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Production and processing of a functional yogurt fortified with microencapsulated omega-3 and vitamin E

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PRODUCTION AND PROCESSING OF A FUNCTIONAL YOGURT FORTIFIED WITH MICROENCAPSULATED OMEGA-3 AND VITAMIN E

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
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Master of Science

in

The Department of Food Science

by
Jose Daniel Estrada Andino
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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ ii

LIST OF TABLES .................................................................................................................... v

LIST OF FIGURES .................................................................................................................. vi

ABSTRACT .............................................................................................................................. viii

CHAPTER 1 INTRODUCTION .................................................................................................. 1

CHAPTER 2 LITERATURE REVIEW ......................................................................................... 3
  2.1 General Information on Yogurt ....................................................................................... 3
  2.1.1 Definition and Classification .................................................................................... 3
  2.1.2 Production and Consumption ................................................................................... 4
  2.1.3 Omega-3 Addition .................................................................................................... 5
  2.2 Omega-3 Fatty Acids ..................................................................................................... 6
    2.2.1 Sources and Chemical Structure ............................................................................ 6
    2.2.2 Oxidation ............................................................................................................... 8
    2.2.3 Health Benefits ....................................................................................................... 9
    2.2.4 Intake ..................................................................................................................... 10
  2.3 Vitamin E ....................................................................................................................... 11
    2.3.1 Chemical Structure ................................................................................................ 11
    2.3.2 Role in Human Metabolism ................................................................................... 12
    2.3.3 Antioxidant Properties in Foods ........................................................................... 12
  2.4 Microencapsulation ....................................................................................................... 13
    2.4.1 Definition .............................................................................................................. 13
    2.4.2 Microencapsulation by Spray Drying .................................................................... 14
    2.4.3 Oxidation of Microencapsulated Fish Oil ............................................................... 15

CHAPTER 3 MATERIALS AND METHODS .......................................................................... 18
  3.1 Emulsion Preparation and Spray Drying of Microencapsulated Fish Oils .................... 18
  3.2 Microencapsulation Efficiency (ME) and Encapsulation Yield (EY) of MMO and MSO ......................................................................................................................... 20
  3.3 Peroxide Value, Anisidine Value, Total Oxidation, Free Fatty Acids, Color and Moisture of PAM, MMO and MSO ........................................................................................................... 21
  3.4 Scanning Electron Microscopy of PAM, MMO and MSO ............................................. 23
  3.5 Production of Functional Yogurt Fortified with Omega-3 and Vitamin E ................. 24
  3.6 Fatty Acid Methyl Esters (FAMEs) Analysis of MMO, MSO and Yogurts .................... 26
  3.7 Determination of Alpha Tocopherol Content Using HPLC .......................................... 28
3.8 Thiobarbituric Acid Values of Yogurts ................................................................. 28
3.9 Lactic Acid Bacteria Counts, pH, Water Holding Capacity and Color of Yogurts ...... 29
3.10 Rheological Analysis of Yogurts ........................................................................... 30
3.11 Confocal Scanning Laser Microscopy of Yogurts’ Structure ............................ 31
3.13 Statistical Analysis ............................................................................................. 32

CHAPTER 4 RESULTS AND DISCUSSION .................................................................. 33
4.1 Microencapsulation Efficiency (ME) and Encapsulation Yield (EY) of MMO and MSO ................................................................. 33
4.2 Peroxide Value, Anisidine Value, Total Oxidation, Free Fatty Acids, Color and Moisture of PAM, MMO and MSO ....................................................... 34
4.3 Scanning Electron Microscopy of PAM, MMO and MSO ...................................... 37
4.4 Alpha Tocopherol Content of Yogurts ................................................................. 39
4.5 Fatty Acid Methyl Esters (FAMEs) Analysis of MMO, MSO and Yogurts ................. 41
4.6 TBA Values of Yogurts ....................................................................................... 47
4.7 Lactic Acid Bacteria Counts, pH, Water Holding Capacity and Color of Yogurts .... 49
4.8 Rheological Analysis of Yogurts ......................................................................... 54
4.9 Confocal Scanning Laser Microscopy of Yogurts’ Structure .............................. 59
4.10 Transmission Electron Microscopy of Yogurts ................................................... 61

CHAPTER 5 CONCLUSIONS ......................................................................................... 65

REFERENCES ............................................................................................................. 68

APPENDIX: CHARACTERISTICS OF PMO AND PSO ............................................... 79

VITA .............................................................................................................................. 80
**LIST OF TABLES**

Table 3.1 PAM, MMO, and MSO emulsions formulations………………………………………19

Table 3.2 Yogurt mix formulations……………………………………………………………………25

Table 4.1 Total oil, surface oil, ME, and EY of MMO and MSO………………………………………33

Table 4.2 Properties of PAM, MMO and MSO………………………………………………………35

Table 4.3 FAMEs composition (%) of MMO and MSO………………………………………………41

Table 4.4 SAFA (%) of yogurts during 4 weeks of refrigerated storage…………………………….42

Table 4.5 MUFA (%) of yogurts during 4 weeks of refrigerated storage…………………………….43

Table 4.6 PUFA (%) of yogurts during 4 weeks of refrigerated storage…………………………….43

Table 4.7 Total ω-3 (%) of yogurts during 4 weeks of refrigerated storage……………………….45

Table 4.8 EPA (%) of yogurts during 4 weeks of refrigerated storage……………………………..45

Table 4.9 DHA (%) of yogurts during 4 weeks of refrigerated storage………………………………46

Table 4.10 Syneresis of yogurts during 4 weeks storage at 4ºC……………………………………….52

Table 4.11 Total color differences of yogurts during 4 weeks of refrigerated storage………………..54

Table 4.12 Initial Flow Behavior Properties of PY, YPAM, YMMO and YMSO…………………..55

Table 4.13 Flow Behavior Properties of PY, YPAM, YMMO and YMSO after 4 weeks Storage……………………………………………………………………………………………………56
LIST OF FIGURES

Fig. 2.1 Chemical structure of eicosapentaenoic acid .......................................................... 7
Fig. 2.2 Chemical structure of docosahexaenoic acid ......................................................... 7
Fig. 2.3 Chemical structure of α-tocopherol ....................................................................... 11
Fig. 3.1 Flow diagram for PAM, MMO and MSO production ............................................ 19
Fig. 3.2 Flow diagram for yogurt processing ...................................................................... 24
Fig. 4.1 Image of PAM ......................................................................................................... 36
Fig. 4.2 Image of MMO ....................................................................................................... 36
Fig. 4.3 Image of MSO ......................................................................................................... 37
Fig. 4.4 Scanning electronic microscopy image of PAM .................................................... 37
Fig. 4.5 Scanning electronic microscopy image of MMO ....................................................... 38
Fig. 4.6 Scanning electronic microscopy image of MSO ....................................................... 38
Fig. 4.7 α-tocopherol content of PY, YPAM, YMMO and YMSO during 4 weeks of refrigerated storage .................................................................................................................. 40
Fig. 4.8 Thiobarbituric acid values of PY, YPAM, YMMO, and YMSO ..................................... 48
Fig. 4.9 Lactic acid bacteria counts of yogurts during 4 weeks storage ............................... 51
Fig. 4.10 Acidity (pH) of yogurts during 4 weeks storage ..................................................... 51
Fig. 4.11 Color lightness values (L*) of yogurts ................................................................. 53
Fig. 4.12 Color green (negative values)/ red (positive values) (a*) of yogurts ..................... 53
Fig. 4.13 Color blue (negative values)/ yellow (positive values) (a*) of yogurts ................. 54
Fig. 4.14 Initial Frequency Sweeps Test of Yogurts: Elastic Modulus (G’) and Viscous Modulus (G’’) vs. Angular Frequency ....................................................................................... 57
Fig. 4.15 Week 4 frequency sweeps test of yogurts: elastic modulus (G’) and viscous modulus (G’’) vs. angular frequency ....................................................................................... 57
Fig. 4.16 Initial stress sweeps test of yogurts: elastic modulus (G’) vs. oscillation stress ...... 58
Fig. 4.17 Week 4 stress sweeps test of yogurts: elastic modulus (G’) vs. oscillation stress........59
Fig. 4.18 Initial confocal scanning laser micrographs of PY, YPAM, YMMO, and YMSO……60
Fig. 4.19 Week 4 confocal scanning laser micrographs of PY, YPAM, YMMO, and YMSO…61
Fig. 4.20 Initial transmission electron micrographs of PY, YPAM, YMMO, and YMSO……62
Fig. 4.21 Week 4 transmission electron micrographs of PY, YPAM, YMMO, and YMSO……63
Fig. 4.22 TEM micrograph of Lactobacillus bulgaricus..............................................64
Fig. 4.23 TEM micrograph of Streptococcus thermophilus............................................64
ABSTRACT

Market and nutritional values of fermented milks can be enhanced by adding microencapsulated polyunsaturated fish oil and vitamin E. Polyunsaturated fatty acids (PUFAs) lower the risk of heart disease and vitamin E is an important lipophilic antioxidant that protects cell membranes from oxidation. This study aimed to develop functional yogurts YMSO and YMMO fortified with α-tocopherol and microencapsulated salmon oil (MSO) or microencapsulated menhaden oil (MMO), respectively and to evaluate their physicochemical properties during 1-mo of storage. Stable emulsions were prepared with 7% red salmon oil (SO) or menhaden oil (MO), 22% arabic gum, 11% maltodextrin, 0.83% α-tocopherol and 59.17% water. The emulsions were spray dried and MSO or MMO were produced. Plain yogurt (PY) was produced and 2% of MSO or MMO were added to three batches of yogurt to produce YMSO or YMMO. PY and yogurt with 22% arabic gum and 11% maltodextrin (YPAM) were used as controls. Yogurts were analyzed for syneresis, pH, color, lactic acid bacteria (LAB), thiobarbituric acid (TBA) value, fatty acid methyl esters composition, α-tocopherol content, rheological properties and microstructure. Triplicate experiments were conducted and data was analyzed at α=0.05. After 1-mo storage, the yogurts pH decreased and syneresis slightly increased regardless of the treatment and all yogurts had a similar reduction in LAB counts from 8 Log CFU/g to 6 Log CFU/g. α-Tocopherol contents of YMMO and YMSO were significantly (P<0.05) higher than those of PY and YPAM during the 4 week storage study. Initial PY, YPAM, YMMO and YMSO α-tocopherol contents were 37.65 ± 3.17, 43.03 ± 0.71, 75.61 ± 0.98, and 72.09 ± 0.18 µg/g yogurt, respectively. All yogurts had low TBA values and YMMO and YMSO had significantly higher (P<0.05) total ω-3 content than the controls. All yogurts were described as pseudoplastic fluids and showed
viscoelastic characteristics during 1 month storage. The study demonstrated that PY can be fortified with fish oil and \( \alpha \)-tocopherol.
CHAPTER 1 INTRODUCTION

Yogurt is one of the oldest fermented milk products known. Fermentation of milk involves the action of microorganisms, principally the lactic acid bacteria. These microorganisms sour the milk by converting the milk sugar lactose to lactic acid (Kagan, 1985). Yogurt gels are built of clusters of aggregated casein particles formed as a result of gradual fermentation of lactose by lactic acid bacteria (Horne, 1999, 2003). The Food and Drug Administration (FDA, 2008) standard of identity for yogurt drinks specifies >8.25% milk solids-not-fat and fat levels to satisfy nonfat yogurt (<0.5%), low-fat yogurt (2%), or yogurt (>3.25%) before the addition of other ingredients (Chandan et al., 2006). Yogurt is among the most common dairy products consumed around the world (Saint-Eve et al., 2006). As the popularity of yogurt products continues to grow, manufacturers are continuously investigating value-added ingredients to entice health-conscious consumers (Allgeyer et al., 2010).

Marine lipids have for the last decades received increasing attention because of their beneficial health effects. Since the first evidences regarding the beneficial health effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on cardiovascular diseases appeared, many studies have demonstrated that these fatty acids have a positive effect especially on myocardial infarction (Schmidt et al., 1992) and other diseases. Salmon and menhaden oils are a good source of polyunsaturated fatty acids (PUFAs), specifically EPA and DHA. Due to a low intake of fish consumers in most of the Western world have an intake of EPA and DHA that is far below the recommended intake. Due to their healthy image and wide consumption, dairy products could be good vehicles for incorporation of fish oil. Foods containing fish oil are very susceptible to oxidation due to the high content of PUFAs (Nielsen et al. 2007).
Vitamin E is a fat-soluble vitamin (Traber, 1999) and recent research suggests that its deficiency is associated with an elevated risk of atherosclerosis and other degenerative diseases. The physiological role of vitamin E centers on its ability to react with and quench free radicals in cell membranes and other lipid environments, thereby preventing PUFAs from being damaged by lipid oxidation (Bramley et al., 2000). Temperature, oxygen and UV light parameters during extraction, encapsulation and storage of vitamin E affect its degradation (Sabliov et al., 2009).

Microencapsulation is described as a technique wherein a bioactive compound is encapsulated by a biopolymer thereby protecting it from oxygen, water, light or other conditions in order to improve its stability and also to change liquid solutions to powders for easier handling (Gharsallaoui et al., 2007). Microcapsules range in size from one micron to seven millimetres and release their contents at a later time by means appropriate to the application (Shekhar et al., 2010). The encapsulating agents used in this study were maltodextrin and arabic gum. Maltodextrins are commonly used as wall material due to their high water solubility and low viscosity. Arabic gum is known in the food industry for its emulsifying properties (Avaltroni et al., 2004). The main objective of this study was to develop a functional yogurt fortified with microencapsulated omega-3 (ω-3) fatty acids and vitamin E. Plain yogurt (PY) fortified (2% w/v) with microencapsulated salmon oil (MSO) or microencapsulated menhaden oil (MMO) was produced and the physical, chemical and microbiological characteristics were evaluated during a 1 month storage period at 4 °C. PY and yogurt with 22% arabic gum and 11% maltodextrin (YPAM) were used as controls. The entire experiment was replicated three times and data was statistically analyzed at α= 0.05.
CHAPTER 2 LITERATURE REVIEW

2.1 General Information on Yogurt

2.1.1 Definition and Classification

Yogurt is a product made from heat treated milk that may be homogenized prior to the addition of lactic acid bacteria (LAB) cultures containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Code of Federal Regulations Section 131.203, 2011). Similarly, Tamime (2002) defined yogurt as a product of the lactic fermentation of milk by addition of a starter culture, which results in a decrease of milk pH to less than or equal to 4.6.

Industrially, yogurts can be largely divided into two types. A set-style yogurt is made in retail containers giving a continuous undisturbed gel structure in the final product (Tamime and Robinson, 1999). On the other hand, stirred yogurt has a delicate protein gel structure that develops during fermentation (Benezech and Maingonnat, 1994). In stirred yogurt manufacture, the gel is disrupted by stirring before mixing with fruit and then it is packaged. Stirred yogurts should have a smooth and viscous texture (Tamime and Robinson, 1999). In terms of rheology, stirred yogurt is a viscoelastic and pseudoplastic product (De Lorenzi et al., 1995). Yoghurts come in a variety of textures (e.g. liquid, set, and smooth), fat contents (e.g. luxury, low-liquid, virtually fat-free) and flavors (e.g. natural, fruit, cereal), can be consumed as a snack or part of a meal, as a sweet or savoury food, and are available all year round. This versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population subgroups (McKinley, 2005).
2.1.2 Production and Consumption

According to the United States Department of Agriculture (USDA), National Agricultural Statistics Service (2008), a total of 1.63 billion lbs. of yogurt were produced in the United States in 1998, and by 2008, production had increased 120% to 3.59 billion lbs annually. In 2009, production of yogurt totaled a record high 3.83 billion lbs and it was the 12th straight year that yogurt production set a new record. According to Lempert (2009), yogurt was one of the 6 traditional snack categories that showed economic growth in 2009, while the National Purchase Diary Group Incorporated (2010), a market research company, announced recently that yogurt is among the top growing snack foods for kids ages 2 to 17. In the food market, claiming health properties is a clear way to differentiate products and, in most cases, hike up prices and improve profit levels. Therefore, the functional food and beverage market has attracted a large number of standard food and drink companies (Granato et al., 2010). Among functional foods, dairy-based functional foods account for nearly 43% of the market, which is almost entirely made up of fermented dairy products. Dairy products containing omega-3, phytosterols, isoflavones, conjugated linoleic acid, minerals, and vitamins also have a prominent role in the development of functional foods (Ozer and Kirmaci, 2010).

Although fermented milk products such as yoghurt were originally developed simply as a means of preserving the nutrients in milk, it was soon discovered that by fermenting with different microorganisms, an opportunity existed to develop a wide range of products (McKinley, 2005). In the Western world, concepts are expanding from the past emphasis on survival, hunger satisfaction, and preventing adverse effects, to an emphasis on the use of foods to promote a well-being state, improving health, and reducing the risk of diseases. These concepts are particularly important in light of the increasing cost of health care, and the steady
increase in life expectancy (Roberfroid, 2007). The USDA, Economic Research Service (2008) reported that for 2007 the United States annual per capita consumption of yogurt, excluding frozen, was 11.5 lbs. This represents a 77% increase from 6.5 lbs per capita in year 2000. Per capita consumption of yogurt is also relatively high in high-income countries, such as Japan and Western Europe, and demand growth is unabated (USDA, 2006). There is likely to be further growth and innovation in the yoghurt sector of the dairy market in terms of nutrition and health, an area with massive growth potential because of its appeal to the health-conscious consumer in the functional yogurt sector (McKinley, 2005) and due to its potential to increase sales (Milk Facts, 2004).

2.1.3 Omega-3 Addition

Eating dairy products, such as yogurt, helps to improve the overall quality of the diet and increases the chances of achieving nutritional recommendations. Research has shown that milk consumption is positively associated with the likelihood of children and teenagers achieving the recommended intakes for vitamin A, folate, vitamin B12, calcium and magnesium (Ballew et al., 2000). It is advantageous to use yogurt as a vehicle for supplementing the diet with heart healthy nutrients (Cueva and Aryana, 2008). Many efforts have been made by the industry to develop food products and nutritional preparations that contain appreciable amounts of ω-3 PUFAs (Avramis and Jacobs, 2008). Yogurt and drinking yogurt have been shown to be especially good vehicles for fish oil enrichment, since the products are very stable towards oxidation (Nielsen et al., 2007).

Several dairy and non dairy product formats with fish oil have been reported. In the presence of such new components, the gel structure and other properties of fermented milks change. Avramis and Jacobs (2008) developed a method of manufacturing a yogurt comprising
ω-3 PUFAs. The objective was to provide a cultured edible product containing ω-3 PUFAs and a source of iron that can easily be manufactured and that does not develop an objectionable off-flavor during 5 weeks of refrigerated storage. This product comprised from 0.05% to 30% of non-encapsulated oil containing at least 0.01% of ω-3 PUFAs by weight of the edible product and at least $10^7$ viable cells of LAB. It was observed by the inventors that the pasteurization or sterilization of a product base containing added ω-3 PUFAs resulted in the immediate development of a pronounced fishy off-flavor and the addition of ω-3 PUFAs prior to fermentation also produced a fishy off-flavor. Addition of the ω-3 PUFAs after fermentation of the pre-mix did not produce significant off-flavor. Moreover, off-flavor formation could be avoided by pre-mixing the ω-3 PUFAs with previously pasteurized or sterilized fruit puree. Akahoshi et al. (2000) described the production of yogurt containing refined fish oil with no fishy odor by masking with specific sweeteners and using an oxygen blocking hermetic package. The refined fish oil used in this product was a commercially available emulsified product in which the fish oil content was 20% and DHA (C22:6 ω-3) content was about 5%. According to this invention, yogurt which contains sufficiently high amount of unsaturated fatty acids such as DHA and EPA (C20:5 ω-3) can be provided without fear of deteriorating the flavor or taste of the yogurt by selecting a specific sweet substance to control the generation of a fishy odor. Regarding texture, ω-3 fortification yielded yogurts with less firmness and higher syneresis.

2.2 Omega-3 Fatty Acids

2.2.1 Sources and Chemical Structure

One of the features of the fats and oils from fish and aquatic mammals is their high content of long chain highly unsaturated fatty acids such as EPA or DHA which are not contained in the fats of terrestrial animals or in vegetable oils (Akahoshi et al., 2000). Salmon oil is a good source
of PUFAs, specifically EPA and DHA. Values reported by the USDA National Nutrient Database (NAL.USDA.gov, 2009) indicate that every 100 g of salmon oil contain 13.02 and 18.23 g of EPA and DHA, respectively. Menhaden is an abundant marine species mainly considered as reduction fishery and it is mainly used for fish oil extraction and fish meal production (International Fishmeal and Fish Oil Organization, 2006). Menhaden oil has an EPA and DHA content of 13.17 and 8.56 g/ g of oil, respectively (NAL.USDA.gov, 2009).

All fatty acids in the ω-3 family share a common characteristic: the first unsaturated bond, corresponding to a double chemical bond, is always situated on the 3rd carbon from the metabolically non-reactive methyl end of the chain (Bourre, 2004). EPA contains 5 double bonds in its carbon chain (Fig. 2.1) while DHA contains 6 double bonds (Fig. 2.2).

![Fig. 2.1 Chemical structure of eicosapentaenoic acid](image1)

![Fig. 2.2 Chemical structure of docosahexaenoic acid](image2)
2.2.2 Oxidation

PUFAs in fish oil are readily oxidized to produce off flavor volatiles when exposed to light, oxygen, pro-oxidants, and high temperatures (McClements and Decker, 2000). Oxidation of these fatty acids is accompanied by the formation of volatile, potent flavor molecules, such as unsaturated aldehydes. In terms of sensory perception of oxidized fish oil 1-penten-3-one, 4-heptanal, 1-octen-3-ne-1, 5-octadien-3-one, 2-4-heptadienal and nonadienal have been identified as important volatiles. These products may have a very low odor threshold and consequently negatively affect sensory properties (Venkateshwarlu et al., 2004). Flavour attributes associated with oxidation products of unsaturated fatty acids include cardboard, paint, oily, rancid, and metallic. A fishy off-flavour note typically results from oxidation of ω-3 PUFAs and is regarded as particularly objectionable in dairy products (Avramis and Jacobs, 2008). Even though fish oil may not smell at the stage when it is added to foods, it often becomes odorous during storage, and exhibits an extremely deteriorated flavor and taste. Consequently, the quality of foods fortified with fish oil usually deteriorates rapidly if not stabilized (Yue et al., 2008).

The peroxide value (PV) is a usual indicator for the determination of primary oxidation products (Chávez-Servín, et al., 2008). According to Gracey et al. (1999), oil with a PV below 5 milliequivalents of peroxide (meq)/ Kg oil can be considered fresh oil or one in which hydroperoxides have degraded into secondary oxidation products, like ketones and aldehydes. The secondary oxidation products are measured by anisidine value (AV). AV is the measure of aldehyde production during oxidation of fats which is used to characterize the oxidative history of fat because aldehydes normally originate from oxidation of unsaturated fatty acids (Beare-Rogers et al., 2001). Peroxides occur in early stages and after reaching a stable concentration they begin to decompose and concentration decreases. This may cause misleading
interpretations, therefore the determination of PV, AV and calculation of total oxidation (TOTOX) values are important for a precise measure of the degree of lipid oxidation. The sum of 2 times the PV and AV is known as the TOTOX value, and this parameter provides a better picture of the overall quality status of oils (Wai et al., 2009). The high PUFAs content in fish oil, in combination with high autolytic activity and moisture, make it very prone to lipolysis. Thus, the oils extracted from fish tissue will tend to have high levels of free fatty acids (FFA) (Aryee et al., 2009). FFA values greater than 3% are considered inedible (Gracey et al., 1999).

2.2.3 Health Benefits

A high intake of EPA and DHA has been associated with reduction of cardiovascular disease (CVD) risk (Prisco et al., 1995), symptoms of rheumatoid arthritis (Kremer et al., 1995), inflammatory bowel disease (Belluzzi et al., 2000), risk of certain cancer forms (Jacobsen, 2010) and risk of developing depression (Mischoulon and Fava, 2000). CVD are ranked as the number one killer in the United States. Coronary heart disease caused approximately 1 of every 6 deaths in the United States in 2006 and the burden of CVD remains high and prevalence and control of traditional risk factors remains an issue for many Americans. In 2010, the total direct and indirect costs of CVD and stroke in the United States were estimated to be $503.2 billion (American Heart Association, 2010). Many scientific publications strongly suggest that regular consumption of significant amounts of PUFAs rich in ω-3 fatty acids can be highly effective in the prevention or treatment of CVD. The mechanisms responsible for the observed effects of ω-3 fatty acids on cardiovascular health are not known with confidence (Kris-Etherton et al., 2002). The ones possibly involved are: decrease in serum triglyceride concentration, atherosclerotic plaque growth retardment and hypotensive, antithrombogenic and anti-inflammatory effects (Connor, 2000).
In addition, ω-3 fatty acids are essential to normal neuronal development and their depletion has been associated with neurodegenerative diseases such as Alzheimer’s disease. In the human eye and retina, the ratio of DHA: EPA is 5: 1 and their presence is necessary for normal eye development (Garter et al., 2008). An average of one in three fatty acids in the nervous system is polyunsaturated. Position 2 of phospholipids is generally occupied by a PUFA, which is usually 20:4ω6 (arachidonic acid), 22:4ω6 (adrenic acid), 22:5ω3 (docosapentaenoic acid) and especially DHA. PUFAs of the ω-3 series have very special roles in cell membranes, especially in the nervous system: all brain cells and organelles contain high levels of these fatty acids, but they are not present in sufficient quantities in modern occidental diets (Bourre, 2004).

2.2.4 Intake
Since the first omega-3 fatty acid advisory, the Food and Drug Administration (FDA) has ruled that intakes of up to 3 g/d of marine ω-3 fatty acids are Generally Recognized as Safe (GRAS) for inclusion in the diet (Stone, 1996). Intake of PUFAs is generally too low in the diet of Western developed countries, and it is recommended that intake of these acids be increased by supplementation of selected foods (Bauch et al., 2006). The intake of total ω-3 fatty acids in the United States is ≈ 1.6 g/d (Kris-Etherton et al., 2000). Of this, α-linolenic acid (18:3ω3) accounts for ≈ 1.4 g/d, and only 0.1 to 0.2 g/d comes from EPA and DHA (USDA, 2002). A number of countries (Canada, Sweden, United Kingdom, Australia, and Japan) as well as the World Health Organization and North Atlantic Treaty Organization have made formal population-based dietary recommendations for ω-3 fatty acids. Typical recommendations are 0.3 to 0.5 g/d of EPA + DHA (Institute of Medicine, 2002). Patients with CVD should be encouraged to increase their consumption of EPA and DHA to ≈ 1 g/d. Although these levels of EPA and DHA intake potentially can be attained through fish consumption, with an emphasis on
fatty fish (i.e., salmon, herring, and mackerel), the required amount of fish intake may be
difficult to achieve and sustain over the long term. For individuals who do not eat fish, have
limited access to a variety of fish, or cannot afford to purchase fish, a fish oil supplement may be
considered (Kris-Etherton et al., 2002). Both EPA and DHA have been made available in caplet
form. According to Garter et al. (2008) consumers do not generally enjoy consuming the caplets
in part because they are large and also because the caplets can develop a fishy rancid type odor
rapidly. Considering the issues disclosed above, ω-3 intake can be enhanced by using
microencapsulation technology to enrich commonly consumed dairy foods.

2.3 Vitamin E

2.3.1 Chemical Structure

Vitamin E is the generic term for tocols and tocotrienols that exhibit vitamin activity similar to
that of α-tocopherol (American Institute of Nutrition, 1987). Tocopherols, that are typically the
main compounds having vitamin E activity in foods, are derivatives of the parent compound
tocol, and have one or more methyl groups at positions 5’, 7’, or 8’ of the ring structure or
chromanol ring. The α (Fig. 2.3), β, γ, and δ forms of tocopherol differ according to the number
and position of the methyl groups. Vitamin E is very non polar and exists mainly in the lipid
phase of foods (Thompson and Hatina, 1979).

![Chemical structure of α-tocopherol](image_url)

Fig. 2.3 Chemical structure of α-tocopherol
2.3.2 Role in Human Metabolism

According to the World Health Organization and the Food and Agriculture Organization of the United Nations (2004), vitamin E is an important component of the cell’s antioxidant defense system in the phospholipid bilayer. It prevents peroxidation of PUFAs in cell and cell components such as endoplasmic reticulum and mitochondria. Oxidation products are associated with numerous diseases and clinical conditions like cardiovascular diseases and neurological disorders. If exposure to free radicals exceeds the protective capacity of the antioxidant defense system, a phenomenon called “oxidative stress” may damage biological molecules. There is considerable evidence that diseases cause an increase in oxidative stress; therefore, consumption of foods rich in antioxidants, which are potentially able to quench or neutralize excess radicals, may play an important role in modifying the development of disease. The recommended daily allowance of α-tocopherol for adult males and females is 15 mg/day and no toxicity or pro-oxidant effect has been reported below 200 mg/day (Institute of Medicine, 2004). The bioavailability of vitamin E compounds is usually quite high in individuals who digest and absorb fat normally (Burton and Traber, 1990).

2.3.3 Antioxidant Properties in Foods

Oxidative products in foods are responsible for initiating oxidative rancidity, which is deteriorative in the processing and storage of lipid containing foods (Al-Ismail and Aburjai, 2004). Synthetic antioxidants such as TBHQ (tertiary butyl hydroquinone), BHA (Butylated hydroanisole), and BHT (butylated hydroxytoluene), alpha tocopherol acetate, and EDTA (ethylene diamine tetra acetic acid) are used for retarding fish oil oxidation. However, the addition of those artificial chemicals is restricted by the FDA because of food safety concerns, not to mention emerging trends for consumer preferences toward more “green” food processing.
applications (Yue et al., 2008). It is therefore of interest to mention vitamin E as an important natural antioxidant which inhibits lipid peroxidation (Lúcio et al., 2009). Vitamin E is being used to maintain food quality and extend shelf life by preventing or delaying oxidation of the labile fatty acids and lipid soluble components (Eitenmiller and Lee, 2004). Despite the growing understanding of the role of vitamin E as oxidant protector in food and biological systems, its mechanism of action is not completely understood yet (Traber and Atkinson, 2007).

Vitamin E is unstable in the presence of heat, oxygen, light, and even some unsaturated fats. Consequently, most processed and convenience foods do not contribute enough vitamin E to ensure an adequate intake (Whitney and Rolfes, 2008). Furthermore, at high concentrations, some antioxidants, such as tocopherol may have the opposite effect and become pro-oxidants (Jacobsen, 2010).

2.4 Microencapsulation

2.4.1 Definition

The encapsulation of active components in powders has become a very attractive process in the last decades (Fuchs et al., 2006). Microencapsulation is a process by which particles of sensitive or bioactive materials are packed into thin films of a coating material (Shahidi and Han, 1993). The main objective is to build a barrier between the component in the particle and the environment (Fuchs et al., 2006). The wall protects the core material against deterioration (Beatus et al., 1985) which can lead to loss of nutritional value and the development of off flavors (Velasco et al., 2000). Microencapsulation is also used to transform liquids into dry, free flowing powders, enhance handling properties, limit losses of volatile materials, and control release of active material (Onwulata et al, 1994). The barrier may protect against oxygen, water, light and avoid contact with other ingredients in a ready meal (Fuchs et al., 2006). In the food
industry, typical core materials are aroma and flavor compounds, essential oils, vitamins, minerals, oils and fats (Rosenborg and Young, 1993). The process of oil microencapsulation consists basically on the preparation of an oil in water emulsion containing the matrix components in the aqueous phase, which is then dried (Velasco et al., 2003). They are commonly microencapsulated by spray-drying which is a well-established technique in the food industry (Jackson and Lee, 1991). Microencapsulated fish oils (MFO) are used as functional ingredients in a growing number of milk and bakery products (Velasco et al., 2003).

2.4.2 Microencapsulation by Spray Drying

Among the different techniques available for the encapsulation of bioactive food ingredients, spray drying is the most important technique (Desai and Park, 2005). The production of oil and fat microcapsules by spray drying requires the core material to be dispersed homogeneously and finely in a continuous phase containing the wall material, forming an emulsion (Sims, 1989). The process involves four stages: preparation of a dispersion or emulsion; homogenization of the dispersion; atomization of the feed emulsion; and dehydration of the atomized particles (Shahidi and Han, 1993). Many different inlet/outlet temperature combinations are used, in most of the cases based on core material, wall material, addition ratios and equipment capacity. Typical inlet/outlet temperatures used in research for microencapsulation include 180/70 °C, 160/60 °C and 210/90 °C for n-octenylsuccinate-derivatized starch and glucose syrup coated fish oil (Serfert et al., 2009; Drusch and Berg, 2008), 210/95 °C for refined menhaden oil (45%) encapsulated with 49.65% corn syrup, 7.5% sodium caseinate, 2% lecithin and 0.85% potassium phosphate (Baik et al., 2004) and 180/80 °C for avocado oil using whey protein isolate and maltodextrin in a 33.3: 66.7 oil to wall solids ratio. The effectiveness of spray-drying is critically dependant on the type of wall material used, which will influence emulsion stability before drying, mechanical
stability and shelf life after drying. For spray drying to yield powder with high quality attributes, the emulsion should have the following properties: small size and narrow distribution of oil droplets stable to agglomeration and coalescence, a high solid content for enabling formation of a continuous matrix as a protective barrier where oil droplets are uniformly distributed and embedded, a low viscosity for easy flow, pump and spray, and a proper ratio of wall and oil materials (Bae and Lee, 2008). Important attributes evaluated in spray dried encapsulated oil powders are: regular shape, surface oil content, microencapsulation efficiency, moisture content, oxidative stability, particle size, wettability, flowability and color. Emulsification and spray drying are used to have a composition of 25-30% oil in matrix (Barrier and Rousseau, 1998) and particle size ranging from 10-100 µm (Konstance et al., 1995). According to Drusch and Berg (2008), depending on the extraction method used, oil load, and spray drying conditions, the non-encapsulated oil (% of the total oil) of fish oil microcapsules ranges from 0.99 ± 0.03 to 13.5%. In conclusion, spray drying is an economical and flexible process for microencapsulation (Velasco et al., 2003).

2.4.3 Oxidation of Microencapsulated Fish Oil

Lipid oxidation in microencapsulated oils is of paramount importance because it results in loss of nutritional value and development of undesirable flavors in a wide range of commercialized food products (Velasco et al., 2000). Although encapsulation itself prevents lipid oxidation (Serfert et al., 2009), in applications like encapsulation of oils rich in PUFA, non-encapsulated core material may strongly impair product acceptability. Oxidation is the main cause of deterioration of lipids and it is characterized by the production of a wide range of hydroperoxides caused by the reaction of atmospheric oxygen with unsaturated lipids. Oxidation of PUFA leads to development of volatile secondary oxidation products. These products may have a very low odor
threshold and consequently negatively affect sensory characteristics of the microcapsules (Venkateshwarlu et al., 2004) and limit the shelf-life of the food products to which they are added. High inlet and outlet air temperature may lead to ballooning and autoxidation of the encapsulated and non-encapsulated core material during the drying process (Drusch and Schwarz, 2006). Lipid oxidation as determined by PV or AV was high in spray dried (210/90 °C) microcapsules with 50% fish oil load. Propanal, a major volatile secondary lipid oxidation product, was increased in samples with 30% oil load spray-dried at 210/90 °C (Drusch and Berg, 2008). Tests of accelerated oxidation at 60 °C of spray dried and fluidized bed agglomerated encapsulated vegetable oil have shown that at time zero after encapsulation, the encapsulated oil is more oxidized than the initial oil. This can be due to the evolution during the different processes. After 8 weeks, the encapsulated oil was not oxidized when compared to non-encapsulated free oil (Fuchs et al., 2006). Augustin and Sanguansri (2003) investigated the effect of encapsulating tuna oil in a mix of protein and carbohydrates (casein/sugar). These two components were pre-heated together to form Maillard reaction products, which are antioxidants. The process improved resistance to oxidation. In a recent study by Serfert et al. (2009), the process of emulsification led to a significant development of lipid hydroperoxides when using fish oil (27% EPA + DHA) without added antioxidants. The hydroperoxide content increased from 4.6 mmol/ Kg oil to 17.8 mmol/ Kg oil. Intense mechanical stress and agitation due to shear and turbulence during the preparation of the emulsion lead to oxygen inclusion. The microcapsules were stored during 56 days at 20 °C and it was reported that the addition of rosemary extract, rich in carnosic acid, to the emulsions retarded autoxidation. At the end of the storage study, the hydroperoxide and propanal content amounted to approximately 20 mmol/ Kg oil and a maximum of 47 µmol/ Kg oil, respectively. Microencapsulation may be used in
combination with antioxidants and/or masking flavors. However, additional measures (packaging, neutral atmosphere) are recommended. The addition of α-tocopherol (100 ppm) delayed the oxidation of fish oil encapsulated with sodium caseinate (25-50% w/w of oil) (Hogan et al., 2003).

In non supplemented control infant formula (CIF) and microencapsulated fish oil-supplemented infant formula (SIF) the initial PVs before storage were 0.55 and 0.85 meq/ Kg, respectively. In both samples stored at 25 °C only slight increases in PV were recorded. PV after 12 months of storage for CIF and SIF were 0.83 meq/ Kg and 2.02 meq/ Kg, respectively (Chávez-Servín, et al., 2008). In a study by Kelly and Keogh (2000) the infant formula produced from microencapsulated powder, at 100% RDA for ω-3 PUFAs, did not deteriorate in sensory shelf life properties over a 5 week storage period even though there was an increase in PV during storage. Furthermore, 3 days storage of breads prepared from commercial and experimentally produced fish oil powders, under ambient conditions showed little increase in PV.
CHAPTER 3 MATERIALS AND METHODS

3.1 Emulsion Preparation and Spray Drying of Microencapsulated Fish Oils

Microencapsulated fish oil powders were produced as described in Fig. 3.1. Purified menhaden oil (PMO) was provided by Omega Protein® Inc. (Houston, TX) and crude salmon oil (SO) was obtained from the USDA/ARS Subartic Research Unit (Fairbanks, AK). SO was purified using chitosan as described by Huang and Sathivel (2010). Five percent (w/v) shrimp chitosan was added to the SO and the mixture was stirred for 1 hour at room temperature. Then the mixture was centrifuged (23,385.6 x g) for 25 min at 4 °C and the supernatant purified salmon oil (PSO) was collected. PMO and PSO (Appendix: Characteristics of PMO and PSO) were used to produce a stable emulsion using a CPX- 500 ultrasonic processor (Cole Parmer Instruments, Vernon Hills, IL). The following ratio of ingredients was mixed with constant stirring using a RCT B S1 magnetic stirrer (IKA Labrotechnik, Janke & Kunkel GmbH & Co., Staufen, Germany): 7% MO or SO, 22% Encapcia™ gum arabic (Colloides Naturels, Bridgewater, NJ), 11% maltodextrin DE 20 (NOW Foods, Bloomingdale, IL), 0.83% Fluka Analytical α-tocopherol (Sigma Aldrich, Co., St. Louis, MO) and 59.17% distilled water (Table 3.1). The ultrasonicator was set to deliver 65,000 J at 80% amplitude to each batch (150 mL/batch) of emulsion in a 250 mL glass beaker constantly cooled to a temperature of approximately 26 °C by an ice bath. The emulsions were kept refrigerated at 5 ± 2 °C for no longer than 20 min in a 1000 mL tightly sealed glass bottle (Pyrex®, Germany) wrapped with aluminum foil. The emulsion was spray dried to produce MMO or MSO in a pilot scale FT80 tall form spray drier (Armfield Ltd., Ringwood, UK) at an inlet temperature of 180 ± 1 °C, compressed air supply of 100 KPa and feed pump, inlet fan, and exhaust fan at speeds of 4 Hz, 32 Hz and 36 Hz, respectively.
Table 3.1 PAM, MMO, and MSO Emulsions Formulations

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Addition Rate</th>
<th>PAM</th>
<th>MMO</th>
<th>MSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (g)</td>
<td>67% / 59.17%</td>
<td>670.0</td>
<td>591.7</td>
<td>591.7</td>
</tr>
<tr>
<td>Gum arabic (g)</td>
<td>22%</td>
<td>220.0</td>
<td>220.0</td>
<td>220.0</td>
</tr>
<tr>
<td>Maltodextrin (g)</td>
<td>11%</td>
<td>110.0</td>
<td>110.0</td>
<td>110.0</td>
</tr>
<tr>
<td>PMO (g)</td>
<td>7%</td>
<td>-</td>
<td>70.0</td>
<td>-</td>
</tr>
<tr>
<td>PSO (g)</td>
<td>7%</td>
<td>-</td>
<td>-</td>
<td>70.0</td>
</tr>
<tr>
<td>α-tocopherol (g)</td>
<td>0.83%</td>
<td>-</td>
<td>8.3</td>
<td>8.3</td>
</tr>
</tbody>
</table>

PAM = Arabic gum-maltodextrin powder, MMO = microencapsulated menhaden oil, MSO = microencapsulated salmon oil, PMO = purified menhaden oil, PSO = purified salmon oil.

A mixture containing 22% gum arabic, 11% maltodextrin, and 67% distilled water was ultrasonicated and spray dried as described above to produce a powder containing arabic gum and maltodextrin (PAM) and it was used for a control yogurt in this study. All powders were stored around 4 to 5 days at -18 ± 3 °C in a walk in blast freezer (Master-Bilt Products, New Albany, MS) in dark tightly sealed glass bottles.

Fig. 3.1 Flow Diagram for MMO and MSO Production
3.2 Microencapsulation Efficiency (ME) and Encapsulation Yield (EY) of MMO and MSO

The method described by Wanasundara and Shahidi (1995) was used to determine the microencapsulation efficiency of MMO and MSO. The total oil (TO) (%) of microencapsulated fish oils contained both encapsulated and surface oils. The total oil was determined by dissolving 100 g of MMO or MSO in 500 ml of a 0.88% (w/v) potassium chloride (Sigma Aldrich, Co., St. Louis, MO) solution in a 2 L glass beaker with constant stirring. A few crystals of 97% tert-butylhydroquinone (Sigma Aldrich, Co., St. Louis, MO) and 1.5 L of 2:1 chloroform (Mallinkrodt Baker Inc., Phillipsburg, NJ): methanol (Sigma Aldrich, Co., St. Louis, MO) solution were added to the mixture which was centrifuged in a Beckman J2-HC centrifuge (GMI Inc., Ramsey MN) at 23,385.6 x g during 5 min at 4 °C. The mixture was transferred to a separatory funnel to separate the chloroform layer supernatant, which was collected in a round bottom flask for subsequent evaporation in a rotary evaporator (Buchi RE-121 Rotavapor, Switzerland) under vacuum at 40 °C to minimize oxidation. The TO (%) of MMO and MSO was calculated gravimetrically. The surface oil content (SO) (%) of the powder was estimated by mixing 2 g of MMO or MSO with 10 mL of 95% n-hexane (Mallinkrodt Baker Inc., Phillipsburg, NJ) during 10 min, filtering the mixture through Whatman No. 4 filter paper (Whatman International Ltd, England), and washing 4 times with 10 mL of hexane. The washed sample in the filter paper was dried in an air oven (VWR™ Model No. 1330FM. Sheldon Manufacturing INC. Cornelius, OR) at 70 °C until hexane evaporation was complete and a constant weight was obtained. The SO (%) was calculated gravimetrically. ME (Eq. (3.1)) was calculated as reported by Shen et al. (2010).
\[ ME = \frac{TO - SO}{TO} \times 100 \]  

(3.1)

EY was determined using Eq. (3.2):

\[ EY = \frac{OM \text{ (g/100 g solids)}}{OE \text{ (g/100 g solids)}} \]  

(3.2)

EY is the ratio of the oil in the microcapsules (OM) (g/100 g of solids) to the oil in the emulsion (OE) (g/100 g of solids).

3.3 Peroxide Value, Anisidine Value, Total Oxidation, Free Fatty Acids, Color and Moisture of PAM, MMO and MSO

The peroxide value (PV), anisidine value (AV), and free fatty acids (FFA) content were tested to determine the lipid oxidation of MMO and MSO. Samples used were obtained from the oil extracted in the TO analysis described in section 3.2. The PV (meq/Kg) and AV were determined following American Oil Chemists Society (AOCS) Official Methods (1997). The acetic acid-chloroform method (AOCS Cd 8-53) was used to determine the PV. Briefly, 2 g of oil were weighed into a 125 mL Erlenmeyer flask and 12 mL of 3:2 acetic acid (Fisher Scientific, Fair Lawn, NJ): chloroform were added under an air hood. The flask was manually swirled until the sample was dissolved and 0.2 mL of a saturated Potassium iodide (Mallinkrodt Baker Inc., Phillipsburg, NJ) solution were added using an Eppendorf® automatic pipette (Eppendorf North America, Hauppauge, NY). The solution was allowed to stand for 1 min with occasional swirling before addition of 12 mL of distilled water. A BioChemika starch solution (Sigma Aldrich, Co., St. Louis, MO) was added (4 drops) and the resulting blue solution was titrated with 0.01 N Na2S2O3 (Sodium thiosulfate) (Mallinkrodt Baker Inc., Phillipsburg, NJ) added drop wise with
constant shaking until the blue color disappeared. The same procedure was carried out for a blank solution. The PV of oils was calculated as indicated in Eq. (3.3) in which $S$ is the volume (mL) of Na$_2$S$_2$O$_3$ used to titrate the sample, $B$ is the volume (mL) of Na$_2$S$_2$O$_3$ used to titrate the blank, $M$ is the molar concentration of the Na$_2$S$_2$O$_3$ solution (N), and $W$ is the weight of the sample in grams.

$$PV = \frac{(S-B) \times M \times 1000}{W}$$  \hspace{1cm} (3.3)

Official method AOCS Cd 18-90 was used to determine AV which is given by Eq. (3.4):

$$AV = \frac{25 \times (1.2 \times As - Ab)}{m}$$  \hspace{1cm} (3.4)

Approximately 1 g of oil ($m$) was weighed into a 25 mL volumetric flask and dissolved to volume with anhydrous 99.8% 2, 2, 4 – trimethylpentane (isooctane) (Sigma Aldrich, Co., St. Louis, MO). The absorbance of the solution ($Ab$) at 350 nm was measured in a cuvette with a Genesys 20 spectrophotometer model 4001/4 (Thermo Fisher Scientific, Fair Lawn, NJ), using a reference cuvette filled with solvent as a blank. Exactly 5 mL of the fat solution were pipetted into a test tube and exactly 5 mL of the solvent were added into a second test tube. By means of an automatic pipette, 1 mL of a 99% p-anisidine (Sigma Aldrich, Co., St. Louis, MO) reagent solution (0.25 g/ 100 mL glacial acetic acid) was added to each tube followed by vigorous shaking in a mini MV-1 vortexer (IKA-Works Inc., Wilmington, NC). After exactly 10 min, the absorbance ($As$) of the solvent in the first test tube was measured at 350 nm, using the solution from the second test tube as a blank in the reference cuvette. TOTOX values (Eq. (3.5)) were calculated as described by Wai et al., (2009).
\[ TOTOX = 2PV + AV \]  

FFA content (\%) was determined using a titration method described by the AOCS Ca 5a-40 Official Method (1997), with slight modifications. Two grams of the oil samples were mixed with 10 ml of heated and neutralized ethanol (Fisher Scientific, Fair Lawn, NJ) solution and 0.4 ml of phenolphthalein (Sigma Aldrich, Co., St. Louis, MO) in a 125 ml flask. The mixture was titrated using 0.1 N Sodium hydroxide (\textbf{NaOH}) (Mallinkrodt Baker Inc., Phillipsburg, NJ) until a faint pink color was retained. The \% FFA (Eq. (3.6)) expressed as oleic acid was calculated as follows:

\[
FFA(\%) = \frac{NaOH (mL) \times N \times 28.2}{Mass(g)}
\]  

(3.6)

N stands for the normality of the NaOH and mass (g) refers to the mass of sample used.

A Lab Scan XE spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA) was used for color analysis of PAM, MMO and MSO. The spectrophotometer was standardized using white and black tiles and results are reported in \( L^* \) (lightness), \( a^* \) (greenness- redness), and \( b^* \) (blueness- yellowness) values. Approximately 2 g of PAM, MMO or MSO were placed in a thin, even layer over a glass fiber sample pad (CEM Corporation, Matthews, NC) for moisture analysis in a Moisture Analyzer Smart System 5 (CEM Corporation, Matthews, NC). Results are reported as percent moisture.

3.4 Scanning Electron Microscopy of PAM, MMO and MSO

The scanning electron microscopy analysis was conducted in the Louisiana State University Socolofsky Microscopy Center. A JSM-6610 high-performance scanning electron microscope (SEM) (JEOL Ltd., Tokyo, Japan) with an acceleration voltage of 5 kV was used for fast
characterization, imaging and measurement of powder structures. Prior to SEM imaging, a thin layer of PAM, MMO or MSO was placed over an aluminum stub for a 25 mA sputter coating with gold in an EMS 550X Sputter Coater (Electron Microscopy Sciences, Hatfield, PA) with a vacuum of $1 \times 10^{-1}$ mbar during 2 min.

3.5 Production of Functional Yogurt Fortified with Omega-3 and Vitamin E

Plain stirred yogurt fortified with $\omega$-3 fatty acids and vitamin E was produced using pilot scale equipment in the Louisiana State University Agricultural Center Dairy Processing Plant (Fig. 3.2).

![Flow Diagram for Yogurt Processing](image)

Fig. 3.2 Flow Diagram for Yogurt Processing
All equipment, utensils and containers were sanitized before use with a 200 ppm Sodium hypochlorite (Eurochem International Corporation, Atlanta, GA) solution. Pasteurized partially skinned milk (Great Value®, Bentonville, AR) was obtained from a local store. Grade A Non-fat dry milk (NFDM) was obtained from Dairy America, Fresno, CA. Extra fine granulated sugar was obtained from Domino Foods, Inc., Yonkers; NY. BioXtra ≥99.0% Sodium benzoate was obtained from Sigma Aldrich, Co., St. Louis, MO; starter cultures of L. bulgaricus (LB-12) and S. thermophilus (ST-M5) were obtained from Chr. Hansen, Milwaukee, WI. Yogurt mix formulations are reported in Table 3.2.

Table 3.2 Yogurt Mix Formulations

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Addition Rate</th>
<th>PY</th>
<th>YPAM</th>
<th>YMMO</th>
<th>YMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (Kg)</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>NFDM (g)</td>
<td>5% / 3%</td>
<td>190.0</td>
<td>114.0</td>
<td>114.0</td>
<td>114.0</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>3%</td>
<td>114.0</td>
<td>114.0</td>
<td>114.0</td>
<td>114.0</td>
</tr>
<tr>
<td>PAM (g)</td>
<td>2%</td>
<td>-</td>
<td>76.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MMO (g)</td>
<td>2%</td>
<td>-</td>
<td>-</td>
<td>76.0</td>
<td>-</td>
</tr>
<tr>
<td>MSO (g)</td>
<td>2%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>76.0</td>
</tr>
<tr>
<td>L. bulgaricus (ml)</td>
<td>1 ml/ 3.8 L</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>S. thermophilus (ml)</td>
<td>1 ml/ 3.8 L</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium benzoate (g)</td>
<td>0.10%</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil, NFDM = non fat dry milk, PAM = Arabic gum-maltodextrin powder, MMO = microencapsulated menhaden oil, MSO = microencapsulated salmon oil.

Yogurt mixes were prepared in 3.9 Kg batches in 8 L stainless steel containers from partially skimmed fluid milk. Mixes for plain yogurt with MMO (YMMO) and MSO (YMSO) were produced and PY and YPAM were used as controls. The NFDM, sugar and MMO, MSO or PAM were added to milk preheated to 45 ± 2 ºC, with constant manual stirring. Preheated mixes were homogenized in a two stage Gaulin 300 DJF 4 2PS homogenizer (APV Gaulin,
Wilmington, MA) at 3447.4 and 10,342.1 KPa in the first and second stages, respectively. Homogenized mixes were batch pasteurized at 85 ± 1 °C for 30 min and then rapidly cooled in an ice bath to 43 ± 1 °C for inoculation with freshly thawed starter cultures. Incubation was carried in an incubator Model 815 (Thermo Scientific, Two Rivers, WI) at 43 ± 1 °C, until a pH around 4.50 was reached. The yogurts were quickly cooled in an ice bath and stored overnight at 4 °C in a walk-in cooler. Stirred yogurt was produced by manually stirring gels using a stainless steel perforated milk stirrer (Nelson Jameson, Marshfield, WI) following a standard procedure of 35 full rotations around the container. Sodium benzoate was added during the stirring process as a preservative. The finished plain stirred yogurt batches were packed and tightly sealed in 5 clear polypropylene containers (1.5 L) (Reynolds Plastic, Richmond, VA). Each container was filled with approximately 550 g of yogurt, wrapped with aluminum foil and refrigerated at 4 °C. Containers were labeled: initial, week 1, week 2, week 3 and week 4 and were removed from the walk in cooler (Vollrath Refrigeration Division, Riverfalls, WI) only at the time of analysis labeled.

3.6 Fatty Acid Methyl Esters (FAMEs) Analysis of MMO, MSO and Yogurts

FAMEs content, expressed as a percentage of the FAMEs detected, was measured by gas chromatography in lipids extracted from MMO and MSO prior to use in yogurt production and in PY, YPAM, YMMO, and YMSO during 1 month of refrigerated storage at 4 °C. Lipids were extracted from yogurts using a modified Folch extraction method (2010). A 50 g sample of yogurt was dissolved in 1 L of 2:1 chloroform: methanol solution and filtered through Whatman No. 4 filter paper. The filtrate was mixed with 200 mL of a 0.66% sodium chloride (Mallinckrodt Baker Inc., Phillipsburg, NJ) solution and subsequently centrifuged at 649.6 x g for 15 min. The chloroform layer containing the lipids was evaporated under vacuum using a rotary evaporator at
40 °C until the evaporation was complete. The extracted lipids were stored in a blast freezer at -20 °C until analyzed.

FAMEs were prepared using the method described by Maxwell and Marmer (1983). Oil samples were methylated for analysis of fatty acid profile. Approximately 20 mg of oil were weighed into a 10 ml glass test tube and 4.5 ml of isooctane, 500 µL of internal standard (10 mg methyl tricosanoate (23:0)/ ml isooctane) and 500 µL 2N potassium hydroxide (1.12 g/ 10 mL methanol) were added. The mixture was vortexed during 1 min and centrifuged to separate the layers for 3 min at 3200 rpm. The lower methanol layer was discarded using a glass pipette, 1 ml of saturated ammonium acetate solution was added and the mixture was vortexed and centrifuged again to neutralize the potassium hydroxide. Deionized water was added (1 ml) and vortexing and centrifugation were repeated to wash ammonium acetate from the isooctane layer. The final lower layer was removed and 2-3 g of anhydrous sodium sulfate were added, the mixture was vortexed and let to sit for 20-30 min prior to a 15 min centrifugation at 3200 rpm. The liquid containing methyl esters in isooctane was transferred with a glass pipette to a 10 mL glass test tube and a 0.5 ml sample was transferred to an amber GC vial. Isooctane (0.5 ml) was added to each sample and samples were stored at -70 °C until analysis in a gas chromatograph coupled to a flame ionization detector (Agilent Technologies Inc, Santa Clara, CA). Separation was made on a FAMEWAX (30 m X 0.32 mm i.d., 0.25 mm film) capillary column (RESTEK, USA). Helium gas was the carrier at a constant flow rate of 2 ml/ min. The inlet and detector temperatures were 250 °C and 280 °C, respectively. The temperature program was as follows: 195 °C with a 0 min hold; increased to 240 °C at 5 °C/min with a 2 min hold followed by a 2 min post run at 240 °C and an average velocity of 64 cm/s. Standards used for identification of peaks were Restek 35077, 35066, 35024 and 35027. The internal standard used was C23:0.
3.7 Determination of Alpha Tocopherol Content Using HPLC

The α-tocopherol concentration of lipids extracted from PY, YPAM, YMMO and YMSO was determined using the method described by Jang and Xu (2009). Lipids were extracted from yogurts using the modified Folch extraction method described in section 2.6. All solvents used were HPLC-grade (Fisher Scientific, Fair Lawn, NJ). A sample (0.2 g) of oil was dissolved in 2 mL of hexane in a glass test tube and vortexed. The mixture was transferred to HPLC vials and 25 μL were injected into the HPLC system for analysis. The HPLC system consisted of Waters (Milford, MA) 510 pumps, a 715 Ultra WISP injector, and fluorescence detectors. Chromatograms were recorded and processed using Waters Millennium chromatography software. Samples were injected into a 25 cm × 4.6 mm diameter 5-μm Supelcosil LC-Si (Supelco, Bellefonte, PA) column. The column was preceded by a 5 cm × 4.6 mm i.d. guard column packed with 40-μm pellicular silica. The mobile phase consisted of 0.5% ethyl acetate and 0.5% acetic acid in hexane at a flow rate of 1.5 mL/ min. The fluorescence detector was set at 290 nm excitation and 330 emission to monitor α-tocopherol. The α-tocopherol concentration was determined and expressed as μg/ g yogurt.

3.8 Thiobarbituric Acid Values of Yogurts

The thiobarbituric acid (TBA) value is an analysis in which secondary oxidation products of the lipid soluble components of yogurt react with TBA forming condensation products the absorbance of which is measured at 530 nm. The analysis was conducted following the procedure described by Hekmat and McHamon (1997). One gram of yogurt was weighed into a glass screw top test tube, 9 ml of a 15% (w/v) trichloroacetic acid (Sigma Aldrich, Co., St. Louis, MO) and 0.375% (w/v) of TBA reagent (Sigma Aldrich, Co., St. Louis, MO) in hydrochloric (0.25N) acid (Sigma Aldrich, Co., St. Louis, MO) solution were added, mixed well
and heated in a boiling water bath (LAB-LINE Instruments Inc., Melrose Park, IL) for 15 min. Samples were quickly cooled to room temperature with running water and centrifuged at 6,311 x g for 15 min at 20 °C. Absorbance was measured at 530 nm and the malonaldehyde (MDA) concentration was calculated with the extinction coefficient of 1.56 x 10^5 M^-1 cm^-1 using Eq. (3.7):

\[ A_{\lambda} = \varepsilon \cdot c \cdot L \]  

(3.7)

Where \( A_{\lambda} \) is absorbance, \( \varepsilon \) is the extinction coefficient, \( c \) is the molar concentration expressed in moles of MDA per Kg of yogurt and \( L \) is the light path (1 cm). TBA was reported as \( \mu \)moles MDA/ Kg yogurt.

### 3.9 Lactic Acid Bacteria Counts, pH, Water Holding Capacity and Color of Yogurts

Lactic acid bacteria (LAB) counts, pH, water holding capacity (WHC) and color analyses of PY, YPAM, YMMO and YMSO were conducted the same day of stirring and packaging and after 1, 2, 3 and 4 weeks of refrigerated storage. LAB counts were determined by making serial dilutions of yogurt samples in 3M™ MRS broth for LAB (3M Microbiology, St. Paul, MN) and plating 1 mL of each dilution in duplicate on 3M™ Petrifilm™ aerobic count plates (3M Microbiology, St. Paul MN). The petrifilms were labeled and incubated in a gravity convection incubator (Thermo Fisher Scientific, Fair Lawn, NJ) under anaerobic conditions using a GasPak system (BD, Franklin Lakes, NJ) at 35 °C during 48 h. All the reddish-brown colony forming units (CFU) on the petrifilm plates were counted on a standard colony counter (Leica Inc., Buffalo, NY) and reported as Log CFU/ g.

The pH was measured using an EcoTestr pH meter (Eutech Instruments, Vernon Hills, IL) at about 6 ± 1 °C. The pH meter was calibrated using commercial pH 4.00 and 7.00
buffer solutions (Thermo Fisher Scientific, Beverly, MA). The WHC of yogurt, expressed as syneresis, was determined using the method described by Cueva and Aryana (2008). Syneresis was determined by emptying 200 g of yogurt into a cheese cloth lined funnel placed on top of a graduated cylinder. The amount of whey collected after 2 h at room temperature was used as an index of syneresis. A Lab Scan XE spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA) was used for color analysis of yogurts. The spectrophotometer was standardized using white and black tiles and results were reported in L*, a*, and b* values. Total color differences (ΔE*) (Eq. (3.8)) were calculated as follows:

\[ \Delta E^* = \sqrt{\Delta L^*}^2 + \Delta a^*^2 + \Delta b^*^2 \]  

(3.8)

ΔL*, Δa*, and Δb* are the differences of the L*, a* and b* values between the storage samples and day 1 samples.

### 3.10 Rheological Analysis of Yogurts

The flow behavior and viscoelastic properties of PY, YPAM, YMMO and YMSO at 5 ºC were determined weekly during 1 month using an AR 2000 ex Rheometer and Universal Analysis (TA Instrument, New Castle, DE, USA) software with fitted plate geometry using plates of 40 mm in diameter. TA instruments states that the AR2000ex can accurately measure torque down to 0.1 μNm. In order to simulate consumer behavior, yogurt samples were gently stirred with a spoon before testing the rheological properties. The flow behavior of yogurts was evaluated with a 1000 μm gap between plates as described by Ozer et al. (1999). The shear stress was measured at
shear rates from 0.00185 to 116 s\(^{-1}\). Since all samples presented a yield stress, the flow behavior was modeled using the Herschel-Bulkley model (Eq. (3.9)).

\[
\sigma = \sigma_o + K (\dot{\gamma})^\eta
\]  

(3.9)

In Eq. (2.8) \(\sigma\) is shear stress (Pa), \(\sigma_o\) is yield stress (Pa), \(K\) is consistency index (Pa.s\(^n\)), \(\dot{\gamma}\) is shear rate (s\(^{-1}\)) and \(\eta\) is the flow behavior index. Logarithms were taken for \(\sigma\) and \(\dot{\gamma}\) and a plot of log \(\sigma\) versus log \(\dot{\gamma}\) was constructed. The resulting line yielded the magnitude of log \(K\) (i.e., intercept) and \(\eta\) (i.e., slope). Viscosity at shear rate 116 s\(^{-1}\) was reported.

Frequency sweep tests were conducted in angular frequencies between 0.1 and 15 Hz and 500 \(\mu\)m gap between plates. The elastic modulus (\(G'\)) and viscous modulus (\(G''\)) of yogurt samples were obtained using the Universal Analysis software. Stress sweep range tests were conducted to determine the gels’ breakage stress (Pa). The analysis was conducted at a constant angular frequency of 1 Hz and oscillation stress from 1 to 65 Pa. A plot of \(G'\) versus oscillation stress was constructed.

### 3.11 Confocal Scanning Laser Microscopy of Yogurts’ Structure

Confocal scanning laser microscopy (CSLM) was used to evaluate the gel structure of fully hydrated yogurt samples. A Leica TCS SP2 spectral confocal microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany) and Leica LCS software were used. Each yogurt sample was placed over a micro slide (Gold Seal® Products, Portsmouth, NH) and covered with a No. 1½ cover glass (Corning®, Lowell, MA) for analysis. Optical sectioning of the yogurt samples was initiated 5 \(\mu\)m beneath the cover glass using the reflectance mode and images at various sections of the slide were digitally captured. The analysis was conducted in the Louisiana State University Socolofsky Microscopy Center.
3.12 Transmission Electron Microscopy of Yogurts

Transmission electron microscopy (TEM) imaging was used to produce micrographs of yogurts. The analysis was conducted in the Louisiana State University Socolofsky Microscopy Center using a JEOL 100CX Transmission Electron Microscope (JEOL USA, Inc., Peabody, MA) with magnification of 10X and acceleration voltage of 80 KV. Images were recorded as photographic negatives, which were scanned at high resolution for digital recording. Samples were prepared for imaging by dissolving 1 drop of yogurt in a 1.5 mL Eppendorf tube (Eppendorf North America, Hauppauge, NY) with 1 mL of distilled water. One drop of the dissolved samples was placed over a CF400-Cu-50 carbon grid (Electron Microscopy Sciences, Hatfield, PA) and was subsequently stained with 1 drop of 2% uranyl acetate (TED PELLA, Inc., Redding, CA) during 1 min. The carbon grid was gently blotted and placed on a Petri dish until used.

3.13 Statistical Analysis

The entire experiment was replicated three times and means and standard deviations were reported. The Statistical Analysis System (SAS Version 8.2, SAS Institute Inc., Cary, NC) software was used to conduct analyses of variance to determine differences among treatment means using the post hoc Tukey's studentized range test.
CHAPTER 4 RESULTS AND DISCUSSION

4.1 Microencapsulation Efficiency (ME) and Encapsulation Yield (EY) of MMO and MSO

Total oil (TO) contents of MMO and MSO were calculated to be 16.28 ± 0.18% and 16.05 ± 0.08%, respectively (Table 4.1). MMO had surface oil (SO) content of 3.52 ± 0.24% which was higher than the 3.14 ± 0.08% measured in MSO (Table 4.1). These results are comparable to Drusch and Berg’s (2008) results which indicated that depending on the extraction method used, oil load and spray drying conditions, the SO of fish oil microcapsules ranges from 0.99 ± 0.03 to 13.5%. SO content is an important attribute evaluated in spray dried encapsulated oil powders as it influences oxidative stability, wettability, flowability and color (Konstance et al., 1995). The ME for the encapsulation process of MMO and MSO were 78.37 ± 1.66% and 80.46 ± 0.48%, respectively (Table 4.1).

Table 4.1 Total oil, surface oil, ME, and EY of MMO and MSO

<table>
<thead>
<tr>
<th>Sample</th>
<th>MMO</th>
<th>MSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>TO (%)</td>
<td>16.28 ± 0.18</td>
<td>16.05 ± 0.08</td>
</tr>
<tr>
<td>SO (%)</td>
<td>3.52 ± 0.24</td>
<td>3.14 ± 0.08</td>
</tr>
<tr>
<td>ME (%)</td>
<td>78.34 ± 1.66</td>
<td>80.46 ± 0.48</td>
</tr>
<tr>
<td>EY (%)</td>
<td>76.92 ± 0.87</td>
<td>75.85 ± 0.38</td>
</tr>
</tbody>
</table>

MMO = microencapsulated menhaden oil, MSO = microencapsulated salmon oil, TO = total oil, SO = surface oil, ME = microencapsulation efficiency, EY = encapsulation yield.

ME reflects not only the non-encapsulated oil present on the surface of microcapsules but also the proportion of oil extracted from near the surface of the capsules (Rusli et al., 2006). A number of studies comparing the ME (Rosenberg and Young, 1993; Dian et al., 1996; Bhandari et al., 1998; Chung et al., 2008; Drusch et al., 2007) of different microencapsulating agents are
readily available. Carbohydrates such as maltodextrin are considered good encapsulating agents because they exhibit low viscosities at high solids contents and good solubility. Most of them lack the interfacial properties required for high microencapsulation efficiency and are generally associated with other encapsulating materials such as proteins or gums (Yoshii et al., 2001). The values obtained in our study are comparable to those reported by McNamee et al. (2001), who reported ME of 72.7 ± 1.3% for soybean oil microencapsulated using gum arabic and maltodextrin with a dextrose equivalent of 18.5. According to Shekhar et al. (2010), high ME is positively influenced by factors such as high moisture removal rate, fast solidification of micro particles and high concentration of coating materials. The EY calculated for our study were 76.92 ± 0.87% and 75.85 ± 0.38% for MMO and MSO, respectively (Table 4.1). Arabic gum stands out among other gums due to its excellent emulsification properties, attributed to the presence of a protein fraction in its structure (Dickinson et al., 2003). EY greater than 90% have been obtained in encapsulation processes by spray drying in which the wall materials were rich in protein (Young et al., 1993). Fuchs et al. (2006) tested the feasibility of encapsulating vegetable oil using conditions similar to the ones used in our study and the results were lower than the ones calculated in our study. An arabic gum: maltodextrin matrix was used and spray drying was carried in pilot scale dryer at inlet/outlet temperatures of 200/ 100 °C. The process resulted in a 65 ± 10% yield and very little oil was lost during processing.

4.2 Peroxide Value, Anisidine Value, Total Oxidation, Free Fatty Acids, Color and Moisture of PAM, MMO and MSO

PV is an indicator of initial lipid oxidation. The PVs (Table 4.2) of MMO and MSO were 3.06 ± 0.05 and 2.12 ± 0.02 meq/ kg oil, respectively. According to Gracey et al. (1999), oil with a PV below 5 meq/ kg can be considered fresh oil or one in which hydroperoxides have degraded into secondary oxidation products, like ketones and aldehydes. The secondary oxidation products
measured as AV (Table 3.2) were determined to be $5.84 \pm 0.06$ and $1.94 \pm 0.07$ for MMO and MSO respectively. The Council for Responsible Nutrition (2006) set a fish oil quality standard of an AV less than or equal to 20. MMO (11.96 ± 0.14) had a higher TOTOX value than MSO (6.19 ± 0.04) (Table 4.2). The FFA values for MMO and MSO (Table 4.2) were well below 3% and were estimated to be $0.69 \pm 0.01\%$ and $1.07 \pm 0.09\%$, respectively. The FFA content of MSO may be influenced by hydrolysis promoted by higher moisture content (Table 4.2) in MSO or in the encapsulated PSO. FFA content greater than 3% is considered inedible (Gracey et al., 1999). Yin and Sathivel (2010) concluded that an increase in FFA content of PMO during storage may be influenced by hydrolysis promoted by the initial moisture content of the oil.

### Table 4.2 Properties of PAM, MMO and MSO

<table>
<thead>
<tr>
<th>Sample</th>
<th>PAM</th>
<th>MMO</th>
<th>MSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV (meq/ Kg oil)</td>
<td>-</td>
<td>3.06 ± 0.05</td>
<td>2.12 ± 0.02</td>
</tr>
<tr>
<td>AV</td>
<td>-</td>
<td>5.84 ± 0.06</td>
<td>1.94 ± 0.07</td>
</tr>
<tr>
<td>TOTOX</td>
<td>-</td>
<td>11.96 ± 0.14</td>
<td>6.19 ± 0.04</td>
</tr>
<tr>
<td>FFA (%)</td>
<td>-</td>
<td>0.69 ± 0.01</td>
<td>1.07 ± 0.09</td>
</tr>
<tr>
<td>Color L*</td>
<td>85.83 ± 0.03</td>
<td>98.83 ± 0.01</td>
<td>88.67 ± 0.01</td>
</tr>
<tr>
<td>Color a*</td>
<td>-1.21 ± 0.02</td>
<td>-0.20 ± 0.02</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>Color b*</td>
<td>4.02 ± 0.00</td>
<td>11.09 ± 0.02</td>
<td>9.76 ± 0.01</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>3.15 ± 0.02</td>
<td>3.06 ± 0.05</td>
<td>3.33 ± 0.03</td>
</tr>
</tbody>
</table>

PAM = Arabic gum-maltodextrin powder, MMO = microencapsulated menhaden oil, MSO = microencapsulated salmon oil, PV = peroxide value, AV = anisidine value, TOTOX = total oxidation, FFA = free fatty acids, L* = degree of darkness to lightness, a* = degree of greenness (-) to redness (+), b* = degree of blueness (-) to yellowness (+).

The color L*, a* and b* values are reported in Table 3.2. MMO had a lighter color than PAM and MSO. Results for a* indicated that MSO had a redder color compared to PAM and MMO. MMO and MSO had significantly higher b* values (more yellowness) which may be attributed to the presence of SO around the microcapsules (Fig. 4.1, 4.2 and 4.3). Moisture contents of
PAM, MMO and MSO were calculated to be 3.15 ± 0.02%, 3.06 ± 0.05%, and 3.33 ± 0.03%. These results are similar to the moisture contents (2.89-3.02 %) reported by Klaypradit and Huang (2008) for tuna oil encapsulated with chitosan. Furthermore, Fuchs et al. (2006) reported a water content of 3.8 ± 1.8 g/ 100 g of dry matter in a spray dried sunflower oil encapsulated with acacia gum and maltodextrin.

Fig. 4.1 Image of PAM. PAM = Powder containing arabic gum and maltodextrin

Fig. 4.2 Image of MMO. MMO = microencapsulated menhaden oil
4.3 Scanning Electron Microscopy of PAM, MMO and MSO

Scanning electron micrographs were used to observe the microstructure of PAM (Fig. 4.4), MMO (Fig. 4.5) and MSO (Fig. 4.6). The structure of spray dried particles can be affected by several factors such as processing conditions which can affect size and shape. The micrographs indicate that the microencapsulation and spray drying processes of PAM, MMO and MSO
yielded mostly irregular particles w/o wrinkles and no extensively fractured particles but with slight agglomeration. Changes of particle size and morphology during spray drying are related to moisture content and drying temperature. In the first stage of spray drying or constant drying stage, the hot air causes an increase of the droplet temperature, which promotes quick liquid evaporation from the droplet surface and a corresponding shrinkage of the droplet (Gharsallaoui
et al., 2007). In our study, the shrinkage phenomenon described above may have caused the formation of the irregular dry crust of the spray dried powders which is also related to a high water evaporation rate. According to Gharsallaoui et al. (2007), spray-drying produces, depending on the starting feed material and operating conditions, a very fine powder (10–50 µm) or large size particles (2–3 mm). Accordingly, the particle sizes observed through SEM for PAM, MMO and MSO were 30-35 µm, 20-25 µm, and 20-25 µm, respectively. These values are comparable to those reported by Fuchs et al., (2006) who reported a mean diameter of 24-37 µm for microencapsulated oil spray dried at inlet/outlet temperatures of 220/100 ºC and mean emulsion flow rate of 24-68 ml/ min. Senatore et al. (2010) reported a spray dried microencapsulated epoxidized linseed oil powder with an average diameter of 16 µm. The higher the provided energy for feed atomization during spray drying, the finer the formed droplets, on the other hand, the size of particles increases when both viscosity and surface tension of the initial liquid are high (Gharsallaoui et al., 2007). No wrinkles were observed on PAM, MMO or MSO; however there are variations in the diameters of the particles and small round aggregates are present around the larger particles. Presence of slight agglomeration is in agreement with the results of Senatore et al. (2010) in which SEM reflected that large particles were covered with smaller particles to form agglomerates. Evidence of fractured particles is very low in PAM (Fig. 4.4), nevertheless a few particles with irregular and porous surface and plenty of small to intermediate-sized particles are observed in MMO (Fig. 4.5) and MSO (Fig. 4.6). SEM analyses suggest that MO and SO are well protected in the matrix of arabic gum and maltodextrin.

4.4 Alpha Tocopherol Content of Yogurts

Means and standard deviations of α-tocopherol content during 4 weeks of storage of yogurt are illustrated in Fig. 4.7. Initial PY, YPAM, YMMO and YMSO α-tocopherol contents were 37.65
$\pm 3.17$, $43.03 \pm 0.71$, $75.61 \pm 0.98$, and $72.09 \pm 0.18 \mu g/ g$ yogurt, respectively. After 4 weeks of storage, $\alpha$-tocopherol contents were reduced by $13.4\%$, $16.4\%$, $19.0\%$ and $23.4\%$, respectively; however YMMO and YMSO $\alpha$-tocopherol contents were still greater than the control yogurts. A number of factors such as oxygen, light, heat, alkali, trace minerals, and hydroperoxides can cause decomposition of vitamin E vitamers (Bramley et al., 2000). The decrease in $\alpha$-tocopherol content during refrigerated storage may be primarily caused by oxygen dissolved in the yogurt matrix, and reaction with hydroperoxides produced by initial lipid oxidation reactions. $\alpha$-Tocopherol is expected to have chain-breaking antioxidant activity in yogurts as well as in human body tissues. Burton et al. (1985) showed that $\alpha$-tocopherol donates its phenolic hydrogen atom to peroxyl radicals arising and in the process becomes an $\alpha$-tocopheryloxyl radical.

![Graph showing $\alpha$-tocopherol content of PY, YPAM, YMMO and YMSO during 4 weeks of refrigerated storage](image)

Fig 4.7 $\alpha$-tocopherol content of PY, YPAM, YMMO and YMSO during 4 weeks of refrigerated storage
The remarkable degree of oxidative protection afforded by small amounts of α-tocopherol may be partly explained by the fact that peroxyl radicals react with α-tocopherol about 10,000 times faster than they react with PUFAs, making it less likely that an oxidative chain reaction will be propagated than that it will be quenched (Buettner, 1993). Furthermore, α-tocopherol is stable at high temperatures if no oxygen is present (Shin et al., 1997).

### 4.5 Fatty Acid Methyl Esters (FAMEs) Analysis of MMO, MSO and Yogurts

Table 4.3 FAMEs composition (%) of MMO and MSO*

<table>
<thead>
<tr>
<th>Fatty Acid Methyl Esters</th>
<th>MMO</th>
<th>MSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0 (myristic)</td>
<td>8.50 ± 0.50</td>
<td>4.82 ± 0.20</td>
</tr>
<tr>
<td>C16:0 (palmitic)</td>
<td>18.89 ± 0.60</td>
<td>14.79 ± 0.40</td>
</tr>
<tr>
<td>C16:1ω7 (palmitoleic)</td>
<td>11.65 ± 0.48</td>
<td>5.83 ± 0.18</td>
</tr>
<tr>
<td>C18:0 (stearic)</td>
<td>3.56 ± 0.03</td>
<td>3.02 ± 0.08</td>
</tr>
<tr>
<td>C18:1 ω9 (oleic)</td>
<td>6.21 ± 0.09</td>
<td>17.59 ± 0.49</td>
</tr>
<tr>
<td>C18:1 ω7 (vaccenic)</td>
<td>3.07 ± 0.04</td>
<td>2.73 ± 0.07</td>
</tr>
<tr>
<td>C18:2 ω6 (linoleic)</td>
<td>1.49 ± 0.03</td>
<td>1.76 ± 0.06</td>
</tr>
<tr>
<td>C18:3 ω3 (alpha-linolenic)</td>
<td>1.67 ± 0.03</td>
<td>1.23 ± 0.02</td>
</tr>
<tr>
<td>C18:4 ω3 (octadecatetraenoic)</td>
<td>3.27 ± 0.06</td>
<td>2.19 ± 0.01</td>
</tr>
<tr>
<td>C20:1 ω9 (eicosenoic)</td>
<td>1.01 ± 0.07</td>
<td>8.18 ± 0.16</td>
</tr>
<tr>
<td>C20:4 ω6 (arachidonic)</td>
<td>1.14 ± 0.01</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td>C20:4 ω3 (eicosatetraenoic)</td>
<td>1.76 ± 0.01</td>
<td>1.61 ± 0.07</td>
</tr>
<tr>
<td>C20:5 ω3 (EPA)</td>
<td>13.93 ± 0.09</td>
<td>9.84 ± 0.36</td>
</tr>
<tr>
<td>C22:1 ω11 (cetoleic)</td>
<td>-</td>
<td>7.08 ± 0.35</td>
</tr>
<tr>
<td>C22:5 ω3 (docosapentaenoic)</td>
<td>2.69 ± 0.06</td>
<td>2.66 ± 0.13</td>
</tr>
<tr>
<td>C22:6 ω3 (DHA)</td>
<td>13.62 ± 0.22</td>
<td>12.48 ± 0.42</td>
</tr>
<tr>
<td><strong>SAFA</strong></td>
<td>32.49 ± 1.18</td>
<td>23.60 ± 0.67</td>
</tr>
<tr>
<td><strong>MUFA</strong></td>
<td>22.26 ± 0.54</td>
<td>42.18 ± 0.89</td>
</tr>
<tr>
<td><strong>PUFA</strong></td>
<td>40.21 ± 0.26</td>
<td>32.37 ± 1.09</td>
</tr>
<tr>
<td><strong>Total ω -3</strong></td>
<td>36.94 ± 0.26</td>
<td>30.01 ± 1.00</td>
</tr>
</tbody>
</table>

FAMEs = fatty acid methyl esters, MMO = microencapsulated menhaden oil, MSO = microencapsulated salmon oil, SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, ω-3 = omega-3. * Only major fatty acids were reported.
MMO and MSO FAMEs compositions are presented in Table 4.3 and only major fatty acids were reported. MMO had a content of saturated (SAFA), monounsaturated (MUFA), polyunsaturated (PUFA), and total ω-3 fatty acids of 32.49 ± 1.18%, 22.26 ± 0.54%, 40.21 ± 0.26%, and 36.94 ± 0.26%, respectively. MMO content of palmitic (C16:0) acid was 18.89 ± 0.60% and was the most abundant of the saturated fatty acids detected. Among the ω-3 fatty acids, EPA (C20:5ω-3) and DHA (C22:6ω-3) were the predominant fatty acids accounting for 13.93 ± 0.09% and 13.62 ± 0.22% of the fatty acids detected, respectively. These values are higher than the EPA (13.17 g/ 100 g oil) and DHA (8.56 g/ 100 g oil) contents reported by the USDA National Nutrient Database (NAL.USDA.gov, 2009) and than those reported by Osman et al. (2007) for menhaden oil in which EPA and DHA accounted for 12.5% and 7.9%, respectively. Values reported by the USDA National Nutrient Database (NAL.USDA.gov, 2009) indicate that every 100 g of salmon oil contains 13.02 g and 18.23 g of EPA and DHA, respectively. The nature and quantity of lipids in fish oils vary according to species and habitats (Shamsudin and Salimon, 2006) and may also be affected by different storage, purification or microencapsulation processes.

Table 4.4 SAFA (%) of yogurts during 4 weeks of refrigerated storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY</td>
<td>61.59 ± 1.17&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>63.23 ± 2.01&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>62.06 ± 0.26&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>63.26 ± 1.08&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>63.62 ± 1.47&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>YPAM</td>
<td>63.69 ± 0.96&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>65.80 ± 1.58&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>64.80 ± 0.99&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>63.40 ± 0.42&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>63.47 ± 0.79&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMMO</td>
<td>60.76 ± 1.43&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>59.80 ± 1.18&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>60.11 ± 0.57&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>61.91 ± 1.20&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>63.44 ± 0.80&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMSO</td>
<td>60.41 ± 0.55&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>59.93 ± 1.11&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>58.69 ± 0.28&lt;sup&gt;aD&lt;/sup&gt;</td>
<td>60.02 ± 0.43&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>59.03 ± 0.36&lt;sup&gt;aB&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup><sup>b</sup> means with different letters along each row are significantly different (P< 0.05). <sup>A</sup><sup>B</sup> means with different letters along a column are significantly different (P< 0.05). SAFA = saturated fatty acids, PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil.
Table 4.5 MUFA (%) of yogurts during 4 weeks of refrigerated storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY</td>
<td>31.43 ± 0.79&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>31.58 ± 1.00&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>31.90 ± 1.32&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>31.56 ± 0.69&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>31.52 ± 1.92&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>YPAM</td>
<td>31.80 ± 0.97&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>29.80 ± 1.21&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>31.36 ± 0.53&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>31.18 ± 1.26&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>31.34 ± 1.39&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMMO</td>
<td>27.00 ± 0.49&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>27.62 ± 0.59&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>27.27 ± 0.29&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>29.10 ± 0.19&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>28.33 ± 0.65&lt;sup&gt;aB&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMSO</td>
<td>30.64 ± 0.16&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>30.69 ± 0.19&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>30.83 ± 0.26&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>30.36 ± 0.17&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>30.71 ± 0.18&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> means with different letters along each row are significantly different (P< 0.05). <sup>AB</sup> means with different letters along a column are significantly different (P< 0.05). MUFA= monounsaturated fatty acids, PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil.

Table 4.6 PUFA (%) of yogurts during 4 weeks of refrigerated storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY</td>
<td>5.03 ± 0.19&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>5.10 ± 0.16&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>5.09 ± 0.10&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>5.02 ± 0.09&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>4.80 ± 0.04&lt;sup&gt;aC&lt;/sup&gt;</td>
</tr>
<tr>
<td>YPAM</td>
<td>5.16 ± 0.03&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>5.14 ± 0.10&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>5.02 ± 0.08&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>5.00 ± 0.12&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>4.94 ± 0.15&lt;sup&gt;aC&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMMO</td>
<td>6.34 ± 0.20&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>6.17 ± 0.33&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>5.99 ± 0.25&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>5.94 ± 0.22&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>6.10 ± 0.35&lt;sup&gt;aB&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMSO</td>
<td>9.44 ± 0.55&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>9.37 ± 0.48&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>9.18 ± 0.40&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>8.65 ± 0.20&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>7.02 ± 0.16&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> means with different letters along each row are significantly different (P< 0.05). <sup>AB</sup> means with different letters along a column are significantly different (P< 0.05). PUFA= polyunsaturated fatty acids, PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil.

SAFA (Table 4.4) were the predominant fatty acids in PY, YPAM, YMMO and YMSO followed by MUFA (Table 4.5) and PUFA (Table 4.6). This composition was expected considering that milk lipids are known to be mainly composed of saturated fatty acids (MacGibbon and Taylor, 2006). Initial SAFA contents for PY, YPAM, YMMO and YMSO were 61.59 ± 1.17%, 63.69 ± 0.96%, 60.76 ± 1.43%, and 60.41 ± 0.55%, respectively. No significant (P>0.05) changes in SAFA content were observed during storage in any of the yogurts (Table 4.4). There were no significant (P>0.05) variations in MUFA contents of yogurts during 4 weeks storage. During the entire storage study, MUFA content of YMMO was significantly (P<0.05) lower than those of PY, YPAM and YMSO. After 4 weeks storage MUFA contents accounted for 31.52 ± 1.92%,
31.34 ± 1.39%, 28.33 ± 0.65% and 30.71 ± 0.18% of the FAMEs detected in PY, YPAM, YMMO and YMSO, respectively (Table 4.5). Initial PUFA contents (%) of PY, YPAM, YMMO and YMSO were 5.03 ± 0.19%, 5.16 ± 0.03, 6.34 ± 0.20%, 9.44 ± 0.55%, respectively (Table 4.6). No significant (P>0.05) changes in PUFA content were observed during 3 weeks of yogurt storage in PY, YPAM and YMSO; however after 4 weeks storage a decrease of 4.57%, 4.26% and 25.64, respectively, was observed. This may be indicative of ongoing oxidative reactions during refrigerated storage of yogurts. PUFA contents of YMMO did not significantly increase or decrease during 4 weeks of refrigerated storage. The differences in the PUFA content changes between YMSO and YMMO during storage may be related to a greater content of transition metal ions in PSO compared to PMO. Transition metals are capable of breaking down unsaturated lipids into alkyl radicals (Reische et al., 1998). The acceleration of lipid oxidation in emulsions is thought to be mainly due to the decomposition of lipid hydroperoxides into peroxyl and alkoxyl radicals by transition metals or other prooxidants. These radicals react with unsaturated fatty acids, which leads to the formation of lipid radicals that react with other lipids and the oxidation chain reaction propagates (McClements and Decker, 2000). Furthermore, added antioxidants or ingredients may act synergistically with endogenous antioxidants. However, pro-oxidant effects of endogenous and added antioxidants may also occur if the total concentration becomes too high (Jacobsen, 2010). According to Rietjens et al., (2002), there is a dualistic behavior of vitamin E which implies that it can function as an antioxidant or prooxidant. Upon oxidative stress, increased levels of α-tocopherol result in higher levels of α-tocopherol radicals which can initiate processes of lipid peroxidation by themselves. A higher α-tocopherol content of PSO compared to PMO (APPENDIX: Characteristics of PMO and PSO) combined with the addition of α-tocopherol in the emulsion preparation, may have promoted oxidation
reactions that resulted in a decrease in PUFA content of YMSO, which was not observed in YMMO. After 4 weeks storage the PUFA contents of PY, YPAM, YMMO and YMSO were 4.80 ± 0.04%, 4.94 ± 0.15%, 6.10 ± 0.35% and 7.02 ± 0.16%, respectively. Yogurt is known to be an excellent delivery vehicle for essential fatty acids (Gerdes, 2007) and results from previous studies have shown that yogurt enriched with ω-3 PUFAs has very good oxidative stability (Nielsen et al., 2007; Nielsen et al., 2009). In our study, YMMO demonstrated to be a stable matrix for maintaining PUFAs content unchanged during 4 weeks of refrigerated storage. YMSO may also be considered a good delivery vehicle for PUFAs; however, in our study, the characteristics of the initial salmon oil may have limited its performance.

Table 4.7 Total ω-3 (%) of yogurts during 4 weeks of refrigerated storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY</td>
<td>1.41 ± 0.12&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>1.36 ± 0.07&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>1.36 ± 0.06&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>1.34 ± 0.09&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>1.33 ± 0.05&lt;sup&gt;aC&lt;/sup&gt;</td>
</tr>
<tr>
<td>YPAM</td>
<td>1.43 ± 0.03&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>1.44 ± 0.03&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>1.39 ± 0.02&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>1.35 ± 0.09&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>1.36 ± 0.11&lt;sup&gt;aC&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMMO</td>
<td>5.18 ± 0.05&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>4.83 ± 0.11&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>4.73 ± 0.13&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>4.67 ± 0.09&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>4.43 ± 0.12&lt;sup&gt;cB&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMSO</td>
<td>6.06 ± 0.30&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>6.01 ± 0.17&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>5.8 ± 0.19&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>5.75 ± 0.23&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>5.18 ± 0.17&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> means with different letters along each row are significantly different (P< 0.05). <sup>AB</sup> means with different letters along a column are significantly different (P< 0.05). ω-3 = omega-3, PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil.

Table 4.8 EPA (%) of yogurts during 4 weeks of refrigerated storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>YPAM</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMMO</td>
<td>2.28 ± 0.09&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>2.20 ± 0.01&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>2.13 ± 0.04&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>2.05 ± 0.02&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>1.87 ± 0.06&lt;sup&gt;dA&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMSO</td>
<td>2.20 ± 0.03&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>2.19 ± 0.13&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>2.08 ± 0.12&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>2.03 ± 0.05&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>1.82 ± 0.11&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> means with different letters along each row are significantly different (P< 0.05). <sup>AB</sup> means with different letters along a column are significantly different (P< 0.05). EPA= eicosapentaenoic acid, PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil. * - stands for not detected.
Table 4.9 DHA (%) of yogurts during 4 weeks of refrigerated storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY</td>
<td>-*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>YPAM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>YMMO</td>
<td>2.18 ± 0.03&lt;sup&gt;aB&lt;/sup&gt;</td>
<td>2.09 ± 0.03&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>1.87 ± 0.05&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>1.83 ± 0.08&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>1.84 ± 0.07&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>YMSO</td>
<td>2.28 ± 0.01&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>2.13 ± 0.04&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>1.93 ± 0.13&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>1.94 ± 0.05&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>1.85 ± 0.07&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> means with different letters along each row are significantly different (P< 0.05). <sup>AB</sup> means with different letters along a column are significantly different (P< 0.05). DHA= docosahexaenoic acid, PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil. * - stands for not detected.

Initial total ω-3 contents of PY, YPAM, YMMO and YMSO were 1.41 ± 0.12%, 1.43 ± 0.03%, 5.18 ± 0.05% and 6.06 ± 0.30%, respectively (Table 4.7). Total ω-3 content did not significantly (P>0.05) vary during the storage of PY and YPAM. On the other hand total ω-3 contents of YMMO and YMSO were significantly reduced 14.48% and 14.52%, respectively after 4 weeks storage. Oxidation, which results in loss of nutritional value, generally occurs faster in emulsions such as yogurt than in bulk oils. It has also been reported that several food ingredients in emulsions contain trace levels of metal ions that serve as catalysts for oxidation reactions (Jacobsen, 2010). The total ω-3 contents of YMMO and YMSO after 4 weeks storage accounted for 4.43 ± 0.12% and 5.18 ± 0.17%, respectively. These ω-3 contents were approximately 3.3 to 3.8-fold higher than total ω-3 contents detected in PY and YPAM after 4 weeks storage. Among the total ω-3 fatty acids in YMMO, EPA (Table 4.8) and DHA (Table 4.9) were the major fatty acids accounting for 2.28 ± 0.09% and 2.18 ± 0.03% of the fatty acids detected, respectively. Similarly, EPA (Table 4.8) and DHA (Table 4.9) were the predominant ω-3 fatty acids in YMSO with an initial content of 2.20 ± 0.03% and 2.28 ± 0.01%, respectively. EPA and DHA were not detected in PY or YPAM at any point of the storage study. EPA, DHA contents decreased in YMMO and YMSO proportionally to the increase in storage time. The reduction in EPA and
DHA content in our study may be attributed to the oxidation reactions induced by the pasteurization process, oxygen dissolved in the yogurt matrix and in the headspace and to the low pH of the product, which may have enhanced oxidation of the microcapsules’ surface oil. Previous studies have shown that the low pH in mayonnaise is one of the most important causes of its rapid lipid oxidation (Jacobsen et al., 2001). It has been reported that several food ingredients in emulsions contain trace levels of metal ions that serve as catalysts for oxidation reactions (Jacobsen, 2010). Moreover, oxidation during heating can significantly increase the degradation of PUFAs, promote the production of undesirable toxic lipid oxidation products and lower the quality of foods fortified with fish oil (Yue et al., 2008). After 4 weeks of refrigerated storage at 4 ºC, EPA and DHA contents in YMMO were 1.87 ± 0.06% and 1.84 ± 0.07% and in YMSO, 1.82 ± 0.11% and 1.85 ± 0.07%, respectively. Microencapsulation of fish oil could improve the oxidative stability of ω-3 fortified yogurt and could significantly enhance its health benefits and positive consumer perception. Results in our study are in accordance to Traber (1999) who assured that α- tocopherol is capable of capturing free radicals and breaking lipid peroxidation chain reactions, thereby preventing the destruction of lipids. In our study, even though there was a slight reduction in EPA and DHA contents, both were still present in high quantity in YMMO and YMSO after 1 month of storage. Moreover, according to Fig. 4.7 there was a greater decrease in the α-tocopherol contents of YMMO and YMSO which may be indicative of the antioxidant role played by Vitamin E during storage which prevented further destruction of lipids.

4.6 TBA Values of Yogurts

The evaluation of the oxidative stability of microencapsulated (Shen et al., 2010) and non-encapsulated (Kaitaranta, 1992) oils has been conducted using thiobarbituric acid reactive
substances analysis. Initial TBA values for PY, YPAM, YMMO and YMSO were 4.08 ± 0.11, 4.08 ± 0.10, 4.15 ± 0.17, 4.61 ± 0.07 μmoles MDA/ Kg yogurt. YMSO TBA values were significantly (P<0.05) higher than those of all other yogurts during the 4 weeks of refrigerated storage (Fig. 4.8). Changes in TBA values of PY, YPAM and YMMO during storage were not significant (P>0.05). There was a significant (P<0.05) 4.76% increase in TBA of YMSO after 4 weeks storage which may have a connection to its 25.64% decrease in YMSO PUFAs content. It is well documented that fish oils and milk fat are prone to oxidation upon heating (Yin and Sathivel, 2010; Al-Rowaily, 2008), nevertheless, Boran et al. (2006) reported that fish oils can also deteriorate to an unacceptable level during refrigerated storage (4 ºC) due to lipid oxidation. Al-Rowaily (2008) reported that refrigerated storage of set yogurt for seven

Fig. 4.8 Thiobarbituric acid values of PY, YPAM, YMMO, and YMSO
days and strained yogurt for 15 days caused a significant difference (P<0.05) in the oxidation parameters of PV, AV and TOTOX values. Lee et al. (2007) reported a slow TBA absorbance increase from 0.083 to 0.10 over the initial 6 days of storage of evening primrose oil (EPO)-enriched yogurt, followed by a dramatic increase up to 0.165 after 15 days. EPO is of special interest due to its high PUFAs content. Overall, the results indicated that lipid oxidation proceeded more rapidly in yogurt with EPO addition than that without EPO, which is in accordance to the trend observed in our study with YMSO. Furthermore, Hekmat and McMahon (1997) studied the overall effect of 30 day storage on yogurt chemical oxidation, as measured by the TBA test. Low fat yogurt exhibited a tendency toward increased chemical oxidation during storage up to 30 days.

4.7 Lactic Acid Bacteria Counts, pH, Water Holding Capacity and Color of Yogurts

Addition of MMO and MSO had no effect on LAB counts (Log CFU/ g yogurt) of yogurts during 4 weeks of storage compared to LAB counts of the controls. A decrease in viable counts of LAB was observed during the storage study of all yogurts, which indicates certain instability of the starter cultures during shelf life (Fig. 4.9). LAB counts remained stable during the first week of storage and decreased from 8.70 ± 0.01, 8.70 ± 0.03, 8.70 ± 0.01, and 8.70 ± 0.04 Log CFU/ g yogurt in PY, YPAM, YMMO, and YMSO, respectively, to 6.3 ± 0.02 Log CFU/ g yogurt in all samples between weeks 1 and 3. Counts remained unchanged between weeks 3 and 4. In a study concerning microbiological evolution of LAB during the shelf life of yogurt, Rotar et al. (2007) reported that the initial concentration of LAB was between $10^6$ – $10^7$ CFU/ g (a concentration considered good for the yogurt to fortify its nutritional value to consumers), and towards the end of the shelf-life study the concentration was very low, around $10^4$ CFU/ g, which is considered inadequate. Dave and Shah (1996) reported that viable counts of starter culture
bacteria in yogurt increased and then declined during a 28 day refrigerated storage study. Simultaneously, there was a gradual drop in pH of yogurt, especially after 20 days of storage. Lee et al., (2007) reported a decreasing trend in mean microbial counts during 15 days of refrigerated storage of yogurt enriched with evening primrose oil.

Starter cultures transform lactose into lactic acid which is responsible of the initial acidification responsible for coagulation at pH ≈ 4.6, and the post acidification during the shelf life. Acidity of PY, YPAM, YMMO, and YMSO, measured as pH, presented a slight decrease from 4.50 ± 0.01 in day 1 to 4.42 ± 0.01, 4.41 ± 0.01, 4.41 ± 0.01, and 4.42 ± 0.01 respectively, after 4 weeks of storage (Fig. 4.10). This is in accordance with results reported by Serra et al. (2009), in which titratable acidity slightly increased after 28 days of cold storage of set and stirred yogurts made from ultra-high pressure homogenization-treated milk. This is a result of the persistent metabolic activity of starters, which has also been called after–acidification because the enzymatic activity of the LAB is reduced but not completely stopped (Rasic and Kurmann, 1978). According to Lee and Lucey (2004), acidification is a key phenomenon that must be controlled in yogurt processing and storage as it directly affects the product’s microstructure and rheological behavior.

Spontaneous syneresis is the result of the breakage of many protein strands and structural rearrangements, which result in the expulsion of whey (Serra et al., 2009). Syneresis produced by funnel drainage does not represent the usual breakage of the yogurt matrix, but reflects the capability of the whole gel structure to retain water. Syneresis of yogurts during storage is presented in Table 4.8. A slight, but not significant (P>0.05), increase in syneresis was observed in all yogurts during storage. The slight increase in syneresis of yogurts during storage may be closely related to whey release caused by gel network rearrangements triggered by changes in
pH. No significant (P>0.05) differences in syneresis were observed due to the addition of PAM, MMO, or MSO to PY throughout the storage study.

Fig. 4.9 Lactic acid bacteria counts of yogurts during 4 weeks storage

Fig. 4.10 Acidity (pH) of yogurts during 4 weeks storage
Significant (P<0.05) differences in syneresis over 5 weeks of storage time were reported by Boeneke and Aryana (2008) in lemon yogurts fortified with folic acid. Similarly, Aportela et al. (2005) found a significant influence of storage time on the syneresis of piña colada yogurt systems fortified with fiber and calcium. The physical attributes of yogurts, including the lack of visual whey separation, are crucial aspects of the quality and overall sensory consumer acceptance (Lee and Lucey, 2010).

Table 4.10 Syneresis of yogurts during 4 weeks storage at 4 °C

<table>
<thead>
<tr>
<th>Storage Time</th>
<th>Initial</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY</td>
<td>53.6 ± 2.6(^a)</td>
<td>54.2 ± 2.3(^a)</td>
<td>54.6 ± 2.6(^a)</td>
<td>56.3 ± 3.3(^a)</td>
<td>58.9 ± 2.8(^a)</td>
</tr>
<tr>
<td>YPAM</td>
<td>59.2 ± 4.7(^a)</td>
<td>59.6 ± 5.9(^a)</td>
<td>60.0 ± 5.0(^a)</td>
<td>59.6 ± 4.4(^a)</td>
<td>60.0 ± 2.0(^a)</td>
</tr>
<tr>
<td>YMMO</td>
<td>57.7 ± 4.6(^a)</td>
<td>59.33 ± 5.2(^a)</td>
<td>61.7 ± 4.0(^a)</td>
<td>61.4 ± 3.5(^a)</td>
<td>63.0 ± 4.4(^a)</td>
</tr>
<tr>
<td>YMSO</td>
<td>56.3 ± 1.8(^a)</td>
<td>56.6 ± 3.9(^a)</td>
<td>58.6 ± 3.8(^a)</td>
<td>59.0 ± 1.4(^a)</td>
<td>59.6 ± 2.6(^a)</td>
</tr>
</tbody>
</table>

\(^a\) means with different letters along a column are significantly different (P<0.05). PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil.

The L\(^*\) (Fig. 4.11) values of PY, YPAM, YMMO and YMSO were not significantly (P>0.05) different from each other during 4 weeks of storage. A similar trend was observed for negative a\(^*\) values (Fig. 4.12) which indicated the presence of a slight greenness in all samples. The significantly (P<0.05) higher yellowness of YMMO and YMSO during the 4 weeks of storage, may be related to the addition of MMO and MSO which were significantly more yellow than PAM (Table 4.2). Color is one of the most important visual attributes in food and changes in physical, chemical or microbiological parameters of yogurt affect storage, shelf life and may cause color deterioration (Coggins et al., 2010). The L\(^*\), a\(^*\) and b\(^*\) values were converted to total color difference (ΔE\(^*\)) values using the initial (Day 1) values of each sample as reference. PY had ΔE\(^*\) values of 0.89 to 1.17, YPAM from 0.87 to 0.98, YMMO from 0.93 to 1.38 and YMSO...
of 0.54 to 1.43 (Table 4.11). The $\Delta E^*$ values had a trend to increase as storage time increased; however significant changes in $\Delta E^*$ values during storage, were only detected for YMMO and YMSO. Considering that $L^*$ values remained unchanged during the 4 weeks of storage, the slight increase in $\Delta E^*$ may be attributed to an increase in $a^*$ and $b^*$ values due to whey release over time and reaction of microcapsule surface oil with the yogurt matrix, respectively. All the $\Delta E^*$ values in our study were well below 3 and it is known that $\Delta E^*$ values less than 3.0 cannot easily be detected by the naked human eye (Caner, 2005).

Fig. 4.11 Color lightness values ($L^*$) of yogurts

$^{ab}$ Same superscripts indicate no significant ($P>0.05$) differences between samples at the same storage time.

Fig. 4.12 Color green (negative values)/ red (positive values) ($a^*$) of yogurts

$^{ab}$ Same superscripts indicate no significant ($P>0.05$) differences between samples at the same storage time.
Fig. 4.13 Color blue (negative values) / yellow (positive values) (b*) of yogurts. The different superscripts indicate significant (P<0.05) differences between samples at the same storage time.

Table 4.11 Total Color Differences of Yogurts During 4 Weeks of Storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1-Week 1</th>
<th>Week 1-Week 2</th>
<th>Week 2-Week 3</th>
<th>Week 3-Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY</td>
<td>0.89 ± 0.18a</td>
<td>0.94 ± 0.17a</td>
<td>1.08 ± 0.22a</td>
<td>1.17 ± 0.15a</td>
</tr>
<tr>
<td>YPAM</td>
<td>0.87 ± 0.13a</td>
<td>0.94 ± 0.17a</td>
<td>0.98 ± 0.18a</td>
<td>0.97 ± 0.17a</td>
</tr>
<tr>
<td>YMMO</td>
<td>0.99 ± 0.15b</td>
<td>0.93 ± 0.14b</td>
<td>0.98 ± 0.23b</td>
<td>1.38 ± 0.13a</td>
</tr>
<tr>
<td>YMSO</td>
<td>0.54 ± 0.14c</td>
<td>0.57 ± 0.12c</td>
<td>0.89 ± 0.21b</td>
<td>1.43 ± 0.12a</td>
</tr>
</tbody>
</table>

Values are means of means ± standard deviations of triplicate determinations. abc Means across the same row with the same superscript are not significantly (P>0.05) different. PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil.

4.8 Rheological Analysis of Yogurts

The flow behavior properties of PY, YPAM, YMMO, and YMSO were measured in day 1, and during 4 weeks of refrigerated storage. The initial and week 4 flow behavior properties of yogurts are reported in Tables 4.9 and 4.10, respectively. The flow behavior index (η) values for PY, YPAM, YMMO and YMSO were all less than 1.0 regardless of storage time, indicating that all yogurts were pseudoplastic fluids (Paredes et al., 1988) with shear thinning behavior. The η values reported are far from Newtonian behavior (η=1), which indicates a greater resistance to
flow and the existence of a more complex structure. The addition of MMO and MSO did not significantly affect consistency index ($K$) values of YPAM, YMMO and YMSO when compared to PY. The slightly higher $K$ values of YPAM, YMMO and YMSO may be related to the high hygroscopicity and water holding capacity of maltodextrins with high dextrose equivalent (DE), like the one used in our study (DE = 18.5). Furthermore, Valim et al. (2009) reported that arabic gum addition led to better water retention properties in cold-set gels, when compared to gels containing only whey proteins. $K$ values did not change ($P>0.05$) during storage and the $K$ values of PY remained higher than those of YPAM, YMMO and YMSO. A higher $K$ value indicates a more viscous consistency (Batista et al., 2006). In a study on fiber enriched plain yogurt, Sanz et al. (2008) reported Herschel-Bulkley model $\eta$ values greater (0.69-0.83) and $K$ values lower (2.37-5.21) than the ones determined in our study. Yield stress ($\sigma_o$) represents the value of the minimum force that must be applied to a sample in order for it to begin to flow (Sanz et al., 2008). The addition of PAM, MMO or MSO did not significantly ($P>0.05$) change the values of yield stress. No significant ($P>0.05$) differences were determined in the apparent viscosities of yogurts at day 1 or week 4. For stirred yogurts it should be noted that steps such as mixing result in a reduction in viscosity that is only partially restored after shearing is stopped (Lee and Lucey, 2010).

Table 4.12 Initial Flow Behavior Properties of PY, YPAM, YMMO and YMSO

<table>
<thead>
<tr>
<th>Sample</th>
<th>PY</th>
<th>YPAM</th>
<th>YMMO</th>
<th>YMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\eta$ ($\text{Pa.s}^{-1}$)</td>
<td>0.11 ± 0.01$^a$</td>
<td>0.11 ± 0.02$^a$</td>
<td>0.12 ± 0.02$^a$</td>
<td>0.12 ± 0.01$^a$</td>
</tr>
<tr>
<td>$K$ ($\text{Pa.s}^{-1}$)</td>
<td>23.92 ± 2.24$^a$</td>
<td>28.59 ± 1.60$^a$</td>
<td>29.35 ± 0.60$^a$</td>
<td>29.70 ± 2.22$^a$</td>
</tr>
<tr>
<td>Yield Stress (Pa)</td>
<td>0.23 ± 0.04$^a$</td>
<td>0.26 ± 0.05$^a$</td>
<td>0.25 ± 0.01$^a$</td>
<td>0.25 ± 0.02$^a$</td>
</tr>
<tr>
<td>Apparent Viscosity (Pa.s)</td>
<td>0.32 ± 0.05$^a$</td>
<td>0.38 ± 0.02$^a$</td>
<td>0.36 ± 0.02$^a$</td>
<td>0.36 ± 0.02$^a$</td>
</tr>
</tbody>
</table>

$^a,b,c,d$ means with different letters across rows are significantly different ($P<0.05$). PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil, $\eta$ = flow behavior index, $K$ = consistency index.
Table 4.13 Flow Behavior Properties of PY, YPAM, YMMO and YMSO after 4 weeks storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>PY</th>
<th>YPAM</th>
<th>YMMO</th>
<th>YMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>η (Pa.s⁻¹)</td>
<td>0.09 ± 0.01ᵃ</td>
<td>0.10 ± 0.02ᵃ</td>
<td>0.11 ± 0.01ᵃ</td>
<td>0.10 ± 0.03ᵃ</td>
</tr>
<tr>
<td>K (Pa.s)</td>
<td>25.92 ± 1.91ᵃ</td>
<td>27.84 ± 2.44ᵃ</td>
<td>27.81 ± 2.51ᵃ</td>
<td>28.92 ± 3.75ᵃ</td>
</tr>
<tr>
<td>Yield Stress</td>
<td>0.26 ± 0.04ᵃ</td>
<td>0.29 ± 0.04ᵃ</td>
<td>0.27 ± 0.01ᵃ</td>
<td>0.29 ± 0.03ᵃ</td>
</tr>
<tr>
<td>(Pa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparent Viscosity (Pa.s)</td>
<td>0.35 ± 0.02ᵃ</td>
<td>0.39 ± 0.01ᵃ</td>
<td>0.36 ± 0.01ᵃ</td>
<td>0.38 ± 0.03ᵃ</td>
</tr>
</tbody>
</table>

ᵃᵇᶜᵈ means with different letters across rows are significantly different (P<0.05). PY = plain yogurt, YPAM = yogurt with arabic gum-maltodextrin powder, YMMO = yogurt with microencapsulated menhaden oil, YMSO = yogurt with microencapsulated salmon oil, η = flow behavior index, K = consistency index.

Stirred yogurt is a viscoelastic fluid that has some of the elastic properties of an ideal solid and some of the flow properties of an ideal liquid. Elastic or storage modulus (G’) indicates the solid like properties. Viscous or loss modulus (G”) reflects the liquid like properties. The viscoelastic properties of PY, YPAM, YMMO and YMSO are shown in Fig. 4.14 and Fig. 4.15. In the range of frequencies analyzed, the form of the spectra indicated a well formed stable gel, with G’ values higher than G”. These results are in accordance to Sendra et al. (2010) who reported that yogurts showed a predominantly elastic behavior (G’ > G”) over the whole range of frequencies tested (0.1-10 rad/s), which corresponds closely to that of a true gel. G’ values of yogurt gels increased as frequency increased and similar results were reported by Lee and Lucey (2004). According to Lucey (2004), a G’ higher than G” can be due to decreased electrostatic repulsion and increased casein-casein interactions. On the other hand, a G” > G’ means that the material has liquid like behavior (Rao, 1999). Storage time led to an increase in G” and a decrease in G’ in all yogurt. The incorporation of PAM, MMO and MSO produced no significant (P>0.05) increase or decrease in the values of both modules in comparison to PY. The interpretation of these results is that the structural organization and molecular interactions of PY are not modified.
by the addition of PAM, MMO or MSO. Similar interpretations were made by Sanz et al.,
(2008).

![Graph showing Frequency Sweeps Test of Yogurts at Day 1: Elastic Modulus (G') and Viscous Modulus (G'"") vs. Angular Frequency]

![Graph showing Week 4 Frequency Sweeps Test of Yogurts: Elastic Modulus (G') and Viscous Modulus (G'"") vs. Angular Frequency]

Fig. 4.14 Frequency Sweeps Test of Yogurts at Day 1: Elastic Modulus (G’) and Viscous Modulus (G’"") vs. Angular Frequency

Fig. 4.15 Week 4 Frequency Sweeps Test of Yogurts: Elastic Modulus (G’) and Viscous Modulus (G’"") vs. Angular Frequency
Stress sweeps are a way to characterize material stability and are normal quality control tests in food applications (Associated Polymer Labs, Inc., 2010). The initial plot of $G'$ versus oscillation stress of PY, YPAM, YMMO, and YMSO (Fig. 4.16) indicates that all yogurts had a similar behavior in the viscoelastic range analyzed. The $G'$ of all yogurts decreased at a stable rate as stress increased; however a steep drop in $G'$ after an oscillation stress of around 50 Pa, indicates an irreversible gel breakage of the samples. A similar behavior was observed in yogurts after 4 weeks storage (Fig. 4.17), nevertheless, PY had a slightly longer linear viscoelastic range than YPAM, YMMO, and YMSO which indicates a slightly lower degree of stability of the latter. The onset of non-linearity is thought to result from coalescence of the emulsion components or emulsion destabilization that may manifest itself in phase separation during storage (Bohlin Instruments Ltd., 2010). Moreover, a lower $G'$ in stress sweeps analysis may be indicative of a decrease in the solid like behavior of stirred yogurts (Sendra et al., 2010). The wider the linear viscoelastic range the more stable the material (Associated Polymer Labs, Inc., 2010).
The week 4 stress sweep test results are similar to the results observed for $K$ values after 4 weeks storage of yogurts (Table 4.10) in which the consistency of yogurts with added PAM, MMO and MSO was lower than PY.

Fig. 4.17 Week 4 Stress Sweeps Test of Yogurts: Elastic Modulus ($G'$) vs. Oscillation Stress

4.9 Confocal Scanning Laser Microscopy of Yogurts’ Structure

Microstructure of yogurt gels has been analyzed using CSLM in previous studies by Lee and Lucey (2004), Hassan et al. (1995, 2003), and Lucey et al. (1998). Casein micelles have the ability to reflect laser beams, and this property can be used to observe casein in milk and dairy products (Tamime et al., 2007). CSLM has minimal sample preparation steps which can enable the monitoring of yogurt microstructure without disturbing the gel. Figure 4.18 presents initial (day 1) CSLM micrographs of PY, YPAM, YMMO, and YMSO. Large casein micelle aggregates (orange) separated by dark whey spaces can be observed in all yogurts. Accordingly, Hassan et al. (1995) described the formation of a well-defined network in yogurt as the formation of wide non-reflecting (serum cavities) and protein clusters connected via thin
strands. Moreover, stirred yogurt has very large clusters of caseins presumably created by the collisions and shearing during the mixing process (Lee and Lucey, 2006). Yogurt micrographs after 4 weeks of storage of yogurts (Fig. 4.19) show a decrease in the size of the dark whey areas and denser protein aggregation in all yogurts compared to initial images (Fig. 4.18). Rearrangement of the protein aggregates may result in a network with a microstructure composed of thickly aggregated protein strands (Hassan et al., 2003). Moreover, whey separation has been related to the initial presence of large whey pores in the microstructure of stirred yogurts. At pH 4.4 the microstructure of yogurt became finer and more porous and smaller non-
reflective zones appeared as a result of the contraction of the casein network (Hassan et al., 1995). Lucey et al. (1998), reported that gels made from milk heated at 80 °C during 30 min had a smooth surface as observed by CSLM but after gelation structural rearrangements started to occur, which resulted in the formation of visible cracks and a rough surface appearance.

4.10 Transmission Electron Microscopy of Yogurts

TEM uses electrons that pass through the sample to obtain high contrast images. Specimens for examination by TEM need to be very thin to obtain good resolution and are stained with heavy

Fig. 4.19 Week 4 confocal scanning laser micrographs of PY (A), YPAM (B), YMMO (C), and YMSO (D)
metal salts (uranyl acetate and lead citrate) before examination (Lewis, 2007). Initial TEM micrographs of PY, YPAM, YMMO and YMSO (Fig. 4.20) show the presence of well coated spherical microcapsules (black arrows) in YPAM, YMMO and YMSO. The micrographs show an open protein structure (dark areas) and small size dispersed fat globules which are characteristic of homogenized dairy products (Tamime et al., 2007). The original thick protein structure of yogurt gels does not allow proper visualization of microcapsule, bacteria or fat structures. For this reason the samples are dissolved and thoroughly vortexed prior to analysis.

Fig. 4.20 Initial transmission electron micrographs of PY (A), YPAM (B), YMMO (C), and YMSO (D)
and yogurt structure conclusions are not drawn from TEM micrographs. The dissolution of the samples may also be the main reason for the reduction in size of PAM, MMO and MSO microcapsules compared to the size ranges reported from SEM images. Presence of undissolved microcapsules in YPAM, YMMO and YMSO was also observed after 4 weeks storage (Fig. 4.21). Bacteria were also observed in all yogurts and micrographs of *L. bulgaricus* (Fig. 4.22) and *S. thermophilus* (Fig. 4.23) are shown below. Structural studies of various fermented dairy
products with TEM have been conducted previously (Mainville et al., 2001; Kalab, 1993; Tamime et al., 2007).

Fig. 4.22 TEM micrograph of *Lactobacillus bulgaricus*

Fig. 4.23 TEM micrograph of *Streptococcus thermophilus*
CHAPTER 5 CONCLUSIONS

This study aimed to develop an ω-3 and vitamin E enriched stirred plain yogurt and to evaluate its characteristics during 4 weeks of refrigerated storage. Cardiovascular diseases claim millions of lives in the United States yearly and the costs related to their treatment are extremely high. Many scientific publications strongly suggest that regular consumption of significant amounts of PUFAs rich in EPA and DHA can be highly effective in the prevention or treatment of cardiovascular diseases. Vitamin E is an important lipophilic antioxidant that protects cell membranes from oxidative stress which is related to the development of many diseases. Fermented dairy foods have many health claims related to them and they are a product category that is constantly showing economic growth. Considering the above, the production of a functional plain stirred yogurt with microencapsulated purified salmon or menhaden oil and vitamin E is a new alternative for the increasing market of health conscious consumers and may contribute to an increase in the ω-3 and vitamin E consumption of the American population. Furthermore, the microencapsulation process has the potential to prevent loss of nutritional value of PUFAs and development of undesirable oxidation products during yogurt storage.

The specific objectives of this study were to: (1) produce free flowing powders of MMO and MSO at a pilot scale; (2) evaluate the physical and chemical characteristics of MMO and MSO prior to addition to PY; (3) produce and describe the pilot scale processing of YMMO and YMSO; and (4) evaluate the microstructure, rheological, physical, and chemical characteristics of YMMO and YMSO during 4 weeks storage at 4 °C. MMO and MSO free flowing powders were produced from PMO and PSO, respectively, through a process that included batch ultrasonic emulsification (65000 J/batch) and spray drying at an inlet temperature of 180 ± 1 °C on a pilot scale spray drier. MMO and MSO had ME of 78.34 ± 1.66 and 80.46 ± 0.48,
respectively which is in agreement with previously published values for microencapsulated oils. Acceptable TOTOX values were estimated for MMO (11.96 ± 0.14) and MSO (6.19 ± 0.04) and color analysis indicated a significantly lighter and more yellowish color of MMO than MSO. Moisture contents of both powders were around 3% which is in accordance with the moisture content of most food grade powders. EPA and DHA were the predominant ω-3 fatty acids detected in MMO and MSO. Yogurt processing was conducted on a pilot scale in the Louisiana State University Dairy Processing Plant and PY and YPAM were used as controls. MMO and MSO were added during the dry ingredient mix, prior to a two stage homogenization and batch pasteurization at 85 ± 1 °C. Inoculated mixes were incubated to pH 4.5 and stored at 4 °C in polypropylene containers during 1 month for analysis. α-Tocopherol contents of YMMO and YMSO were significantly (P<0.05) higher than those of PY and YPAM during the 4 week storage study, Initial PY, YPAM, YMMO and YMSO α-tocopherol contents were 37.65 ± 3.17, 43.03 ± 0.71, 75.61 ± 0.98, and 72.09 ± 0.18 µg/ g yogurt, respectively. Yogurt FAMEs composition indicated that yogurt lipids are mainly composed of SAFA and MUFA, however the total ω-3 content of PY was significantly (P<0.05) increased by addition of MMO and MSO. Changes in TBA values of PY, YPAM and YMMO during 4 weeks storage were not significant (P>0.05) and YMSO TBA values were significantly (P<0.05) higher than those of all other yogurts during storage. The low TBA values of PY, YPAM, YMMO and YMSO were indicative of only a minor progress in the secondary reactions of lipid oxidation. MMO and MSO addition had no significant (P>0.05) effect on LAB counts, post acidification behavior, syneresis, L*, and a* values of enriched yogurts compared to PY and YPAM. Values for b* were significantly (P<0.05) higher in YMMO and YMSO. The Herschel Bulkley flow behavior analysis of yogurts indicated that all gels had a yield stress and were non Newtonian (η<1) fluids with shear thinning
behavior. The evaluation of viscoelastic properties through frequency sweep tests demonstrated that YMMO and YMSO were well structured stable gels (G’>G’’) not prone to syneresis or phase separation during shelf life. CSLM and TEM micrographs allowed the visualization of protein network microstructure changes and microcapsule presence during the 4 week storage of yogurts.

Market and nutritional values of yogurt and fermented dairy products can be enhanced by adding microencapsulated polyunsaturated fish oils and vitamin E. In this study, the fatty acid composition and oxidative stability of PMO and PSO were not compromised and the majority of the characteristics of yogurts remained unaffected. This value added functional plain stirred yogurt appeals to a wide variety of consumers; therefore it may have the potential to increase sales in the yogurt market.
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APPENDIX: CHARACTERISTICS OF PMO AND PSO

<table>
<thead>
<tr>
<th>Item</th>
<th>PMO</th>
<th>PSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV, milliequivalents/ kg oil</td>
<td>2.87 ± 0.09</td>
<td>2.41 ± 0.05</td>
</tr>
<tr>
<td>AV</td>
<td>5.39 ± 0.27</td>
<td>1.10 ± 0.11</td>
</tr>
<tr>
<td>TOTOX</td>
<td>11.13 ± 0.44</td>
<td>5.92 ± 0.22</td>
</tr>
<tr>
<td>FFA, %</td>
<td>0.62 ± 0.01</td>
<td>0.92 ± 0.02</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>0.18 ± 0.01</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Color L*</td>
<td>77.95 ± 0.02</td>
<td>66.85 ± 0.03</td>
</tr>
<tr>
<td>Color a*</td>
<td>7.6 ± 0.01</td>
<td>8.15 ± 0.33</td>
</tr>
<tr>
<td>Color b*</td>
<td>18.56 ± 0.02</td>
<td>55.99 ± 0.08</td>
</tr>
<tr>
<td>α-tocopherol, µg/ ml oil</td>
<td>558.35 ± 15.88</td>
<td>616.72 ±12.37</td>
</tr>
</tbody>
</table>

PMO= purified menhaden oil, PSO= purified salmon oil, PV= peroxide value, AV= anisidine value, TOTOX= total oxidation, FFA= free fatty acids, L* = degree of darkness to lightness, a* = degree of greenness (-) to redness (+), b* = degree of blueness (-) to yellowness (+).
VITA

Jose Daniel Estrada was born in December 1986 in Tegucigalpa, Honduras. He earned his Bachelor of Science in Food Agroindustry in Zamorano University, Honduras, in December 2007. He joined the Department of Food Science at Louisiana State University in August 2009 where he is currently a candidate for a master’s degree in food science. He will receive his degree in August 2011.