Developing a strawberry yogurt fortified with marine fish oil¹

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ABSTRACT

Fortified dairy products appeal to a wide variety of consumers and have the potential to increase sales in the yogurt industry and help increase intake of long-chain n-3 fatty acids. The objectives of this study were to develop a strawberry yogurt containing microencapsulated salmon oil (MSO; 2% wt/vol) and evaluate its characteristics during 1 mo of storage. Unpurified salmon oil (USO) was purified (PSO) and both USO and PSO were analyzed for peroxide value (PV), anisidine value (AV), total oxidation, free fatty acids (FFA), and moisture content. A stable emulsion was prepared with 7% PSO, 22% gum arabic, 11% maltodextrin, and 60% water. The emulsion was spray-dried to produce MSO. The MSO was added to strawberry-flavored yogurt (SYMSO) before pasteurization and homogenization, and a control (SY) without MSO was produced. Both yogurts were stored for 1 mo at 4°C and we determined the quality characteristics including acidity (pH), syneresis, thiobarbituric acid (TBA), fatty acid methyl ester composition, color, and lactic acid bacteria (LAB) count. The entire experiment was replicated 3 times. Total oxidation (unitless) of USO, PSO, and MSO was calculated to be 20.7 ± 1.26 , 10.9 ± 0.1 , and 13.4 ± 0.25 , respectively. Free fatty acid contents were $1.61 \pm 0.19\%$, $0.59 \pm 0.02\%$, and $0.77 \pm 0.02\%$ for USO, PSO, and MSO, respectively. Eicosapentaenoic acid and docosahexaenoic acid were the predominant polyunsaturated fatty acids in MSO and in SYMSO, but neither was detected in SY. Fortification of SY with MSO had no significant effect on yogurt pH or syneresis. A decrease in concentration of lactic acid bacteria was observed during the storage of all yogurts. Thiobarbituric acid values significantly increased as storage time increased and SY had a significantly lighter (higher L*) and less yellow (lower b*)

color than SYMSO. Although some slight differences were observed in the color and oxidation of SYMSO compared with SY, the study demonstrated that SY could be fortified with salmon oil.

Key words: marine fish oil, n-3, yogurt, microencapsulation

INTRODUCTION

Yogurt is a product made from heat-treated milk; it may also be homogenized before the addition of lactic acid bacteria (LAB) containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (US FDA-DHHS, 2011). According to the USDA National Agricultural Statistics Service (USD-NASS, 2008), 739,355,563 kg of yogurt were produced in the United States in 1998, and by 2008, annual production had increased 120% to 3.59 billion lbs. 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 NPD Group (2010), a market research company, announced recently that yogurt is among the top growing snack foods for children from 2 to 17 yr of age. The production of yogurt with microencapsulated marine n-3 fatty acids may be an alternative for the increasing market of health-conscious consumers and may contribute to an increase in n-3 consumption in the population. In a recent study, Blasbalg et al. (2011) quantified changes in the apparent consumption of n-3 and n-6 fatty acids in the United States during the 20th Century. The authors concluded that the increase in consumption of linoleic acid from soybean oil has decreased human tissue concentrations of eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3). It is known that the recommended intakes for n-3 fatty acids are not uniform (Whelan and Rust, 2006). In 2004, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommended an intake of EPA and DHA of at least 500 mg/d (Cunnane et al., 2004).

Cardiovascular disease (CVD) is ranked as the number one killer in the United States. In 2010, the

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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 polyunsaturated fatty acids (PUFA) rich in n-3 fatty acids can be highly effective in the prevention or treatment of CVD. Intake of PUFA is generally low in the diets of Western (developed) populations, and increased intake of these acids by supplementation of selected foods is recommended (Bauch et al., 2006). Salmon oil is a good source of PUFA, specifically EPA and DHA. Values reported by the USDA National Nutrient Database (USDA, 2009) indicate that 100 g of salmon oil contains 13.02 and 18.23 g of EPA and DHA, respectively.

A wide range of dairy and nondairy foods has been fortified with n-3 fatty acids. The main ways to deliver these fatty acids to foods are through direct addition of fish oil or algal oil and bio-delivery through meat and poultry products (Whelan and Rust, 2006). Most attempts to add DHA or EPA directly to foods have been unsuccessful because these fatty acids are unstable and rapidly give rise to a fishy odor and taste upon oxidation, making the food unpalatable. Microencapsulation is a process by which particles of sensitive materials are packed into a film of a coating material (Sathivel and Kramer, 2010), and this process could be used to decrease oxidation of salmon oil. The direct addition of microencapsulated salmon oil (MSO) to yogurt may not affect the original physical, chemical, and microbiological characteristics of the yogurt. Therefore, the objectives of this study were to develop a strawberry yogurt containing MSO (SYMMSO; MSO at 2% wt/vol) and evaluate its physical, chemical, and microbiological characteristics during 1 mo of storage.

MATERIALS AND METHODS

Extraction and Purification of Oils

Salmon oil was obtained from fresh red salmon (*Oncorhynchus nerka*) heads kept refrigerated at 4°C. The salmon oil was extracted as described by Sathivel (2005), with modifications. The heads were weighed and ground using an industrial meat grinder (Northern Industrial, Burnsville, MN). The ground heads were cooked in 2-L beakers at 75°C for 30 min in a water bath (Lab-Line Instruments Inc., Melrose Park, IL) with constant electric stirring (model 5VB-C2, Eastern Mixers, Clinton, CT). The cooked, ground heads were centrifuged using a Beckman J2-HC centrifuge (GMI Inc., Ramsey, MN) at $7,957.6 \times g$ for 30 min at 4°C. After centrifugation, unpurified salmon oil (USO) was collected and purified using chitosan, as described by

Huang and Sathivel (2010). Chitosan bears hydroxyl and amino groups, which are excellent functional groups for use as adsorbents for a variety of complex compounds (Quignard et al., 2000). Huang and Sathivel (2010) reported that chitosan could be used as an adsorbent for removal of FFA and peroxides from unpurified salmon oil. Five percent (wt/vol) shrimp chitosan was added to the USO and the mixture was stirred for 1 h at room temperature. Then, the mixture was centrifuged ($23,385.6 \times g$) for 25 min at 4°C and purified salmon oil (PSO) was collected. The PSO was characterized and stored in a blast freezer at -20°C until use.

Preparation of MSO

Microencapsulated salmon oil was produced as described by Pu et al. (2011). A stable emulsion was prepared with a CPX-500 ultrasonic processor (Cole Parmer Instruments, Vernon Hills, IL) using the following ratio of components: 7% PSO:22% gum arabic:11% maltodextrin:60% water. The emulsion was spray-dried in a FT80 tall form spray drier (Armfield Ltd., Ringwood, UK) at an inlet temperature of 180°C to produce MSO.

Microencapsulation Efficiency and Encapsulation Yield

The method described by Wanasundara and Shahidi (1995) was used to determine the microencapsulation efficiency of MSO. One hundred grams of MSO was dissolved in 500 mL of a 0.88% (wt/vol) KCl solution. A few crystals of *tert*-butylhydroquinone and 1.5 L of a 2:1 chloroform:methanol solution were added. The mixture was centrifuged at $23,385.6 \times g$ for 5 min at 4°C and transferred to a separatory funnel to separate the chloroform:methanol layer filtrate, which was collected in a round-bottomed flask for subsequent evaporation in a rotary evaporator (Buchi RE-121 Rotavapor, Flawil, Switzerland) at 40°C to reduce oxidation. The total oil (TO; %) of MSO was calculated gravimetrically.

To extract the surface oil (SO) from MSO, 2 g of the microencapsulated powder was mixed with 10 mL of hexane for 10 min. The mixture was filtered through Whatman 4 filter paper and washed with 4×10 mL of hexane. The filter paper containing the washed sample was oven-dried (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. The microencapsulation efficiency (ME) was calculated as follows:

$$ME = \frac{TO - SO}{TO} \times 100.$$

Encapsulation yield (*EY*) was determined using the following formula:

$$EY = \frac{OM \text{ (g/100 g)}}{OE \text{ (g/100 g)}},$$

where *OM* represents grams of total PSO in the 100 g of (dry weight) microcapsules and was calculated based on the *TO* (%) and moisture content of MSO; *OE* represents the PSO in the emulsion and was calculated using the moisture content and PSO load of the emulsion described above.

Peroxide Value, Anisidine Value, FFA, and Moisture of USO, PSO, and MSO

The USO, PSO, and MSO samples were analyzed the day before being used for encapsulation and yogurt production, respectively. The peroxide value (**PV**) and anisidine value (**AV**) were determined following American Oil Chemists Society (**AOCS**) Official Methods (AOCS, 1997). The acetic acid-chloroform method (AOCS method Cd 8–53) was used to determine the PV. The PV of oils were reported in milliequivalents of peroxide per 1,000 g of oil. The AV of the oil was determined using AOCS method Cd 18–90. The total oxidation (**TOTOX**) was calculated based on the PV and AV using the following equation: $TOTOX = 2PV + AV$.

Free fatty acids were measured in USO, PSO, and MSO as described by AOCS method Ca 5a-40 (AOCS, 1997), with slight modifications. Two grams of the oil samples were mixed with 10 mL of heated and neutralized ethanol solution and 0.4 mL of phenolphthalein in a 125-mL flask. The mixture was titrated using 0.1 *N* NaOH until a faint pink color was retained. The percentage FFA as oleic acid was calculated as follows:

$$FFA \text{ (\%)} = \frac{NaOH \text{ (mL)} \times N \times 28.2}{Mass \text{ (g)}},$$

where *N* = normality of NaOH and mass (g) refers to the mass of sample used. The moisture content of USO and PSO was determined using a Moisture Meter (CA-21 Moisture Meter, Cosa Instrument Corporation, Houston, TX). The moisture content of MSO was measured using a Moisture Analyzer Smart System 5 (CEM Corporation, Matthews, NC). Results are reported as percentage moisture.

Preparation of Strawberry-Flavored Yogurt Containing MSO

Strawberry-flavored yogurt with MSO (**SYMISO**) was produced by adding 2% MSO with the dry in-

gredients before pasteurization and homogenization. Strawberry yogurt was produced in the Louisiana State University Department of Dairy Science Creamery. The dry ingredients, including MSO (Table 1), were incorporated into 2% fat milk in 18.9-L stainless steel containers before pasteurization. The mixture was homogenized at 3,447.4 and 10,342.1 kPa in the first and second stages, respectively, using a Gaulin 300 DJF 4 2PS homogenizer (APV Gaulin, Wilmington, MA) and then batch pasteurized for 30 min at $85 \pm 1^\circ\text{C}$. The mixture was cooled to 43°C before addition of lactic cultures of *Lactobacillus bulgaricus* (LB-12, Chr. Hansen, Milwaukee, WI) and *Streptococcus thermophilus* (ST-M5, Chr. Hansen) at a rate of 1 mL/3.78 L of milk each. Inoculated mixes were poured into 8-L stainless steel pots and placed in a low temperature incubator (model 815, Precision Scientific, Asheville, NC) at $43 \pm 1^\circ\text{C}$ until the pH reached 4.5. Samples were cooled immediately and stored at 4°C in a walk-in refrigerator. Strawberry with other natural flavors (Target Flavors Inc., Brookfield, CT), strawberry puree (Sensient Flavors, Fenton, MO), and FD&C red 40 powder color (Sensient Colors) were added during the stirring procedure, after overnight aging of yogurt mixes. Sodium benzoate was used as a preservative. A control (**SY**) without MSO was produced and both yogurts were stored for 1 mo at 4°C . Yogurts were analyzed for fatty acid methyl esters (**FAME**), acidity, syneresis, thiobarbituric acid value (**TBA**), color, and LAB counts at d 1 and wk 1, 2, 3, and 4.

FAME of MSO, SYMSO, and SY

Fatty acid methyl esters were measured by GC in MSO before use in yogurt production and at d 1 and wk 4 for SYMSO and SY. Contents of FAME, expressed as a percentage of total FAME measured, in MSO and yogurts were determined using standard GC methods. Lipid was extracted from yogurt samples using a modified Folch extraction method in which 50 g of yogurt was homogenized in 1 L of 2:1 chloroform:methanol solution and then filtered through no. 4 filter paper. A 0.66% NaCl solution (200 mL) was added before centrifugation at $649.6 \times g$ for 15 min. The chloroform layer containing the lipids was evaporated using a rotary evaporator.

The FAME were prepared using the method described by Maxwell and Marmer (1983). Approximately 20 mg of oil was methylated for analysis of fatty acid profile on a GC coupled to a flame ionization detector (Agilent Technologies Inc., Santa Clara, CA). Separation was made on a FAMEWAX (30 m $\times$ 0.32 mm i.d., 0.25 mm film) capillary column (Restek, Bellefonte, PA). Helium gas was the carrier at a constant flow rate of

Table 1. Yogurt formulation in manufacture of strawberry yogurt (SY) and strawberry yogurt with microencapsulated salmon oil (SYMSO)

Ingredient	Addition rate	Control (SY)	SYMSO
2% fat milk, L		3.8	3.8
Nonfat dry milk, g	5% or 3%	190.0	114.0
Sugar, g	5%	190.0	190.0
Microencapsulated salmon oil, g	2%	—	76.0
<i>Lactobacillus bulgaricus</i> , mL	1 mL/3.8 L	1.0	1.0
<i>Streptococcus thermophilus</i> , mL	1 mL/3.8 L	1.0	1.0
Strawberry with other natural flavors, mL	0.30%	11.4	11.4
Strawberry puree, g	5%	190.0	190.0
Red 40 color, mL	0.005%	0.19	0.19
Sodium benzoate, g	0.10%	3.8	3.8

2 mL/min. The inlet and detector temperatures were 250°C and 280°C, respectively. The temperature program was as follows: initial temperature at 90°C with 8-min hold, increased to 175°C at 10°C/min with 10-min hold, increased to 190°C at 4°C/min with 10-min hold, increased to 210°C at 5°C/min with 5-min hold, and then increased to 250°C at 20°C/min with 8-min hold. Standards used for identification of peaks were Supelco 37, Bacterial Acid Methyl Esters Mix, Marine Oil #1, and Marine Oil #3 (Supelco, Bellefonte, PA) and the internal standard used was C23:0.

pH, Syneresis, TBA Value, and Color of SYMSO and SY

The pH of the yogurts was measured using an EcoT-estr pH tester (Eutech Instruments, Vernon Hills, IL) at about 8°C. The meter was calibrated using commercial pH 4.00 and 7.00 buffer solutions. The water-holding capacity of yogurt, expressed as syneresis, was determined using the method described by Cueva and Aryana (2008). The release of whey in the yogurt samples was measured by pouring 200 g of yogurt at 4°C onto fine cheesecloth placed on top of a funnel. The amount of whey collected in a graduated cylinder after 2 h was used as an index of syneresis. Analysis of TBA of SY and SYMSO samples was done following the procedure described by Hekmat and McMahon (1997). One gram of plain yogurt was weighed into a glass screw-top test tube; then, 9 mL of 15% (wt/vol) TCA and 0.375% (wt/vol) TBA reagent, and 0.25 N HCl solution were added, mixed well, and heated in a boiling water bath for 15 min. Samples were quickly cooled to room temperature with running water and centrifuged at $6,311.2 \times g$ for 15 min at 20°C. Absorbance was measured at 530 nm and the malonaldehyde (MDA) concentration was calculated with the extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\cdot\text{cm}^{-1}$ using the following equation: $A\lambda = \epsilon \times c \times L$, where $A\lambda$ is absorbance, ϵ is the extinction coefficient, c is the molar concentration expressed in

moles of MDA per kg of yogurt, and L is the light path in centimeters. A Lab Scan XE spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA) was used for color analysis of yogurts and MSO. Results are reported in L^* (lightness), a^* (red-green), and b^* (yellow-blue) values.

LAB Microbial Counts

Serial dilutions of yogurt were made using sterile de Man, Rogosa, and Sharpe broth for LAB (3M Microbiology, St. Paul, MN). Petrifilm aerobic count plates (3M Microbiology) were used for plating, and incubation was carried out in a Gaspak system at 35°C for 48 h.

Statistical Analysis

Mean values and standard deviations of triplicate determinations were reported. Analysis of variance was carried out to determine the difference among treatments means (SAS version 8.2, SAS Institute Inc., Cary, NC) using the post hoc Tukey studentized range test.

RESULTS AND DISCUSSION

PV, AV, FFA, and Moisture Contents of USO, PSO, and MSO

Peroxide value is an indicator of initial lipid oxidation. The PV content of USO, PSO, and MSO was 10.13 ± 0.7 , 5.16 ± 0.05 , and 6.32 ± 0.11 mEq/kg, respectively. In the purification process, chitosan effectively adsorbed primary oxidation products. According to Gracey et al. (1999), oil with PV <5 mEq/kg can be considered fresh oil or one in which hydroperoxides have degraded into secondary oxidation products, such as ketones and aldehydes. The secondary oxidation products were measured by AV. The AV of USO was low (0.43 ± 0.14) and no further reduction ($P >$

0.05) occurred with chitosan purification. However, AV increased significantly ($P < 0.05$) after microencapsulation to 0.72 ± 0.03 (MSO). The TOTOX values (unitless; $\text{TOTOX} = 2\text{PV} + \text{AV}$) of USO, PSO, and MSO were calculated to be 20.7 ± 1.26 , 10.88 ± 0.1 , and 13.36 ± 0.25 , respectively. Lipid oxidation in oil-in-water emulsions has been studied and the interaction between lipid hydroperoxides located at the droplet surface and metals originating in the aqueous phase is the most common cause of oxidative instability (McClements and Decker, 2000). Kolanowski et al. (2006) reported that spray-drying results in higher PV and that oxidative stability of fish oil is not improved after the process. All oils had acceptable FFA values: 1.61 ± 0.19 , 0.59 ± 0.02 , and $0.77 \pm 0.02\%$ for USO, PSO, and MSO, respectively. Oils with FFA values $>3\%$ are considered inedible (Gracey et al., 1999). The moisture content of USO and PSO was $0.18 \pm 0.01\%$ and $0.16 \pm 0.01\%$, respectively, and that of MSO was $2.44 \pm 0.12\%$ (Table 2).

SO, TO, Microencapsulation Efficiency, and Encapsulation Yield of MSO

Measurements of TO ($13.9 \pm 0.45\%$) and SO ($3.4 \pm 0.18\%$) content in MSO showed microencapsulation efficiency of $75.5 \pm 2.66\%$. The SO content is an important attribute evaluated in spray-dried encapsulated oil powders because it influences oxidative stability, wettability, flowability, and color (Konstance et al., 1995). Microencapsulation efficiency 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). Carbohydrates such as maltodextrin have typically been used as encapsulating agents (DeZarn, 1995). These materials are considered good encapsulating agents because they

exhibit low viscosities at high solid contents and good solubility. Most of them also 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 microencapsulation efficiency of $72.7 \pm 1.3\%$ for soybean oil microencapsulated using gum arabic and maltodextrin with a dextrose equivalent of 18.5. They suggest that this dextrose equivalent is the most suitable partial replacer for gum arabic as it is easily solubilized and bland in flavor, and as some preliminary work has shown, the dried encapsulates manufactured with this wall material are most rapidly reconstituted in water. The encapsulation yield calculated for this study was $81.5 \pm 0.47\%$, indicating that gum arabic and maltodextrin were able to effectively encapsulate the PSO. Gum arabic stands out among other gums because of its excellent emulsification properties, which are attributed to the presence of a protein fraction in its structure (Dickinson et al., 2003). Results are shown in Table 2.

FAME in MSO, SY, and SYMSO

The fatty acid composition of lipids extracted from MSO, SY, and SYMSO are given in Table 3. Polyunsaturated fatty acids in MSO accounted for 32.7% of the fatty acids detected, and EPA and DHA were the predominant fatty acids, accounting for 62.6% of the total PUFA content. Initially, SY and SYMSO lipids contained 1.76 and 7.50% of total n-3 fatty acids, respectively. After 4 wk of storage, total n-3 fatty acids in SY and SYMSO lipids accounted for 1.47 and 5.82%, respectively. The percentage reduction of total n-3 content after 4 wk of refrigerated storage was 16.5 and

Table 2. Physicochemical characteristics of unpurified (USO), purified (PSO), and microencapsulated (MSO) salmon oil

Item ¹	Salmon oil		
	USO	PSO	MSO
PV, mEq/kg of oil	10.13 ± 0.7	5.14 ± 0.04	6.32 ± 0.11
AV	0.43 ± 0.14	0.58 ± 0.04	0.72 ± 0.03
TOTOX	20.7 ± 1.26	10.86 ± 0.06	13.36 ± 0.25
Free fatty acids, %	1.61 ± 0.19	0.61 ± 0.02	0.77 ± 0.02
Moisture, %	0.18 ± 0.01	0.16 ± 0.01	2.44 ± 0.12
Total oil, %	—	—	13.86 ± 0.45
Surface oil, %	—	—	3.4 ± 0.18
Microencapsulation efficiency, %	—	—	75.49 ± 2.66
Encapsulation yield, %	—	—	81.53 ± 0.47
Color L*	—	—	97.75 ± 0.12
Color a*	—	—	1.37 ± 0.17
Color b*	—	—	12.13 ± 0.09

¹PV = peroxide value; AV = anisidine value; TOTOX = total oxidation ($\text{TOTOX} = 2\text{PV} + \text{AV}$).

Table 3. Fatty acid methyl ester (FAME) composition of microencapsulated salmon oil (MSO), strawberry yogurt (SY), and SYMSO (strawberry yogurt with MSO)¹

FAME, % of total fatty acids	MSO	SY		SYMSO	
		Initial	Wk 4	Initial	Wk 4
C6:0 (caproic)	— ²	1.49 ^a	1.48 ^a	1.15 ^a	1.22 ^a
C8:0 (caprylic)	—	1.06 ^a	1.04 ^a	0.82 ^a	0.89 ^a
C10:0 (capric)	—	2.67 ^a	2.56 ^b	2.08 ^a	2.24 ^a
C12:0 (lauric)	—	3.29 ^b	3.45 ^a	2.57 ^b	2.76 ^a
C14:0 (myristic)	4.29	11.04 ^a	10.99 ^a	9.92 ^b	10.23 ^a
C15:0 (pentadecanoic)	0.63	1.12 ^a	1.13 ^a	0.98 ^a	1.02 ^a
C16:0 (palmitic)	14.05	30.03 ^a	30.03 ^a	26.7 ^a	27.28 ^a
C16:1n-7 (palmitoleic)	4.34	1.68 ^a	1.59 ^b	2.61 ^a	2.34 ^b
C18:0 (stearic)	2.47	14.95 ^a	14.92 ^a	12.19 ^b	12.73 ^a
C18:1n-9 (oleic)	16.85	23.59 ^a	23.51 ^a	22.11 ^a	22.37 ^a
C18:1n-7 (vaccenic)	3.19	3.07 ^b	3.18 ^a	2.97 ^a	2.94 ^a
C18:2n-6 (linoleic)	2.09	4.32 ^a	4.44 ^a	3.98 ^a	3.96 ^a
C18:3n-3 (α -linolenic)	1.67	0.86 ^a	0.59 ^b	0.81 ^a	0.77 ^b
C18:4n-3 (octadecatetraenoic)	3.10	0.9 ^a	0.88 ^a	0.79 ^a	0.73 ^a
C20:1n-11 (gadoleic)	7.56	—	—	1.82 ^a	1.69 ^b
C20:1n-9 (eicosenoic)	3.27	—	—	0.84 ^a	0.82 ^a
C20:4n-3 (eicosatetraenoic)	1.89	—	—	—	—
C20:5n-3 (eicosapentaenoic)	10.24	—	—	2.9 ^a	2.11 ^a
C22:1n-11 (cetoleic)	8.31	—	—	1.77 ^a	1.66 ^b
C22:5n-3 (docosapentaenoic)	2.33	—	—	0.63 ^a	0.49 ^b
C22:6n-3 (docosahexaenoic)	10.24	—	—	2.37 ^a	1.72 ^b
Saturated fatty acids	21.44	65.65 ^a	65.60 ^a	56.41 ^a	58.37 ^a
Monounsaturated fatty acids	45.83	28.34 ^a	28.28 ^a	32.12 ^a	31.82 ^a
Polyunsaturated fatty acids	32.72	6.08 ^a	5.91 ^b	11.48 ^a	9.78 ^b
Total n-3	29.48	1.76 ^a	1.47 ^b	7.50 ^a	5.82 ^b
Total n-6	2.75	4.32 ^b	4.44 ^a	3.98 ^a	3.96 ^a

^{a,b}Different superscripts indicate a statistical difference ($P < 0.05$) between initial and wk-4 means of SY or SYMSO, separately.

¹Values are means of triplicate determinations and rows with values for samples of less than 1% in all columns are not included.

²Not detected.

22.4% for SY and SYMSO, respectively. Among PUFA in SYMSO lipids, EPA and DHA were the predominant fatty acids and neither was detected in SY. Considering that SYMSO had a fat content (estimated by the Babcock method) of $2.0 \pm 0.2\%$, a serving (200 g) of SYMSO at d 1 may contain 0.21 ± 0.01 g of EPA + DHA. Typical recommendations are 0.3 to 0.5 g/d of EPA + DHA (Institute of Medicine, 2002) and patients with CVD are encouraged to increase their consumption of EPA and DHA to approximately 1 g/d (Kris-Etherton et al., 2002). The intake of total n-3 fatty acids in the United States is approximately 1.6 g/d (Kris-Etherton et al., 2000). Of this, α -linolenic acid accounts for approximately 1.4 g/d, and only 0.1 to 0.2 g/d comes from EPA and DHA (USDA, 2002). Moreover, the mean intake of α -linoleic acid, EPA, DHA, and total marine n-3 fatty acids in US women 54.6 $\pm$ 7.0 yr old, was reported to be 1.11, 0.04, 0.12, and 0.18 g/d, respectively (Djoussé et al., 2011).

pH of SY and SYMSO

Fortification of strawberry yogurt with MFO had no significant effect on yogurt pH. The initial pH of SY

and SYMSO was 4.5 ± 0.0 , which is very similar to the pH values of SY (4.4 ± 0.0) and SYMSO (4.4 ± 0.0) measured after 4 wk of refrigerated storage. This is in accordance with results reported by Serra et al. (2009) in which titratable acidity increased slightly after 28 d of cold storage of set and stirred yogurts. This is a result of the persistent metabolic activity of starters, which has also been called postacidification (Beal et al., 1999). Ott et al. (2000) highlighted that pH correlates very well with most of the flavor attributes of yogurt. Moreover, they concluded that the intensity of yogurt flavor perception is driven by pH.

Syneresis of SY and SYMSO

Spontaneous syneresis is the expulsion of whey from yogurts because of structural rearrangements in the gel network (Serra et al., 2009). Structure rearrangements in yogurt have been previously reported by Hassan et al. (1995), Lucey et al. (1998), and Singh and Muthukumarappan (2008). Syneresis results are reported in Table 4. A slight, but not significant ($P > 0.05$), trend to increasing syneresis was observed in SY and SYMSO during storage. The mean values for syneresis in SY

ranged from 43.2 ± 7.02 initially to 48.4 ± 3.19 in wk 4. A similar trend was observed in SYMSO, in which syneresis increased from 40.1 ± 2.08 to 45.2 ± 1.73 from d 1 to wk 4, respectively. No significant differences were found in syneresis with fortification of MSO versus no addition (SY) throughout the storage study, except in wk 2, when syneresis was significantly higher ($P < 0.05$) in SY. This may be indicative of a similarity in the nature of the bonds involved in the gel structure formation of both yogurt samples. 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.

TBA Values of SY and SYMSO

Polyunsaturated fatty acids, as well as dairy products, are known to be susceptible to oxidation upon processing and storage, resulting in development of rancid off-flavors and taste. The lipid oxidation of SY and SYMSO during storage was evaluated by TBA analysis and the results are presented in Table 4. The results showed that lipid oxidation in yogurt was significantly increased in yogurts stored for 4 wk compared with initial values. The initial TBA values for SY and SYMSO were 0.05 ± 0.02 and 0.12 ± 0.01 mol of MDA/kg of yogurt, respectively. The TBA values increased proportionately to storage period and were different ($P < 0.05$) between SY and SYMSO in all the storage periods evaluated. By wk 4, TBA in SY underwent a 2.0-fold increase to 0.1 ± 0.01 , whereas TBA in SYMSO underwent a 2.2-fold increase to 0.26 ± 0.01 mol of MDA/kg of yogurt. Al-Rowaily (2008) studied the effect of processing methods and refrigerated storage of set and strained yogurt on lipid oxidation. The results indicated that lipid oxidation was significantly increased by milk batch pasteurization at 85 to 90°C for 2 min. Refrigerated storage of set yogurt for 7 d and strained yogurt for 15 d caused a significant difference in peroxide, anisidine, and TOTOX values. Lee et al. (2007) reported a slow TBA absorbance increase from 0.083 to 0.10 over the initial 6 d of storage of evening primrose oil (EPO)-enriched yogurt, followed by a dramatic increase up to 0.165 after 15 d. Evening primrose oil is of special interest due to its high PUFA content. Overall, the results of the Lee et al. (2007) study indicated that lipid oxidation proceeded more rapidly in yogurt with EPO than in that without EPO, in accordance with the trend observed in our study with SY and SYMSO. Furthermore, Hekmat and McMahon (1997) studied the overall effect of 30 d of storage on yogurt chemical oxidation, as measured by the TBA test. Low-fat yogurt exhibited a tendency toward increased chemical oxidation during storage

Table 4. Analysis of physicochemical characteristics and lactic acid bacteria growth during 4 wk of storage of strawberry yogurts

Item ¹	Strawberry yogurt (SY)					Strawberry yogurt with microencapsulated salmon oil (SYMSO)				
	Initial	Wk 1	Wk 2	Wk 3	Wk 4	Initial	Wk 1	Wk 2	Wk 3	Wk 4
pH	$4.5 \pm 0.04^{A,a}$	$4.4 \pm 0.1^{A,a}$	$4.4 \pm 0.1^{A,a}$	$4.4 \pm 0.0^{A,a}$	$4.4 \pm 0.0^{A,a}$	$4.5 \pm 0.0^{A,a}$	$4.5 \pm 0.1^{A,a}$	$4.4 \pm 0.1^{A,a}$	$4.4 \pm 0.0^{A,a}$	$4.4 \pm 0.0^{A,a}$
Syneresis, mL	$43.2 \pm 7.02^{A,a}$	$46.6 \pm 5.52^{A,a}$	$47.4 \pm 1.76^{A,a}$	$46.2 \pm 3.34^{A,a}$	$48.4 \pm 3.19^{A,a}$	$40.1 \pm 2.08^{A,a}$	$39.8 \pm 3.88^{A,a}$	$39.4 \pm 1.05^{A,b}$	$40.4 \pm 3.4^{A,a}$	$45.2 \pm 1.73^{A,a}$
TBA, mol of MDA/kg of yogurt	$0.05 \pm 0.02^{B,b}$	$0.05 \pm 0.00^{B,b}$	$0.06 \pm 0.00^{AB,b}$	$0.08 \pm 0.01^{AB,b}$	$0.1 \pm 0.01^{A,b}$	$0.12 \pm 0.01^{D,a}$	$0.13 \pm 0.01^{D,a}$	$0.18 \pm 0.01^{C,a}$	$0.23 \pm 0.00^{B,a}$	$0.26 \pm 0.01^{A,a}$
Color L*	$81.9 \pm 0.04^{B,a}$	$85.4 \pm 0.42^{A,a}$	$85.1 \pm 0.35^{A,a}$	$85.0 \pm 0.03^{A,a}$	$84.1 \pm 0.25^{A,a}$	$79.7 \pm 0.15^{B,b}$	$84.1 \pm 0.62^{A,b}$	$84.3 \pm 0.34^{A,b}$	$84.6 \pm 0.24^{A,b}$	$82.5 \pm 0.71^{A,b}$
Color a*	$9.1 \pm 0.04^{C,b}$	$10.1 \pm 0.5^{BC,a}$	$11.2 \pm 0.32^{AB,a}$	$12.0 \pm 0.3^{A,a}$	$11.9 \pm 0.63^{A,a}$	$9.6 \pm 0.11^{B,a}$	$9.2 \pm 0.25^{B,b}$	$9.5 \pm 0.06^{B,b}$	$9.6 \pm 0.09^{B,b}$	$11.4 \pm 0.88^{A,a}$
Color b*	$4.1 \pm 0.01^{C,b}$	$5.5 \pm 0.13^{A,b}$	$5.5 \pm 0.08^{A,b}$	$5.4 \pm 0.05^{AB,b}$	$4.8 \pm 0.38^{B,b}$	$6.8 \pm 0.07^{B,a}$	$8.4 \pm 0.24^{A,a}$	$8.2 \pm 0.62^{AB,a}$	$8.2 \pm 0.82^{AB,a}$	$7.7 \pm 0.33^{AB,a}$
LAB, log cfu/g	$8.69 \pm 0.01^{A,b}$	$8.69 \pm 0.02^{A,b}$	$7.44 \pm 0.04^{B,a}$	$6.32 \pm 0.06^{C,a}$	$6.29 \pm 0.04^{C,a}$	$8.77 \pm 0.02^{A,a}$	$8.75 \pm 0.02^{A,a}$	$7.41 \pm 0.06^{B,a}$	$6.34 \pm 0.07^{C,a}$	$6.31 \pm 0.04^{C,a}$

^{A–D}Different uppercase superscripts depict statistical difference ($P < 0.05$) between means within a row within SY or SYMSO.

^{a,b}Different lowercase superscripts depict statistical difference ($P < 0.05$) between means of SY and SYMSO measurements taken at the same storage time.

¹TBA = thiobarbituric acid; MDA = malonaldehyde; LAB = lactic acid bacteria.

up to 30 d. Oxidation can cause rancidity and thus negatively affect the sensory properties of taste and aroma (Kolanowski et al., 1999). Inoue et al. (1998) did not report any significant effect of malonaldehyde concentrations ranging from 5 to 10×10^{-5} mmol/mL on the sensory evaluation of frozen yogurt during 6 mo of storage. The results in our study are well below TBA thresholds for rancid odor detection in meats, which have been estimated to be 0.5 to 1 mg/kg (Tarladgis et al., 1960) and 2 mg/kg (Greene and Cumuze, 1981). To the best of our knowledge, a threshold limit for dairy products is not available in literature.

Color of MSO, SY, and SYMSO

The L^* (97.75 ± 0.12), a^* (1.37 ± 0.17), and b^* (12.13 ± 0.09) values of MSO are shown in Table 2 and those for SY and SYMSO are reported in Table 4. The initial L^* (lightness) value for SY (81.9 ± 0.04) was slightly but significantly higher ($P < 0.05$) than that for SYMSO (79.7 ± 0.15). The L^* values showed an increase after the first week of storage and SY values were higher than SYMSO during the entire study. An increase in a^* values was observed during 30 d of storage, from 9.1 ± 0.04 to 11.9 ± 0.63 in SY and from 9.6 ± 0.11 to 11.4 ± 0.88 in SYMSO, indicating an increase in the redness of the yogurts. Gel stirring and acidity changes in yogurt detected during refrigerated storage may be the cause of changes in a^* values because they may cause tissue structure changes that result in leakage of natural pigments (carotenoids) to the yogurt matrix. The control yogurt (initial $b^* = 4.1 \pm 0.01$) was less yellow than SYMSO (initial $b^* = 6.8 \pm 0.07$). The significantly ($P < 0.05$) greater yellowness of SYMSO can be attributed to the addition of MSO, which has a yellowish color imparted by the nonencapsulated salmon oil. To determine if color differences between samples could be detected by the naked eye, 12 untrained panelists were selected from among the staff of the Louisiana State University Department of Food Science. Panelists were requested to evaluate the appearance of the samples, which were identified with random 3-digit codes. No differences in color were detected in the appearance sensory assessment due to addition of 2% MSO to the SY formulation (data not shown).

LAB Counts of SY and SYMSO

Addition of MSO resulted in slightly higher ($P < 0.05$) initial LAB counts in SYMSO. A decrease in viable counts of LAB was observed during storage of SY

and SYMSO, which indicates low stability of the starter culture during shelf life. The initial counts of LAB were 8.69 ± 0.01 and 8.77 ± 0.02 log cfu/g; by wk 4, the counts decreased to 6.29 ± 0.04 and 6.31 ± 0.04 log cfu/g in SY and SYMSO, respectively (Table 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 and 10^7 cfu/g (a concentration considered good for the yogurt to fortify its nutritional value to consumers), but decreased to around 10^4 cfu/g (inadequate level) toward the end of the shelf-life study. Dave and Shah (1996) reported that viable counts of starter culture bacteria in yogurt increased and then declined during a 28-d refrigerated storage study. Lee et al. (2007) reported a decreasing trend in mean microbial counts during 15 d of refrigerated storage of yogurt enriched with EPO.

CONCLUSIONS

In the purification process, chitosan effectively adsorbed primary oxidation products but did not lower secondary oxidation products as measured by AV. The total oxidation of salmon oil was significantly increased after the emulsification and spray-drying process. The addition of MSO (2% wt/vol) to SY had no effect on the pH and syneresis of SYMSO. No significant differences were observed in LAB counts of SY and SYMSO; however, a decrease in LAB counts was observed during the storage of yogurts. Eicosapentaenoic acid and DHA were the predominant PUFA in MSO and they were abundant in SYMSO. Oxidation of SY and SYMSO (TBA values) increased as storage time increased; however, the low concentrations of malonaldehyde may not have had significant effects on sensory perception during 4 wk of storage. The addition of MSO to yogurt did not greatly modify pH, color, or water-holding capacity characteristics of SY. The results from our study demonstrate that SYMSO can be produced by direct addition of MSO before homogenization and pasteurization. To determine the potential for commercialization of strawberry yogurt fortified with microencapsulated fish oil, further research is suggested to evaluate its sensory quality and correlation to physicochemical characteristics.

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