is a measure of yellowness (positive values) and blueness (negative values). Results from the tristimulus (L* a* b*) color test reported in Table 5 indicate statistical changes in color of surimi over storage time ($P<0.05$). With the addition of flaxseed, salmon, BEO and blend oils, the L* value ranged from 89-91 as compared to the control (no oil) with an L* value of 85. The lightness, or L* value, of experimental treatments were statistically different from control sample. Surimi gels with added flaxseed oil, salmon oil and blend measured lighter in color than the control. Measurements of a* over time, between control and treatment showed significant difference ($P<0.05$) although no difference was observed between day 3 and 6 for treatment 3, day 1 and 3 for treatment 4 and 5, and day 3 and 6 for treatment 8. This slightly negative value indicates very little greenish hues. Positive b* values indicate yellowish coloration while negative b* values represent bluish coloration. Statistical difference was observed in the b* values between the control and experimental treatments over time

($P<0.05$). The values for control were the least significant and values obtained for the experimental treatment ranged from 5.63-10.91.

The whiteness value (Figure 5) was the highest ($P<0.05$) for gels with blend of salmon and BEO (treatment 5 and 6) followed closely by treatment with salmon oil only. The whiteness of heat-set surimi gel tends to remain constant within storage time for each treatment. This variation in whiteness between treatments may be as a result of pigmentations in the oils used.

Table 4

Texture Profile Analysis[a] of Heat-set Surimi Gels Developed with the Addition of Flaxseed Oil, Salmon Oil, and Bay Essential Oil (BEO), and Blend (blend was flaxseed: salmon: BEO, 4:1:1)

	Experimental Treatment Codes[b]							
Storage Time (Days)	1	2	3	4	5	6	7	8
Resilience								
1	0.40±0.03^{a}	0.40±0.01^{ba}	0.40±0.00^{a}	0.40±0.00^{a}	0.33±0.01^{c}	0.40±0.01^{a}	0.40±0.00^{a}	0.37±0.00^{ba}
3	0.40±0.00^{a}	0.40±0.00^{a}	0.40±0.01^{a}	0.40±0.00^{a}	0.33±0.00^{bc}	0.40±0.00^{a}	0.40±0.01^{a}	0.40±0.01^{c}
6	0.40±0.01^{a}	0.40±0.01^{a}	0.40±0.00^{a}	0.40±0.01^{a}	0.36±0.01^{ba}	0.40±0.01^{a}	0.40±0.01^{a}	0.40±0.01^{a}
Cohesiveness								
1	0.73±0.00^{cb}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}
3	0.70±0.00^{c}	0.70±0.00^{c}	0.75±0.00^{b}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}
6	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.70±0.00^{c}	0.73±0.01^{cb}
Springiness								
1	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}
3	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.97±0.1^{b}	0.90±0.00^{b}	0.90±0.00^{b}
6	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	1.23±0.60^{a}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}	0.90±0.00^{b}
Gumminess								
1	21.70±1.00^{m}	27.97±0.1^{ced}	24.83±1.30^{kjl}	23.83±0.60^{l}	27.57±0.90^{fed}	25.30±1.20^{ikhjl}	27.97±1.50^{ced}	25.53±0.20^{ikhjg}

Table 4

Cont.

3	22.37±0.90^{m}	29.37±0.7^{cb}	28.65±0.30^{cbd}	26.87±1.00^{fheg}	25.80±0.90^{ikhjg}	24.90±0.50^{kjl}	27.53±0.30^{fed}	25.13±1.00^{ikjl}
6	24.27±0.20^{kl}	29.73±0.40^{b}	31.43±0.10^{a}	26.63±1.10^{ifheg}	27.07±0.50^{feg}	28.0±70.30^{ced}	26.87±0.40^{fheg}	26.13±0.60^{ifhjg}
Chewiness								
1	21.90±0.70^{bdc}	25.53±0.60^{bdc}	26.00±1.71^{bdc}	21.40±0.53^{dc}	24.27±1.00^{bdc}	22.87±0.91^{bdc}	25.27±1.53^{bdc}	22.47±0.13^{bdc}
3	20.43±0.82 ^{dc}	26.63±0.58^{bdc}	26.70±0.59^{bdc}	24.40±1.11^{bdc}	23.20±0.81^{bdc}	23.97±2.78^{bdc}	24.88±0.38^{bdc}	22.77±1.25^{bdc}
6	20.00±0.19^{d}	27.27±0.42^{bac}	28.90±0.36^{ba}	24.00±1.01^{bdc}	24.67±0.61^{bdc}	25.57±0.04^{bdc}	24.63±0.72^{bdc}	23.97±0.77^{bdc}

[a]Data are given as mean values ± standard deviation (n = 3). Different letters within the same row indicate significant differences (Duncan Test, P<0.05) between mean values. [b]Experimental Treatment codes are shown in Table 1.

Table 5

Color Properties[a] of Heat-set Surimi Gels Developed with the Addition of Flaxseed Oil, Salmon Oil, and Bay Essential Oil (BEO), and Blend (blend was flaxseed: salmon: BEO, 4:1:1)

	Experimental Treatment Codes[b]							
Storage Time (Days)	1	2	3	4	5	6	7	8
L*								
1	85.21±0.27^{i}	89.54±0.26^{g}	89.41±0.22^{hg}	90.90±0.10^{ed}	91.54±0.02^{bac}	91.18±0.06^{dc}	91.90±0.06^{a}	91.51±0.38^{bc}

Table 5

Cont.

3	83.76±0.23^{j}	89.48±0.10^{hg}	89.31±0.05^{hg}	90.63±0.38^{ef}	91.42±0.11^{bc}	90.50±0.03^{f}	91.46±0.25^{bc}	91.34±0.40^{bc}
6	83.53±0.29^{j}	89.49±0.29^{hg}	89.13±0.04^{h}	90.72±0.16^{ef}	91.58±0.15^{ba}	90.92±0.13^{ed}	91.26±0.18^{bdc}	91.28±0.05^{bdc}
a*								
1	-3.05±0.11^{e}	-3.50±0.01^{h}	-2.39±0.04^{a}	-3.71±0.05^{ji}	-3.77±0.04^{jk}	-2.56±0.02^{cbd}	-2.56±0.03^{cb}	-3.69±0.05^{i}
3	-3.16±0.04^{f}	-3.64±0.02^{i}	-2.50±0.06^{cb}	-3.78±0.04^{jk}	-3.83±0.01^{lk}	-2.57±0.06^{cd}	-2.48±0.05^{b}	-3.79±0.03^{k}
6	-3.31±0.04^{g}	-3.66±0.01^{i}	-2.49±0.02^{cb}	-3.89±0.06^{l}	-3.97±0.02^{m}	-2.63±0.03^{d}	-2.53±0.02^{cb}	-3.80±0.03^{k}
b*								
1	3.06±0.21^{f}	10.37±0.18^{b}	5.65±0.12^{ed}	10.92±0.10^{a}	10.80±0.16^{a}	5.81±0.13^{d}	5.76±0.09^{d}	10.17±0.17^{b}
3	2.17±0.15^{g}	10.18±0.09^{b}	5.63±0.08^{ed}	10.91±0.04^{a}	10.91±0.09^{a}	5.76±0.05^{d}	5.71±0.09^{ed}	10.15±0.17^{b}
6	2.35±0.21^{g}	9.88±0.08^{c}	5.68±0.13^{ed}	10.67±0.17^{a}	10.88±0.06^{a}	5.58±0.11^{ed}	5.57±0.09^{ed}	9.83±0.03^{c}

[a]Data are given as mean values ± standard deviation (n = 3). Different letters within the same row indicate significant differences (Duncan Test, $P<0.05$) between mean values. [b]Experimental Treatment codes are shown in Table 1.

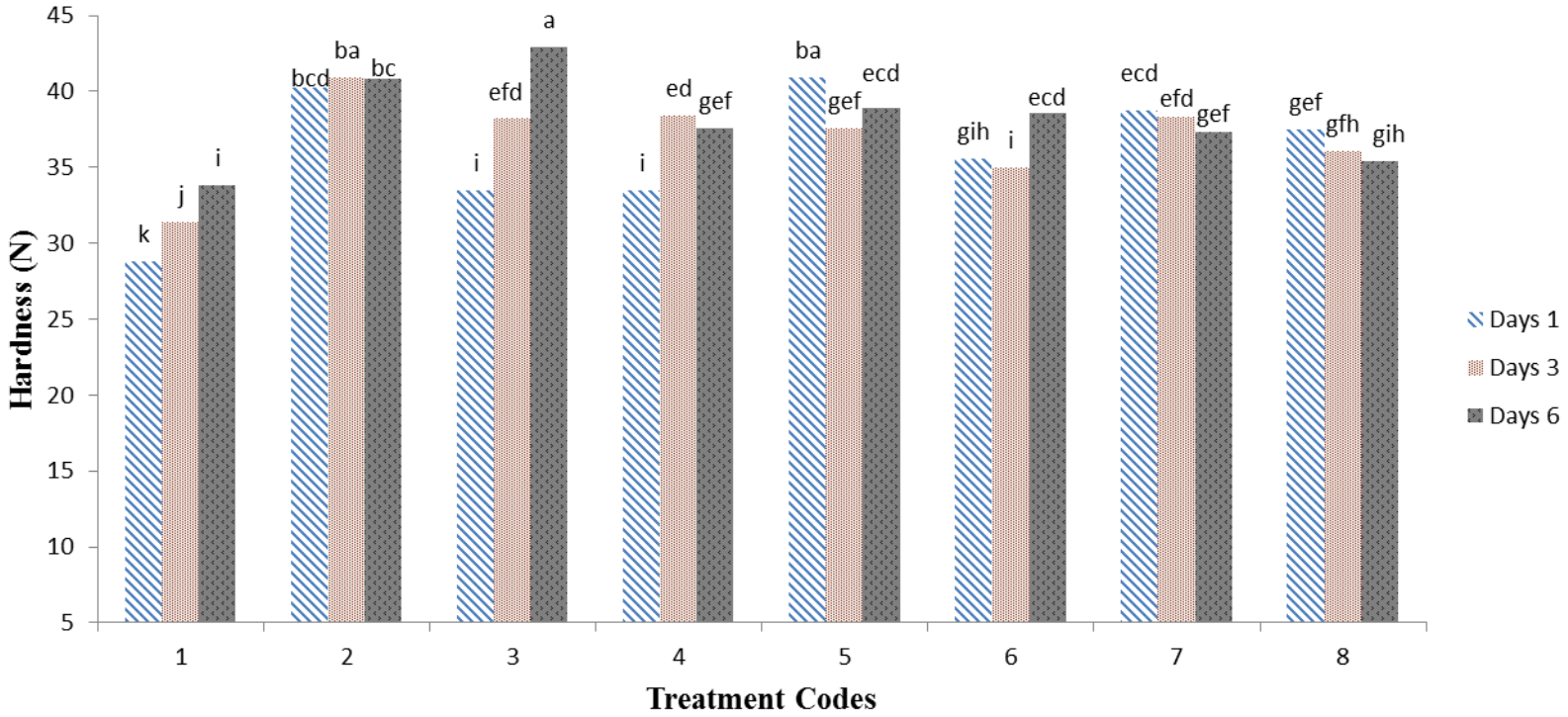


Figure 4. Hardness[a] of heat-set surimi gels developed with the addition of flaxseed oil, salmon oil, and bay essential oil (BEO), and blend (blend was flaxseed: salmon: BEO, 4:1:1).

[a]Data are given as mean values ± standard deviation (n = 3). Different letters on the top of data bars indicate significant differences (Duncan test, $P<0.05$) between mean values. Experimental Treatment codes are shown in Table 1.

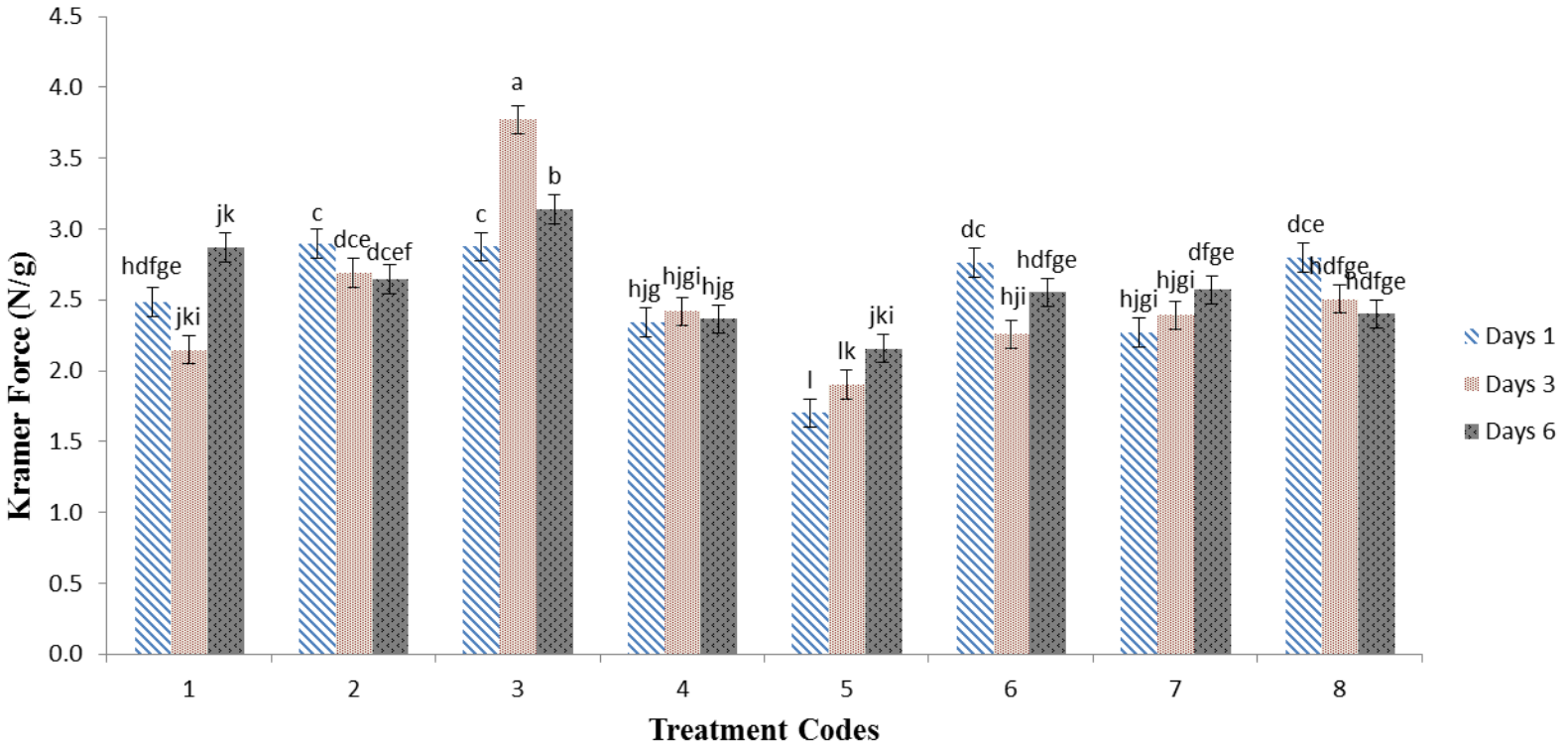


Figure 5. Kramer shear force[a] values of heat-set surimi gels developed with the addition of flaxseed oil, salmon oil, and bay essential oil (BEO), and blend (blend was flaxseed: salmon: BEO, 4:1:1).

[a]Data are given as mean values ± standard deviation (n = 3). Different letters on the top of data bars indicate significant differences (Duncan test, $P<0.05$) between mean values. Experimental Treatment codes are shown in Table 1.

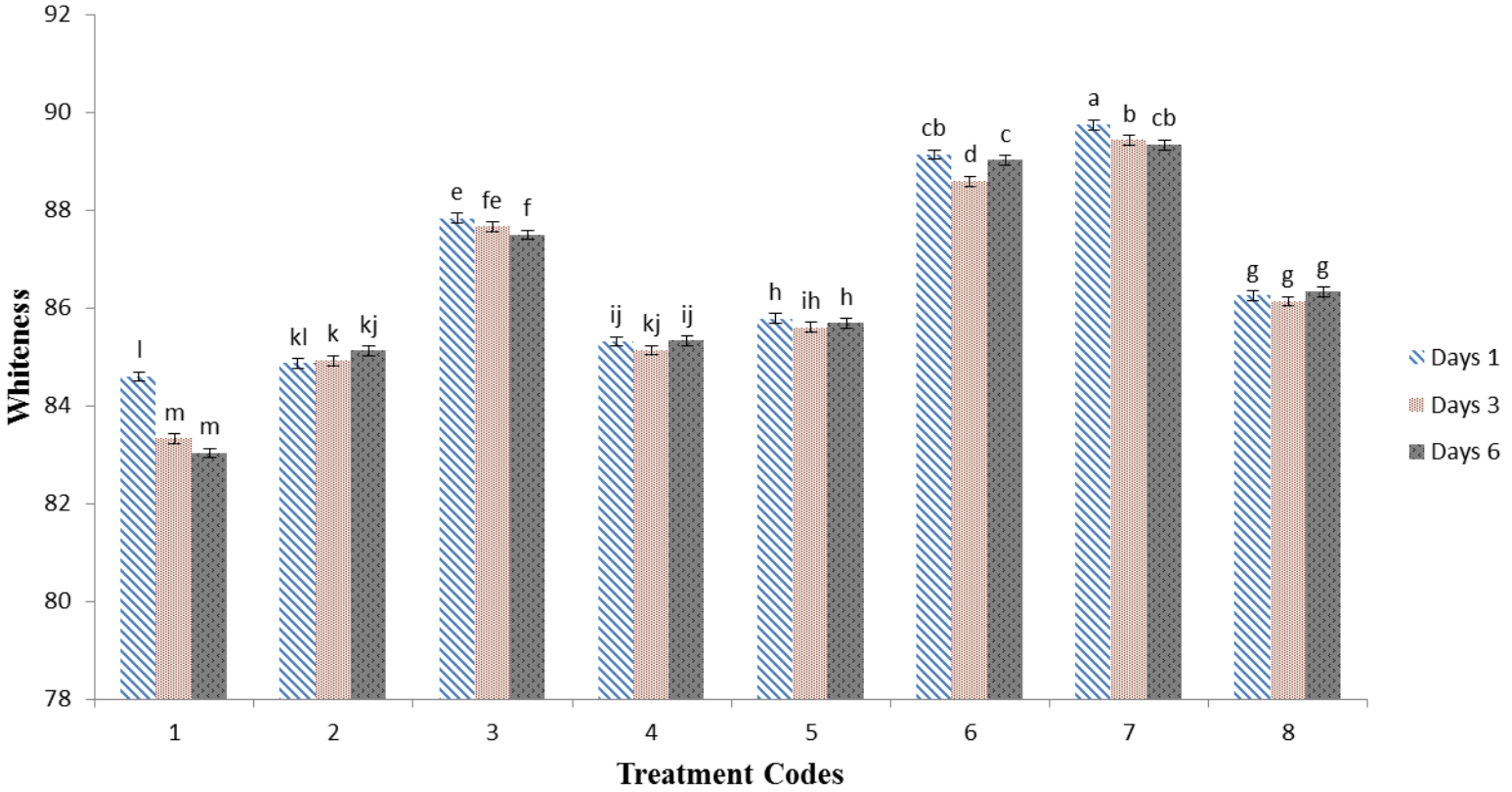


Figure 6. Whiteness[a] of heat-set surimi gels developed with the addition of flaxseed oil, salmon oil, and bay essential oil (BEO), and blend (blend was flaxseed: salmon: BEO, 4:1:1).

[a]Data are given as mean values ± standard deviation (n = 3). Different letters on the top of data bars indicate significant differences (Duncan test, $P<0.05$) between mean values. Experimental Treatment codes are shown in Table 1.

4.2 Objective 2

4.2.1 Chemical characterization of fatty acid composition and their oxidation

The fatty acid content of heat-set surimi with and without (control) addition of oil is represented in Table 6a. The fatty acid content was determined on day 1 and day 6. The major fatty acid in flaxseed oil is ALA– α-linolenic (18:3 ω-3) (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2006); therefore, experimental surimi gels formulated with flaxseed oil and blend contained the highest ($P<0.05$) amount of ALA as compared to other experimental surimi gels. For the control sample, the ALA was below detectable level. Storage time had little or no effect on the ALA content for treatment 4. Surimi gels developed with salmon oil had the greatest ($P<0.05$) level of EPA–eicosapentaenoic (20:5 ω-3) and DHA–docosahexaenoic (22:6 ω-3) as compared to other surimi gels whereas DHA was detected at the highest ($P<0.05$) level in experimental surimi gels developed with salmon oil. (Przybylski, 2005) reported that surimi with added flaxseed oil, a rich source of ALA, had expectedly greater amounts of ALA than salmon oil.

The major fatty acid groups (ω-3 PUFA, ω-6 PUFA, SFA and UFA) in heat-set surimi gel with and without the addition of flaxseed or salmon oils are shown in Table 6b. The main ω-3 PUFAs detected in heat-set surimi gels was ALA, DHA and EPA, whereas the main ω-6 PUFAs detected was LA. The concentration of ω-6 PUFAs ranged from 0.01-0.06% for all gel samples, with the control sample having the least concentration and was below detectable level for day 6. It was observed that the ω-3 fatty acid content of all treatment increased ($P<0.05$) over storage time except for treatment 3 (50g salmon oil) which decreased over storage time. Furthermore, samples containing flaxseed oil recorded the highest content of ω-3 fatty acids. Difference was observed in the ω-3 FAs and ω-6 FAs content for control and all other samples.

All Samples were observed to contain significantly more ω-3 PUFAs and less ω-6 PUFAs ($P<0.05$). Also the UFA content of all samples was observed to increase ($P<0.05$) over storage time but decreased for sample 3. On the other hand, the SFA content of sample 3 increased over time as well as that of other samples. Total UFAs were similar between groups of flaxseed oil and its blends; salmon oil and its blends; however, the UFAs and SFAs for heat-set surimi gels were lower ($P<0.05$) in salmon oil added samples. The addition of BEO did not alter the fatty acid profile of different treatments and also over storage time.

4.2.1.1 Lipid oxidation of heat-set surimi gels

Malondialdehyde (MDA) is the main secondary product of lipid oxidation and is measured with the thiobarbituric reactive substances (TBARS) assay (Nielsen, & Jacobsen, 2009). Results from TBARS analysis are shown in Figure 6. Results indicated significant differences in MDA concentrations between experimental treatments ($P<0.05$) which is dependent on storage time. Concentrations of MDA in heat-set surimi gels ranged from 0.5mg/kg - 2.00 mg/kg over a 6-day storage period. Experimental treatment containing only flaxseed oil had the highest MDA concentration value ($P<0.05$) between treatment and storage time with the control sample (no added oil) having the least value. On the other hand, treatments with blend of flaxseed oil and BEO showed a reduction in the MDA concentration. Between storage times, greatest difference was observed in treatment 3 and 4. Oxidation rate was the lowest in treatment 4 and 5 when compared to other experimental treatments. This is likely to be as a result of lower amount of long chain ω-3 PUFAs, EPA and DHA and BEO addition.

Table 6a

Major Fatty Acid Content[a] (%) of Heat-set Surimi Gels Developed with the Addition of Flaxseed Oil, Salmon Oil, and Bay Essential oil (BEO), and Blend (blend was flaxseed: salmon: BEO, 4:1:1)

	Experimental Treatment Codes[b]							
Storage Time (Days)	1	2	3	4	5	6	7	8
LA								
1	0.00±0.00^{o}	0.06±0.00^{f}	0.01±0.00^{j}	0.06±0.00^{e}	0.06±0.00^{b}	0.01±0.00^{l}	0.01±0.00^{m}	0.00±0.00^{n}
6	BDL	0.07±0.00^{a}	0.01±0.00^{k}	0.06±0.00^{d}	0.06±0.00^{c}	0.01±0.00^{i}	0.01±0.00^{h}	0.05±0.00^{g}
EPA								
1	0.01±0.00^{l}	0.01±0.00^{m}	0.05±0.00^{b}	0.01±0.00^{o}	0.01±0.00^{j}	0.04±0.00^{f}	0.04±0.00^{g}	0.05±0.00^{c}
6	0.00±0.00^{p}	0.01±0.00^{i}	0.04±0.00^{e}	0.01±0.00^{n}	0.01±0.00^{k}	0.05±0.00^{d}	0.05±0.00^{a}	0.01±0.00^{h}
DHA								
1	0.01±0.00^{m}	0.01±0.00j	0.06±0.00^{a}	0.01±0.00^{n}	0.01±0.00^{i}	0.05±0.00^{f}	0.05±0.00^{g}	0.06±0.00^{b}
6	0.01±0.00^{p}	0.01±0.00^{h}	0.05±0.00^{e}	0.01±0.00^{l}	0.01±0.00^{k}	0.06±0.00^{c}	0.06±0.00^{d}	0.01±0.00^{o}
ALA								
1	0.00±0.00^{o}	0.18±0.00^{f}	0.01±0.00^{j}	0.23±0.00^{b}	0.21±0.00^{e}	0.01±0.00^{m}	0.01±0.00^{n}	0.17±0.00^{g}
6	BDL	0.27±0.00^{a}	0.01±0.00^{l}	0.23±0.00^{c}	0.23±0.00^{d}	0.01±0.00^{k}	0.01±0.00^{i}	0.17±0.00^{h}

[a]Data are given as mean values ± standard deviation (n = 3). Different letters within the same row indicate significant differences (Duncan Test, P<0.05) between mean values. ALA– α-linolenic (18:3 ω-3), EPA–eicosapentaenoic (20:5 ω-3), DHA–docosahexaenoic (22:6 ω-3), and LA–linoleic (18:2 ω-6). [b]Experimental Treatment codes are shown in Table 1. BDL: Below Detection Limits. Detection limits vary by fatty acid but usually > 0.0005%

Table 6b

Major Fatty Acid Groups[a] (%) of Heat-set Surimi Gels Developed with the Addition of Flaxseed Oil, Salmon Oil, and Bay Essential Oil (BEO), and Blend (blend was flaxseed: salmon: BEO, 4:1:1)

	Experimental Treatment Codes[b]							
Storage Time (Days)	1	2	3	4	5	6	7	8
ω-3								
1	0.02±0.00^{o}	0.19±0.00^{f}	0.12±0.00^{h}	0.24±0.00^{b}	0.22±0.00^{e}	0.10±0.00^{m}	0.09±0.00^{n}	0.11±0.00^{i}
6	0.01±0.00^{p}	0.28±0.00^{a}	0.10±0.00^{l}	0.24±0.00^{c}	0.24±0.00^{d}	0.11±0.00^{k}	0.11±0.00^{j}	0.19±0.00^{g}
ω-6								
1	0.00±0.00^{o}	0.06±0.00^{f}	0.01±0.00^{j}	0.06±0.00^{e}	0.06±0.00^{b}	0.01±0.00^{l}	0.01±0.00^{m}	0.00±0.00^{n}
6	BDL	0.07±0.00^{a}	0.01±0.00^{k}	0.06±0.00^{d}	0.06±0.00^{c}	0.01±0.00^{i}	0.01±0.00^{h}	0.05±0.00^{g}
UFA								
1	0.02±0.00^{o}	0.30±0.00^{f}	0.17±0.00^{h}	0.40±0.00^{d}	0.34±0.00^{e}	0.15±0.00^{m}	0.13±0.00^{n}	0.16±0.00^{j}
6	0.01±0.00^{p}	0.41±0.00^{a}	0.15±0.00^{l}	0.40±0.00^{c}	0.40±0.00^{b}	0.16±0.00^{k}	0.17±0.00^{i}	0.29±0.00^{g}
SFA								
1	0.02±0.00^{o}	0.05±0.00^{m}	0.07±0.00^{g}	0.05±0.00^{n}	0.05±0.00^{k}	0.08±0.00^{e}	0.08±0.00^{f}	0.09±0.00^{b}
6	0.01±0.00^{p}	0.05±0.00^{i}	0.09±0.00^{c}	0.05±0.00^{l}	0.05±0.00^{j}	0.09±0.00^{d}	0.09±0.00^{a}	0.06±0.00^{h}

[a]Data are given as mean values ± standard deviation (n = 3). Different letters within the same row indicate significant differences (Duncan Test, $P<0.05$) between mean values. ω-3– Omega-3 fatty acids, ω-6–Omega-6 fatty acids, UFA–unsaturated fatty acids, and SFA–saturated fatty acids. [b]Experimental Treatment codes are shown in Table 1. BDL: Below Detection Limits. Detection limits vary by fatty acid but usually > 0.0005%

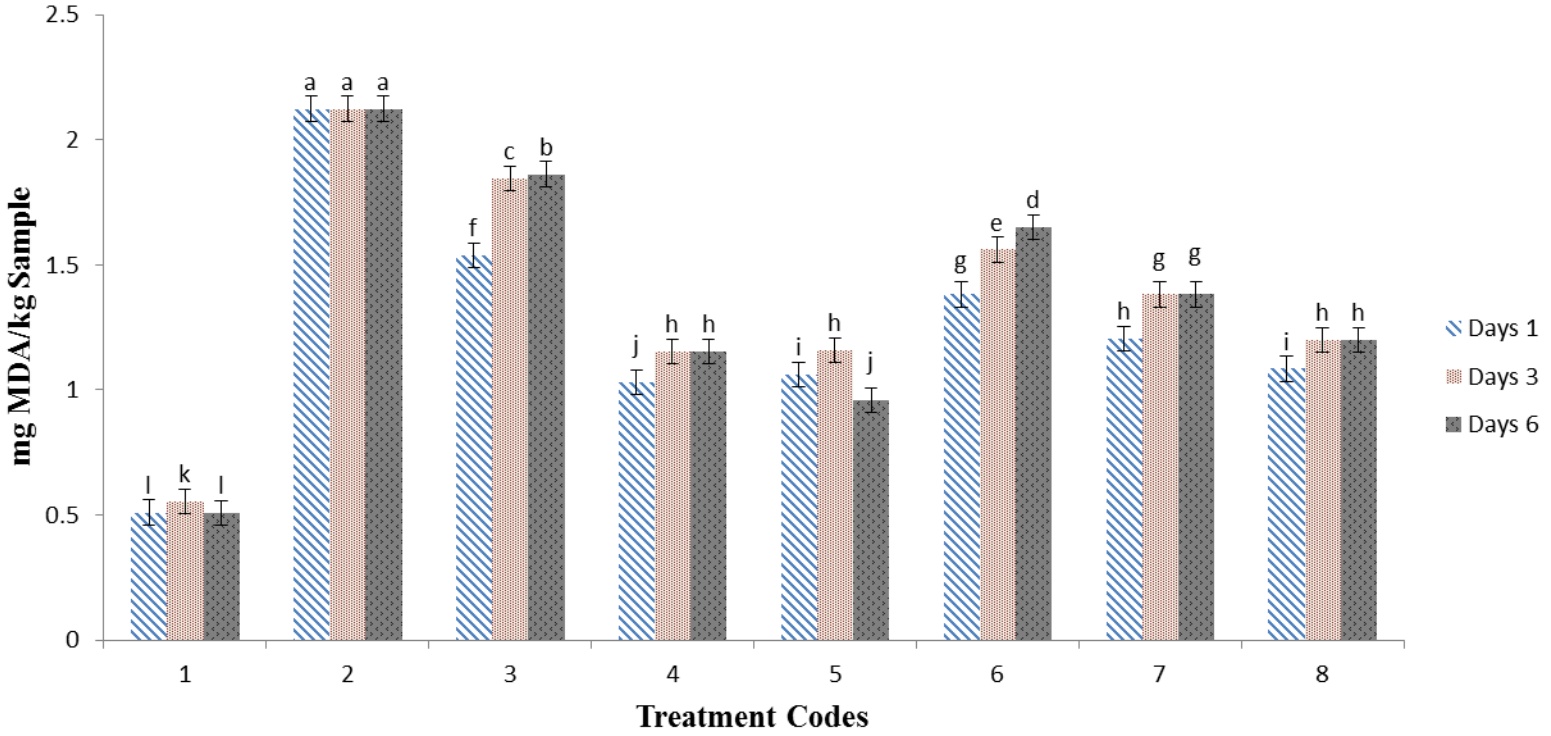


Figure 7. Thiobarbituric reactive substances (TBARS)[a] values of heat-set surimi gels developed with the addition of flaxseed oil, salmon oil, and bay essential oil (BEO), and blend (blend was flaxseed: salmon: BEO, 4:1:1).

[a]Data are given as mean values ± standard deviation (n = 3). Different letters on the top of data bars indicate significant differences (Duncan test, $P<0.05$) between mean values. Experimental Treatment codes are shown in Table

4.2.1.2 Radical Scavenging Activity

Free radicals are part of the propagation of autoxidation and are considered the main reactants. To test essential oil's capacity to neutralize free radicals, DPPH radical scavenging activity was measured. Figure 7 shows the DPPH radical scavenging activity of BEO and it was found to have very high scavenging activity. The scavenging activity was 11.21±1.30% at 0.006255% concentration. An increase in concentration significantly increase the radical scavenging activity ($P<0.05$). At 0.0125, 0.025, 0.05 and 0.1% concentrations the scavenging activity was 22.67±2.04, 36.75±1.47, 51.77±1.67 and 64.78±2.06% respectively. 0.5% and 1% of BEO showed higher scavenging activity of 92.53±0.44 and 96.04±0.21% respectively.

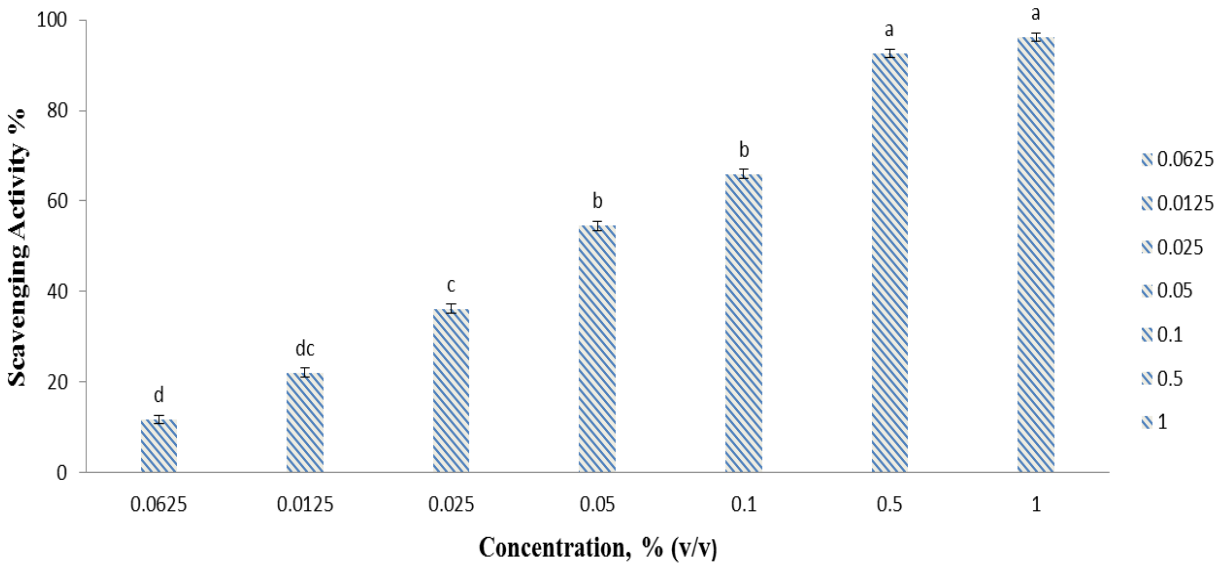


Figure 8. DPPH free radical scavenging activity of BEO. Data are given as mean values ± standard deviation (n = 3). Different letters within the same row indicate significant differences (Duncan Test, $P<0.05$) between mean values.

CHAPTER 5

Discussion and Future Research

In this study, the physicochemical properties of surimi seafood nutrified with ω-3 PUFAs-rich oils from flaxseed, salmon, and oxidation rate of fatty acid composition were determined. Surimi gels were stabilized with BEO during storage time at 4 °C for 6 days.

The proximate composition of heat-set surimi gel as expected was significantly different ($P<0.05$) from the control. Again, the changes observed in moisture with the addition of flaxseed and salmon oil were similar to results in a study by Hsu, & Chiang (2002). Total ash and fat content of control samples were similar to crab flavored surimi gels reported in Pietrowski, Tahergorabi, Matak, Tou, & Jaczynski (2011). However, protein and ash (wet weight basis) were generally the same between treatments.

Heat-set surimi gels treated with BEO and control samples had an initial pH ranging from 6.69 to 6.94. These values increased during the storage period. Results obtained in this study are similar to that reported by Da Silveira et al. (2014) except for the first day of storage; the pH of control samples was significantly higher ($P < 0.05$) than in those treated with BEO. This pH increase might be related to the microbial growth during the storage period. Jay (2000) reported that when the supply of simple carbohydrates is depleted, species like *Pseudomonas*, as well as psychrotrophic Gram-negative bacteria, use proteinaceous compounds as energy source producing ammonia, and thereby increasing the food's pH. (Tassou, Nychas, & Skandamis, 2004) reported that the activity of antimicrobial agents is affected by the pH of a specific food. In general, EOs inhibits microbial growth through the damage caused in the cell membrane by its compounds, which disturbs the cell pH and inorganic ion balance (Burt, 2004; Shylaja, & Peter, 2004).

The water activity of meat products lies in the upper range of a_w-scale for foods because of their high moisture content. Fresh meat has the highest water activity ($a_w > 0.99$). During processing of the meat to meat products, the water activity of the products drops depending largely on the common salt content. E.g. frankfurter-types sausages generally have a water activity of 0.96 to 0.97 (Rodel, Scheuer, & Wagner, 1990). Kim, & Park (2008) reported that addition of salt during processing helps to control microbial growth by lowering the water activity of the product. Water activity is an indicator of the level of microbial activity in food products. Results obtained in this study shows that the water activity declined between treatments over storage time when compared with the control.

The Texture Profile Analysis (TPA) measures certain textural properties which include: springiness-the ratio of the product's height on second compression to the original compression distance which measures the ability of the product to retain its shape and size after compression; cohesiveness-the ratio of the second compression to the first compression that measures resistance of the product during chewing; hardness-the peak height on the first compression that measures product's resistance during a bite; gumminess-the product of hardness and chewiness. This measures the strength required in chewing; chewiness-the product of gumminess and springiness which is a measure of the energy used in chewing; resilience-the measure of a product's ability regains its original position (Cardoso, Mendes, Pedro, & Nunes, 2008). Previous studies of oil incorporation in surimi show mixed results with some reporting a decrease in gel strength and hardness, and others reporting an increase in these measures (Cardoso, Mendes, & Nunes, 2007; 2008; Sánchez-Alonso, Haji-Maleki, & Borderias, 2007). Dickinson, & Chen (1999); Wu et al. (2009); Yost, & Kinsella (1992) postulates that oil may occupy the void spaces of the protein matrix restraining the protein matrix against movement and

possibly interacting with the matrix to increase gel strength. They attributed the increase in gel strength to the substitution of water with the same amount of oil used in their study. Cardoso, Mendes, Vaz-Pires, & Nunes (2009) reported that surimi with lower protein content is known to produce less firm gels, which affects the textural properties of gels. In this study, protein concentration was constant. This may explain the increase in hardness, chewiness and gumminess within storage time. Similar results for cohesiveness were reported by Pérez-Mateos, Boyd, & Lanier (2004), although addition of BEO results in a slight increase. These results likely vary due to the differences in oil types used and amount added to surimi. Again, results obtained in this study are similar to the report of Cardoso et al. (2009) which showed an increase in hardness, gumminess, and chewiness during refrigerated storage of surimi gel. They acknowledged that increase in hardness over time may be attributed to water loss during storage.

Table 4 and Figure 4 shows the tristimulus color values (L* a* b*) and a plot of the whiteness values respectively. L* is a scale, 0-100, of blackness and whiteness with 0 being more black and 100 being more white. The value a* correlates with redness (positive values) and greenness (negative values), and b* is a measure of yellowness (positive values) and blueness (negative values). In terms of consumer acceptability of surimi seafood products, the white coordinate, or high L*, is considered the most desirable hue from the color spectrum. Similar results were reported by Pérez-Mateos et al. (2004) stating that with the addition of menhaden oil, fish oil concentrate, and purified marine oil L* value was improved as compared to no oil (control). The enhancement of L* value has been attributed to the dispersion of light that results from the emulsion created when oil is comminuted with fish muscle proteins and water (Pérez-Mateos et al., 2004; Park, & Lin, 2005). Whiteness was calculated using L*, a*, and b* as previously described. Overall whiteness was the lowest for control and highest for treatment 6.

Treatment 1, 3 and 4 were significantly less white than treatment 2, 5 and 7. This trend is consistent with current literature (Pietrowski et al., 2011; Pérez-Mateos, Lanier, & Boyd, 2006; Chen, & Jaczynski, 2007b). However, Pérez-Mateos et al. (2006) suggested that lipid droplet suspension in the surimi gel scatters light, thus increasing light reflection and subsequently L* values. Negative a* values represent a greenish color. Addition of bay essential increased L* value significantly ($P<0.05$) but generally did not affect the a* and b* values. However, no significant change was observed during storage time. This is in accordance with Pérez-Mateos et al. (2006).

The thiobarbituric reactive substances (TBARS) assay makes use of light spectrophotometry to measure red pigmentation produced when a secondary product of lipid oxidation called malondialdehyde (MDA), reacts with thiobarbituric acid (TBA) (Wang et al. 2002). Pietrowski et al. (2011); Pérez-Mateos et al. (2004) reported that the concentration of MDA has been used to predict oxidative stability of surimi seafood products. (Pérez-Mateos et al. 2004) further reported that surimi gels incorporated with ω-3 PUFAs may be more susceptible to oxidation due to the high degree of unsaturation, hence, undesirable in seafood-derived products due to development of undesirable flavors, rancidity, and possible changes in taste, texture, shelf life and appearance (McClements, & Decker, 2000). Lipid oxidation was evident in this study, but the addition of BEO reduced the rate of oxidation to an acceptable level. There was no significant change over time in MDA concentrations of treatment 1. Furthermore, Schormuller (1969) proposed that the maximum level of TBARS indicating good quality of seafood products is less than 5 mg MDA/kg, but they may be consumed up to 8 mg MDA/kg. In the present study, TBARS for all of the samples were much lower than the proposed limit. Therefore, examining changes in TBARS values between samples and over time is an indication

of oxidative stability. Sebranek, Sewalt, Robbins, & Houser (2005) reported similar antioxidant activities of rosemary essential oils regarding MDA generation in refrigerated sausages.

The antioxidant activity of BEO was determined using DPPH radical scavenging test. BEO was found to have highly effective DPPH radical scavenging activity at 0.5 and 1% concentrations. Gas chromatography analysis of BEO revealed its different chemical composition constituting 1, 8-cineol (40%) and linalool (12.5%). Therefore, the strong scavenging activity of BEO is possibly due to the presence of these chemical compounds. Studies by Lee, Umano, Shibamoto, & Lee (2005) showed promising result in antioxidant activities of 1, 8-cineole and linalool as individual components. Politeo et al. (2007); Cherrat et al. (2014) reported that BEO has almost similar activity to standard BHT in terms of DPPH radical scavenging. Therefore, the strong activity of BEO may be as a result of presence of other minor and major components and the synergistic effect between these components.

Fish and its derivatives are recommended for consumption by health authorities, not only for their high-quality protein content, but also due to their high concentration of polyunsaturated fatty acids (PUFAs) like the ω-3 series of fatty acids which are highly beneficial for human health. Linolenic acid (ALA, 18:3ω-3), eicosapentaenoic acid (EPA, 20:5ω-3), and docosahexaenoic acids (DHA, 22:6ω-3) were the main ω-3 PUFAs, which are potentially beneficial in reducing the risk of cardiovascular diseases and inflammation (Kris-Etherton, Harris, Appel, & Committee, 2002; Morris et al., 2003). Linoleic (LA, 18:2ω6) was the main ω-6 PUFAs present in heat-set surimi gels. Sell, Beamer, Jaczynski, & Matak (2014) reported that the total UFAs present in surimi franks were similar between groups and with SFAs, were lower in cooked surimi franks nutrified with salmon oil. Furthermore, Pietrowski et al. (2011) reported

that surimi gels developed with flaxseed oil had the most ($P<0.05$) UFAs. These results are similar to those obtained in this study.

Alaska pollock surimi was used for development of heat-set surimi gels with flaxseed, salmon and a blend of oils. The surimi gels were treated with BEO to stabilize occurrence of oxidation which may lead to spoilage over storage time at 4^0C. Evaluation of quality parameters showed that although textural properties varied, pH, water activity, color and TBARS values were generally within the acceptable range during the 6-day refrigerated storage period. BEO showed effective antioxidant effect on lipid oxidation of heat-set surimi gels thus will enable the manufacture of functional foods using heat-set surimi gels. The incorporation of BEO may allow food manufacturers to nutrify surimi seafood with beneficial ω-3 rich oils without affecting product quality while extending storage time.

However, future studies should be conducted to evaluate the microbial load of heat-set surimi gels as well as sensory evaluation to determine acceptability by consumers in order to know the market potential of this type of product.

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