Oxidatively Derived Volatile Compounds in Microencapsulated Fish Oil Monitored by Solid-phase Microextraction (SPME)

RÓSA JÓNSDÓTTIR, MARGRÉT BRAGADÓTTIR, AND GUDMUNDUR ÖRN ARNARSON

ABSTRACT: The stability of microencapsulated fish oil was studied during storage at 4 °C for up to 20 wk. Different coating mixtures consisting of gelatin or caseinate in blends with carbohydrates (sucrose, lactose, maltodextrin) were investigated. Oxidative stability of the microencapsulated fish oil was monitored by analysis of volatile compounds using gas chromatography olfactometry (GC-O) or GC flame ionization (GC-FID) (SPME-HS-GC/O or GC/FID and HS-GC/MS), Oxipres test, thiobarbituric acid-reactive substances (TBARS), and sensory analysis. Coating mixture of caseinate and lactose showed slightly better stability than the sucrose and maltodextrin caseinate mixtures. Combination of fish gelatin and maltodextrin did not show as good oxidative stability as the coating blend of caseinate, lactose, and lecithin. Hexanal, 2-nonenal and 2,4-decadienals were selected as quality indicators to monitor the lipid oxidation during storage of the samples. SPME-GC-O analysis of these indicators showed that they were representative for the oxidation occurring in the microencapsulated fish oil. SPME-GC–FID analysis was sensitive enough to detect oxidative changes during storage. Oxidative stability test, TBARS results, and sensory analysis were in agreement with the SPME, indicating that SPME (polydimethylsiloxane/divinylbenzene [PDMS/DVB] fiber) can be a useful tool for rapid analysis of lipid oxidation in microencapsulated fish oil.

Keywords: microencapsulated fish oil, SPME, lipid oxidation, volatile compounds, gas chromatography–olfactometry (GC-O)

Introduction

Epidemiological studies suggest that a diet high in long chain n-3 polyunsaturated fatty acids (PUFAs) may have beneficial effects on human health (Uauy and Valenzuela 2000; Larsson and others 2004; Schmidt and others 2004). However, fish oils are very sensitive to oxidation due to the high degree of unsaturated n-3 PUFAs. Most of the oxidatively derived volatile components identified in fish oil are derived from the n-3 fatty acid family like 2,4,7-decatrienals characterized by fishy and cod liver oil–like flavors (Lindsay 1990). 1-Penten-3-one (pungent, green odor), 4-*cis*-heptenal (fishy odor), 2,4-(*trans, trans*)- heptadienal, and 2,6-(*trans, cis*)-nonadienal (cucumber odor) have been characterized as very potent odorants, contributing to the unpleasant rancid and fishy off-flavor in bulk fish oil, fish oil–enriched mayonnaise, and fish oil–enriched milk emulsion (Karahadian and Lindsay 1990; Jacobsen and others 1999, 2000; Hartvigsen and others 2000; Venkateshwarlu and others 2004).

Microencapsulation can be used to protect PUFAs from oxidation (Shahidi and Han 1993). In microencapsulation, minute particles of ingredients (for example, acidulants, fats, and flavors) are coated with materials that may change their properties. Various methods for encapsulation of fish oils have been used, for example, spray-drying (Keogh and others 2001; Hogan and others 2003; Kagami and others 2003; Kolanowski and others 2004), freeze-drying (Heinzelmann and others 2000a, 2000b), molecular inclusion (Yoshii and others 1996), and enzymatic gelation (Cho and others 2003). When spray-drying is used for microencapsulation, the core material (for example, fish oil) is dispersed in a solution containing the wall material. The wall material must have good emulsifying properties to create a stable emulsion with small droplets (Risch and Reineccius 1988), low viscosity at high concentrations, as well as good drying and film-forming properties (Reineccius 1988). Mixtures of whey proteins as the emulsifying and film-forming material and maltodextrins as matrix-forming material have been found to be effective wall systems for microencapsulation of volatiles (Sheu and Rosenberg 1995). Milk proteins, usually in mixtures with lactose or maltodextrin, have been used in microencapsulation of fish oils (Keogh and others 2001; Hogan and others 2003; Kagami and others 2003). Gelatin is a widely used coating material because of its film-forming and other properties (Shahidi and Han 1993). There has been some interest in replacing porcine or bovine gelatin with fish skin gelatin, as well as interest in replacing gelatin by milk proteins and vice versa. Lipid oxidation in oil-in-water emulsions has been studied extensively and reviewed by McClements and Decker (2000). The emulsifier type has been found to play an important role in the oxidative stability of emulsions, and among the most promising ones are whey protein isolates (Fomuso and others 2002; Hu and others 2003; Osborn and Akohl 2003).

Various methods have been used for isolating and analyzing volatile components from seafood. Static headspace and dynamic headspace sampling (DHS) are widely used isolation methods, and DHS has been used to identify volatile compounds responsible for off-flavors in fish oil (Karahadian and Lindsay 1989; Adios and others 2002). Because of their low level, it is difficult to detect these compounds; however, by using a gas chromatography-olfactometry (GC-O) technique, it is possible to detect compounds that have very low odor thresholds.

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Table 1—Composition of the different mixtures of microencapsulated fish oil

	Fish oil	Protein		Carbohydrate		Emulsifier		Acacia
Mixture[a]	%	Type	%	Type	%	Type	%	%
CALA	20	Caseinate	39.8	Lactose	39.8	Span 80	0.4	0
CAMD	20	Caseinate	39.8	Maltodextrin	39.8	Span80	0.4	0
CASU	20	Caseinate	39.8	Sucrose	39.8	Span 80	0.4	0
GMS	30	Gelatin	17.3	Maltodextrin	34.7	Span 80	0.6	17.4
CALL-O	30	Caseinate	31.8	Lactose	31.7	Lecithin	6.5	0
CALL-T	30	Caseinate	31.8	Lactose	31.7	Lecithin	6.5	0

[a]CALA = caseinat-lactose; CALL-O = caseinate with lactose; CALL-T = caseinate with lactose with 1000 mg/kg DL-α-tocopherol; CAMD = caseinate-maltodextrin; CASU = caseinate-sucrose; GMS = gelatin, gum acacia, and maltodextrin.

The application of solid phase microextraction (SPME) as a static headspace sampling method has been increasing in food analysis in the past year (Wardencki and others 2004). SPME has been widely used for analyzing odorous volatile compounds (Snyder and others 1998; Doleschall and others 2001; Shin and others 2003) including oxidatively derived volatile compounds in vegetable oils and oil-in-water emulsions (Vichi and others 2003; Beltran and others 2005; Kanavouras and others 2005).

The objective of this study was to compare the stability of microencapsulated fish oil coated with caseinate and carbohydrates coating mixtures. To our knowledge, a comparison of the protection provided by different types of carbohydrates such as lactose and maltodextrins in mixtures with proteins against oxidation has not previously been reported in the literature. In addition, this study compares the progress of oxidation of microencapsulated fish oil coated with gelatin-based coating blend. Furthermore, the objective was to use SPME as a simple, solvent-free, and convenient technique, to monitor the lipid oxidation in microencapsulated fish oil.

Materials and Methods

Samples

Two experiments were done to study the oxidative stability of microencapsulated fish oil. In the 1st experiment, the coating materials were mixtures containing caseinate and different types of carbohydrates. In the 2nd experiment, a cod skin gelatin was used as a coating material in a mixture with gum acacia and maltodextrin and compared with caseinate encapsulated oil that showed the best storage stability in the 1st experiment. The fish oil (18% eicosapentaenoic acid [EPA], 12% docosahexaenoic acid [DHA]) was obtained from Lysi Ltd (Reykjavik, Iceland). An oil-in-water emulsion containing the fish oil and coating materials was prepared by homogenizing with a Polytron PT 1300 D homogenizer (Kinematica, Lucerne, Switzerland) at 20000 rpm for 5 min. The proportions of fish oil, protein, carbohydrate, emulsifier, and acacia in the different mixtures are shown in Table 1. The emulsions were spray-dried with use of a Büchi B-191 Mini Spray Dryer with an inlet temperature of 175 °C and an outlet temperature of 100 °C to 110 °C. The emulsions were prepared in 130-mL portions and spray-dried immediately after preparation.

Caseinate and different types of carbohydrates

The coating materials in the 1st experiment were mixtures containing caseinate and different types of carbohydrates: lactose (CALA), sucrose (CASU), or maltodextrin (CAMD). The caseinate (sodium caseinate, EM 6) was purchased from DMV-Intl. (Veghel, The Netherlands). The lactose was purchased from Friedrich Ingredients (Konstanz, Germany), and the maltodextrin with a dextrose equivalent (DE) value of 20, from AVEBE (Veendarn, The Netherlands). The sucrose was food grade and was purchased at a local supermarket. The powdered samples of microencapsulated fish oils were vacuum-packed and kept in the dark at 4 °C for 0, 4, 12, and 20 wk. A control sample for sensory analysis was fresh sample of microencapsulated fish oil taken at the beginning of the shelf life test and stored at –75 °C until tested.

Coating materials and antioxidant

In the 2nd experiment, a high-molecular-weight fish skin gelatin obtained from Norland Products Ltd (New Brunswick, N.J., U.S.A.) was used as the coating material in a mixture with gum acacia (NMD Oslo, Norway) and maltodextrin (GMS) and compared with caseinate that showed the best storage stability in the prior experiment, that is, caseinate with lactose (CALL). The GMS emulsion was stabilized with Span 80 (Fluka Chemie AG, Buchs, Switzerland). The CALL emulsion was stabilized with Lecithin S (Cargill, Hamburg, Germany), and an extra sample was produced containing 1000 mg/kg DL-α-tocopherol from BASF Aktiengesellschaft (Ludwigshafen, Germany) in the fish oil (CALL-T). The samples were vacuum-packed and kept in the dark at 4 °C for 0, 6, and 20 wk.

All samples were analyzed by using headspace solid phase microextraction (HS-SPME) followed by gas chromatography-olfactometry (GC-O) and flame ionization detection (GC-FID), followed by sensory analysis. Stability test by Oxipres was done in the 1st experiment, but thiobarbituric acid-reactive substances (TBARS) were measured in the 2nd. Gas chromatography-mass spectrometry (GC-MS) analysis was performed on samples after 20 wk of storage. Other measurements included free fat, droplet size of emulsions, grain size, viscosity, and vacuole ratio.

Free fat

Surface oil (free fat) was determined by extraction with chloroform (NARL 1978).

Microscopy

Particle size of the spray-dried capsules was determined using Leica DM RA2 light microscope (Leica Microsystems, Wetzlar, Germany). The capsules were dispersed in corn oil (about 30 mg/mL of oil), and 1 drop of this dispersion was placed on a 25 × 75 × 1.0 mm microscope slide (SuperFrost/Plus, Menzel–Gläser, Germany), covered with a 0.17-mm cover glass (Menzel–Gläser, Germany), and viewed in the microscope. Particle size (d1.0) was given as mean diameter as measured using the light microscope. The ratio of capsules containing vacuoles (air bubbles) was counted in at least 5 images from the particle size measurements. The total sum of capsules counted in these images was never less than 300.

Oxidative stability

The oxidative stability of microencapsulated fish oil was measured electronically under oxygen pressure (0.5 MPa) in an Oxipres apparatus (Mikrolab Aarhus A/S, Højbjerg, Denmark). Samples (25 g)

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were weighed into reaction flasks (125 mL), and the pressure signal was recorded at 30 °C. The induction period was determined graphically as a cross-section of the line during the induction period and the 2nd line along the pressure decline. Each sample was measured in at least duplicate, and the results are presented as mean values.

Thiobarbituric acid-reactive substances (TBARS)

TBARS were determined by the extraction procedure described by Vyncke (1975) with few modifications. The sample size was reduced to 0.4 - 0.5 g, and the turbidity of the sample solution at 600 nm was subtracted from its absorbance at 530 nm. TBARS, expressed as μmol malondialdehyde/kg of sample (μmol MDA/kg), was calculated using malondialdehyd-bis-(diethyl acetate) as standard.

Sensory analysis

Sample were evaluated using a 5-point category scale with a description of intensity of rancid odor at each score (ISO 1985, 1987): 0 = none; 0.5 = thresholds or just detectable; 1 = slight; 2 = little; 3 = moderate; 4 = strong.

The Icelandic Fisheries Laboratories (IFL) sensory panel was trained in odor analysis to assess microencapsulated fish oil in 2 sessions. Rancid cod liver oil was used in training the panel to estimate intensity of rancid odor. Members had several years of experience in evaluating rancidity of fish, fish oils, and vegetable oils and have been trained according to international standards (ISO 1993) including detection and recognition of odors. Sensory assessments were carried out by 8 to 12 assessors (age range, 30 to 55 y).

In each session, 8 samples were evaluated (4 different samples each in duplicate), including internal standard (control sample). The same control sample was used to calibrate the panel in each evaluation. The control sample was fresh sample of microencapsulated fish oil taken at the beginning of the shelf life test and stored at –75 °C until tested.

The order of presentation of samples to the panelists was balanced to minimize possible carryover effects between samples. All observations of the microencapsulated fish oil were conducted under standardized conditions, with as little interruption as possible, at room temperature, and under white fluorescent light. The powder of microencapsulated fish oil was presented to the panelists in small, transparent, disposable plastic cups covered with an aluminum foil.

Headspace solid phase microextraction (HS-SPME)

The SPME device and fibers (polydimethylsiloxane [PDMS] and polydimethylsiloxane/divinylbenzene [PDMS/DVB]) were purchased from Supelco (Bellefonte, Pa., U.S.A.). In the 1st experiment, a non-polar PDMS (100 μm) fiber was used, and in the 2nd experiment, a semi-polar PDMS/DVB (65 μm) fiber. The fibers were conditioned before use in the GC injection port as recommended by the manufacturer. A blank analysis was performed to verify that no extraneous compounds were desorbed from the fiber.

The microencapsulated powdered sample was weighted (2.5 g) into a 25-mL sample vial and dissolved in 5 g of saturated aqueous NaCl solution to facilitate and improve the extraction efficiency. A magnetic stirrer was put into the sample vial, sealed, and stirred. Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 0.5 mL of 100 mg/kg aqueous solution of the standard to the sample. The vial was placed in an 80 °C water bath; the SPME fiber was inserted through the spectrum of the sample vial and allowed to equilibrate with the headspace volatiles for 45 min. The fiber was then retracted into the barrel of the syringe and immediately inserted into the injector of the gas chromatograph. Duplicate analyses of each sample were done.

Gas chromatography-olfactometry

The volatile compounds on the SPME fibers were thermally desorbed for 2 min in the GC using splitless mode, with helium as the carrier gas at linear velocity of 22.9 cm/s. The volatiles were separated on a DB-5ms column (30 m × 0.25-mm inner dia × 0.25 μm, J&W Scientific, Folsom, Calif., U.S.A.). Measurements were performed on a GC (HP 5890, Hewlett-Packard, Palo Alto, Calif., U.S.A.). Helium was used as a carrier gas and the following temperature program was used: 50 °C for 7 min, 50 °C to 120 °C at 5 °C/min, and from 120 °C to 220 °C at 10 °C/min. The injector temperature was 250 °C and the detector temperature was 280 °C. The end of the column was split 1:1 between flame ionization detector (FID) and an ODO-1 olfactory detector outlet (SGE Intl. Pty. Ltd, Australia). Nitrogen, bubbled through water to add moisture, was used to drive the sample up to the sniffer. Two persons describing the odor sniffed the effluent. Intensity (quality and duration/retention times) of each odor was determined using an intensity from 0 to 5, 0 = not present; 5 = very strong. The assessors were trained in recognizing characteristic oxidatively derived odors by injecting into the GC-O, mixtures of standard compounds dissolved in ether and sniffing the effluent.

Gas chromatography-mass spectrometry

After 20 wk of storage, samples were prepared in the same way as for the GC-O measurements except that the volatile compounds were collected on 250 mg Tenax 60/80 (Alltech, Ill., U.S.A.) in stainless-steel tubes (Perkin-Elmer, Buchinghamshire, U.K.) for the combined ATD 400 and GC-MS measurements. Volatile compounds were thermally desorbed (ATD 400, Perkin-Elmer) from the Tenax tubes and separated with the same type of column and the same conditions as for the GC-O measurements. The mass detector ion range was 35 to 300 m/z. These measurements were done for identification of the volatiles.

Identification and quantification of the volatile compounds

Identification of the volatiles was done by matching retention indices (RI), calculated according to Van den Dool and Kratz (1963) based on ethyl esters (i.e., RI of ethyl pentanoate is 500) and verified by the database Flavornet (Acree 1997), and mass spectra of samples with authentic standards (Sigma-Aldrich Chemical Co., St. Louis, Mo., U.S.A.). Tentative identifications were based on the MS library data in the HP GCD ChemStation software (Hewlett Packard). Semi-quantitative estimation of concentration of components was done by calculating the peak area ratio (PAR), that is, the ratio between the total ion count of each peak and internal standard.

The linearity was investigated over the range of 125 to 2000 ppb for hexanal, 4-*cis*-heptenal, 1-octen-3-ol, 2,4-heptadienal, heptanoic acid ethyl ester (C_7, internal standard), 2-nonenal, and 2,4-decadienal. The compounds were accurately weighed and dissolved in a stock solution of 10 mL of paraffin oil. Five sets of concentrations were prepared with a range of values form 125 to 2000 ppb. Results from the collections of standards were used to prepare a calibration curve for each standard compound. All collections were made in triplicate. Detection limits were determined as 3× noise in which the noise was determined from 6 blank GC-FID analyses and the magnitude of baseline fluctuation determined over a period of 1 min. The distance between minimum and maximum in this period was defined as baseline noise. The response calculated as 3× noise was converted to concentration by use of the calibration curve prepared as described before.

Statistical analysis

The Number Cruncher Statistical software (NCSS 2000 and Pass

Table 2—Free fat, vacuole ratio, and mean capsule diameter of caseinate-lactose (CALA), caseinate-sucrose (CASU), caseinate-maltodextrin (CAMD), caseinate-lactose-lecithin (CALL), and gelatin-maltodextrin (GMS)

	CALA	CASU	CAMD	CALL	GMS
Free fat (% ± SD)	3.3 ± 0.25	2.7 ± 0.07	2.8 ± 0.15	—[a]	2.8 ± 0.27
Vacuole ratio (% ± SD)	12 ± 2.6	19 ± 4.4	19 ± 3.2	14 ± 3.0	37 ± 6.1
Capsule diameter (μm ± SD)	9.9 ± 6.3	10.2 ± 6.3	8.9 ± 6.5	32 ± 8.2	46 ± 8.8

[a]Failed because lecithin was extracted as well as fish oil.

Trial, Kaysville, Utah, U.S.A.) was used for statistical analysis. Significant differences were determined by analysis of variance (ANOVA), and Duncan's Multiple-Comparison Test was used to determine the statistical difference between groups. An effect was considered significant at the 5% level ($P < 0.05$).

Results and Discussion

Optimization and validation of SPME method

The sampling time and temperature were optimized using 2^2 fractional factorial design with 3 replicates. The sample used for optimization was microencapsulated fish oil that had been oxidized for 5 d at 30 °C in the Oxipres apparatus. From the results of optimization, the sampling time of 45 min and temperature of 80 °C was determined. The precision of the method expressed as relative standard deviation (RSD) was determined by carrying out 7 analyses over a 6-mo period using the PDMS fiber. The sample was microencapsulated fish oil that had been stored at –75 °C. The RSD of peak areas were 4.9%, 8.4%, and 12.5% for hexanal, 2-nonenal and 2,4-decadienal, respectively. The RSD for the odor intensity measured by GC-O were 9.3%, 3.8%, and 14.4% in the same order. The limit of detection (LOD) of the FID detector was 35 ppb, 14 ppb, and 12 ppb for hexanal, 2-nonenal and 2,4-decadienal, respectively, when using the PDMS fiber. The LOD for the PDMS/DBV fiber was lower because of better sensitivity or 9 ppb, 6 ppb, and 2 ppb for hexanal, 2-nonenal and 2,4-decadienal, respectively.

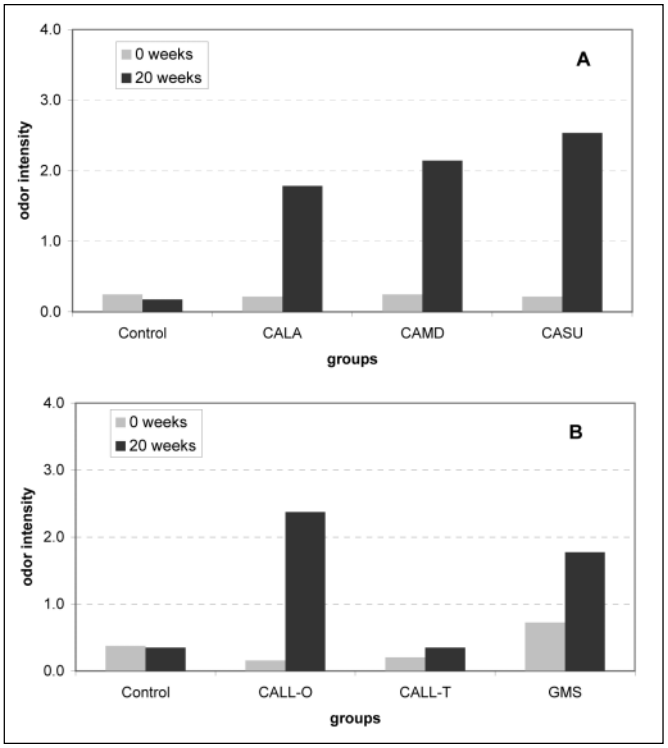

Figure 1—Evaluation of rancid odor by sensory analysis of the following: (A) caseinate-lactose (CALA), caseinate-sucrose (CASU), and caseinate-maltodextrin (CAMD) and (B) gelatin, gum acacia, and maltodextrin (GMS) and caseinate with lactose with (CALL-T) or without tocopherol (CALL-O)

Caseinate and different types of carbohydrates

In this experiment, the coating materials were mixtures of caseinate and different types of carbohydrates. The ratio of capsules containing vacuoles was significantly smaller in the caseinate/lactose (CALA) containing capsules ($P < 0.05$) compared with the sucrose-containing capsules (CASU) and the caseinate/maltodextrin (CAMD) containing capsules (Table 2). The CALA-containing capsules had significantly greater free fat (solvent extractable fat) compared with both CASU and CAMD containing capsules ($P < 0.05$), whereas there was no difference between CASU and CAMD. No significant difference was seen in mean capsule diameter of the samples.

The CALA sample appeared to be less rancid compared with the other samples after 20 wk of storage at 4 °C, although not significant ($P > 0.05$) (Figure 1A). The oxidative stability of the samples as evaluated by the Oxipres test at 30 °C, where the course of oxidation was monitored by changes of oxygen pressure, showed somewhat different behavior (Figure 2). The oxygen pressure of the CASU sample began to decrease before the other samples but more slowly and in 2 steps. The induction periods, before rapid decline in oxygen pressure, were 81, 114, and 116 h for the CASU, CAMD, and CALA, respectively. This apparent difference between the stability of the coating materials indicates less stability of the CASU sample and higher stability of the CAMD and CALA samples.

In the 1st experiment, the nonpolar PDMS fiber, coated with a 100-μm layer, was used for the HS-SPME. The volatiles were separated by gas chromatography and detected by flame ionization detector and by sniffing (olfactometry) because GC-O is an important tool to determine the key odor compounds in food or flavor

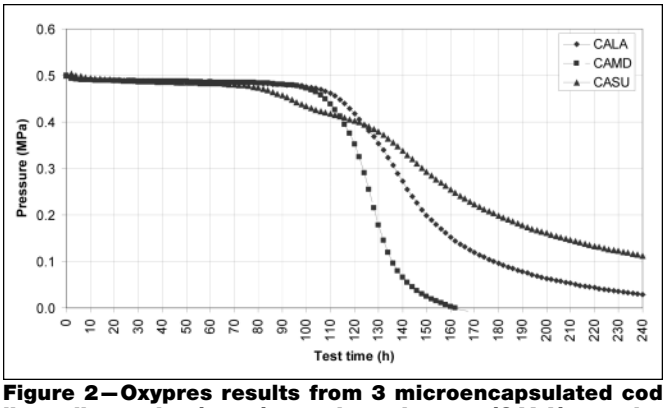

Figure 2—Oxypres results from 3 microencapsulated cod liver oil samples (n = 2): caseinate-lactose (CALA), caseinate-sucrose (CASU), and caseinate-maltodextrin (CAMD)

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samples (Högnadóttir and Rouseff 2003). The GC-MS analysis was performed to identify and verify the presence of the key odor components detected by GC-O. In the beginning of storage time, the highest odor scores were given for rancid, potato-like, and cucumber-like odors in all samples. The odor intensity of these odors increased during storage time in addition to other volatile compounds detected giving odors like grass, earthy, flowery, mushroom, and many described as rancid (Table 3). Aldehydes were the most abundant group of volatiles, and many of them can be associated with lipid oxidation. Alcohols and ketones detected in the samples can also be connected to the lipid oxidation process. Aldehydes and ketones have low flavor thresholds in oil and thus have high impact on flavor, although they are not present in high concentrations (Lindsay 1990). Most of the detected volatiles have previously been reported in fish oils and fish oil–enriched mayonnaise (Hsieh and others 1989; Karahadian and Lindsay 1989, 1990; Horiuchi and others 1998; Hartivgsen and others 2000).

The rancid, potato-like odor detected in all the fresh samples was identified as *cis*-4-heptenal. The cucumber-like odor was also detected in all the fresh samples. According to RI of standard and odor evaluation by GC-O, it is possibly 2,6-nonadienal although the compound could not be identified by GC-MS. During storage, 2-none-

Table 3—Volatile compounds identified in microencapsulated fish oil by using solid-phase microextraction (SPME), gas chromatography–olfactometry (GC-O), and gas chromatography-mass spectrometry (GC-MS) after 20 wk of storage at 4 °C

Compound	Odor description	Odor intensity[a]			ID means[b]	RI DB-5ms[c]
		CALA	CASU	CAMD		
Acetic acid		x	x	x	MS	193
Butanal		—	x	x	MS	202
2-Butenal		x	x	x	MS	229
Pentanal	Caramel, vanilla	2.5	—	3.0	MS, 1, 2	237
1-Butanol		—	x	x	MS	238
1-Penten-3-ol		x	x	x	MS	247
3-Methylbutanal	—	—	x	—	MS, 1, 2	266
Unknown	Heavy, milk-like	3.0	2.5	1.8	2	274
3-Hydroxy-2-butanone	—	x	x	x	MS, 1, 2	275
3-Penten-2-one	—	x		x	MS	312
2-Pentenal, (E)	—	x	x	x	MS	321
Unknown	Flowery	1.5	2.0	—	2	325
1-Pentanol	—	—	x	x	MS	331
2-Penten-3-ol	—	—	—	x	MS	335
1-Hexen-3-ol	—	—	x	—	MS	345
Unknown	Flowery, geranium	3.0	4.0	3.0	2	347
Hexanal	Grass	3.5	2.0	1.5	MS, 1, 2, 3	369
2-Hexenal, (E)	—	x	x	x	MS	430
Unknown	Candy	2.0	2.0	—	2	437
Unknown	Earthy, flowery	4.0	3.0	3.8	2	458
cis-4-heptenal	Rancid, potato-like	5.0	5.0	5.0	MS, 1, 2, 3	500
Heptanal	Rancid	3.5	—	4.0	MS, 1, 2, 3	507
Benzaldehyde	—	—	x		MS	558
1-Octen-3-ol	Mushroom	5.0	4.0	3.8	MS, 1, 2, 3	579
2,4-Heptadienal	—	x	x	x	MS	598
Decane	—	x	x	x	MS	599
Octanal	—	x	x	x	MS	601
2,4-Heptadienal, (E,E)	Citrus, fresh	x	—	x	MS, 1, 2	624
2-Ethyl-1-hexanol	—	x	x	x	MS	630
Unknown	Sweet	2.0	—	—	2	650
Unknown	Heavy, sweet, creamy	2.0	—	3.0	2	663
3,5-Octadiene-2-one	—	x	x	x	MS	663
1-Octanol	—	x	x	x	MS	673
Unknown	Heavy	—	2.5	—	2	676
Unknown	Spicy-like	3.0	3.0	2.0	2	687
2-Nonanone	—	x	x	x	MS	692
Nonanal	—	x	x	x	MS	705
Unknown	Rancid	3.0	2.0	4.0	2	716
2-Nonenal	Cucumber	4.3	5.0	5.0	MS, 1, 2, 3	759
Octanoic acid	—	x	—	x	MS	766
Unknown	Burnt	—	—	2.0	2	784
2-Decanone	—	x	x	x	MS	786
Unknown	Celery	3.5	3.0	—	2	797
Hexadecane	—	x	—	—	MS	794
Decanal	Rancid	x	1.5	2.0	MS, 1, 2	800
2-Decenal	Rancid	3.0	2.8	2.5	MS, 1, 2	845
Hexadecanal	Tallow	2.0	2.3	—	MS, 1, 2	843
Undecanal	Tallow	3.0	3.0	3.0	MS, 1, 2	884
Unknown	Caramel, burnt, sweet	—	1.5	—	2	895
2,4-Decadienal	Rancid	3.0	4.5	3.3	MS, 1, 2	930

[a]Odor intensity calculated as average of 2 assessors (x = identified only by GC-MS).
[b]Identification means: MS = mass spectra; 1 = authentic standards; 2 = odor identification; 3 = odor identification and RI references.
[c]Calculated ethyl ester retention index on DB-5ms capillary column.

nal was detected, giving a cucumber-like odor similar as 2,6-nonadienal but more intense. 2,6-nonadienal and 2-nonenal elute close to each other on the GC column making the separation of their individual odor difficult by GC-O, but they are easily separated by GC-FID and GC-MS. However, the amount of 2,6-nonadienal was below detection limit of the GC-FID in the samples, which rendered the monitoring of this compound during storage impossible.

Cis-4-heptenal was only detected by GC-O in the microencapsulated samples but was below the detection limit of the GC-FID and therefore could not be selected as a possible quality marker for lipid oxidation. *Cis*-4-heptenal is derived from lipid oxidation of n-3 PUFA and it is known to have a very low odor threshold in water (0.04 ppb) (McGill and others 1974). *Cis*-4-heptenal, which can be formed from retro-aldol reaction of *trans*, *cis*-2,6-nonadienal (Josephson and Lindsay 1987), has previously been associated with fishy off-flavors in oxidized fish oil (Karahadian and Lindsay 1989).

Hexanal, 2,4-heptadienal and 2,4-decadienal, which cause oxidized, rancid, and painty flavors in fish oils (Karahadian and Lindsay 1989), were detected in all the stored samples. The negative flavor impact of these oxidation products has been shown to be greater from emulsions than the flavor impact of the same compounds in bulk oil (Jacobsen 1999). This has been explained by lower sensory threshold values for these compounds when released from water than from oil (Druaux and Voilley 1997). The flavor threshold of hexanal and 2,4-decadienal have been determined in oil-water emulsion as 0.19 mg/kg and 0.26 mg/kg, respectively (Dixon and Hammond 1984).

Based on our results and on literature references (Karahadian and Lindsay 1990; Jacobsen and others 1999, 2000; Hartvigsen and others 2000), the following oxidatively derived compounds were selected as quality markers for the microencapsulated fish oil: hexanal, 2-nonenal, and 2,4-decadienal. The development, expressed as peak area ratio (PAR), of the selected volatiles in the microencapsulated fish oil during storage is shown in Figure 3, that is, hexanal (a), 2-nonenal (b), and 2,4-decadienal (c), together with the total peak area (d). The results show that the stability of the microencapsulated fish oil was influenced by the carbohydrate used for encapsulation. After 4 wk of storage, an increased level was only seen for 2-nonenal in the CAMD microencapsulated fish oil. Increased concentration was detected for all the selected volatiles and total amount of volatiles after 12 and 20 wk of storage in all groups with storage time except for CASU were the concentration unexpectedly decreased after 20 wk. This is difficult to explain, but the Oxipres test indicated that the oxidation process appeared to be different in this sample. After 20 wk of storage, a significant difference was observed between CAMD and CASU samples in the content of 2-nonenal, 2,4-decadienal, and in total peak area.

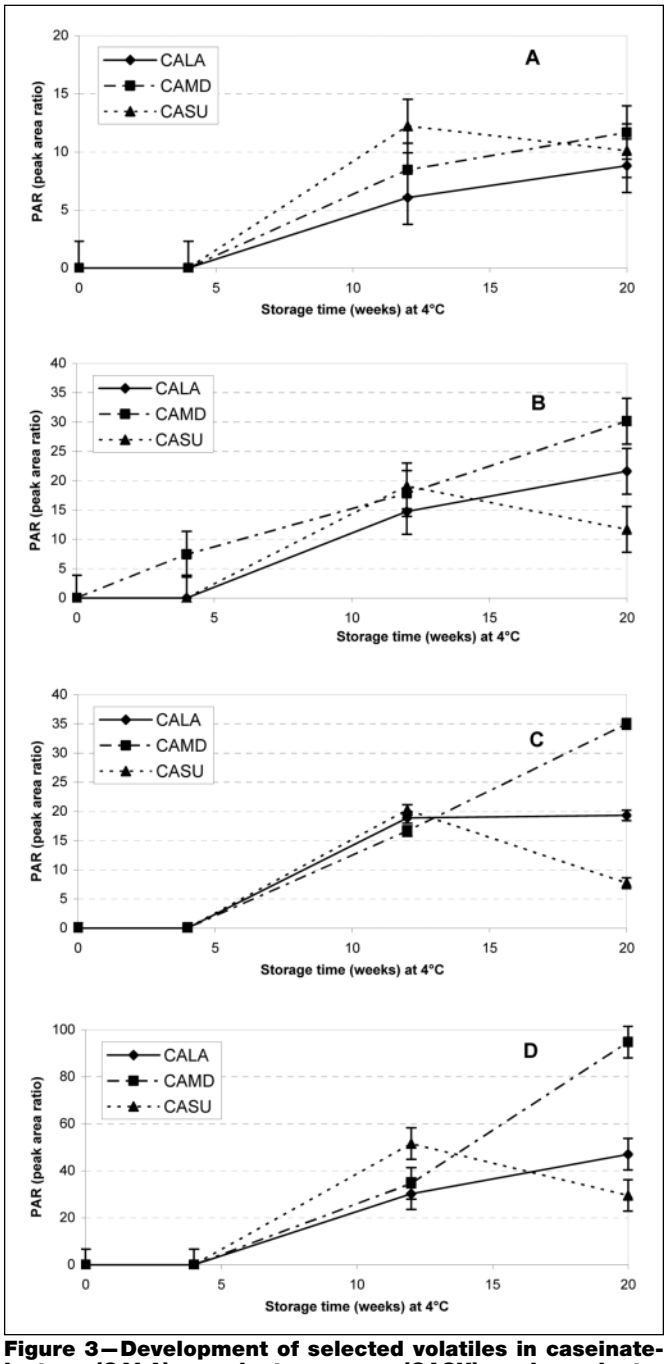

Figure 3—Development of selected volatiles in caseinate-lactose (CALA), caseinate-sucrose (CASU), and caseinate-maltodextrin (CAMD) analyzed by SPME-PDMS fiber and GC-O. (A) hexanal; (B) 2,-nonenal; (C) 2,4-decadienal, and (D) total area.

Coating materials and antioxidant

In the 2nd experiment, fish skin gelatin in a mixture with gum acacia and maltodextrin (GMS) was used as a coating material and compared with caseinate-lactose encapsulated fish oil, which showed slightly better stability than CAMD in the Oxipres experiment. Lecithin was used as the emulsifier for caseinate-lactose encapsulated fish oil instead of Span 80, which was used in the 1st experiment. The fish oil in the caseinate-lactose sample contained either no added tocopherol (CALL-O) or 1000 mg/kg α-tocopherol (CALL-T).

Organoleptic evaluation of rancid odor revealed that the GMS sample had higher initial values than other samples ($P < 0.05$), or approximately 0.7, whereas the control, CALL-O, and CALL-T samples had approximately 0.2 to 0.4 for rancidity scores. At the end of the shelf life test, after 20 wk of cool storage, the rancidity scores had increased to approximately 2 (little rancid odor) for the GMS and CALL-O samples, whereas the CALL-T sample containing tocopherol as antioxidant had much lower rancidity scores ($P < 0.05$). The CALL-T sample had similar rancidity scores as the control sample and was stable throughout the shelf life test (Figure 1b).

The results for the TBARS were in agreement with the sensory results (Figure 4). The initial value for the GMS sample was approximately 86 μmol MDA/kg and much higher than for the other samples ($P < 0.05$), which had TBARS values between 22 and 33 μmol MDA/kg, for CALL-T and CALL-O, respectively. During cold storage, the

TBARS increased only in the CALL-O sample, from 33 to 109 μmol MDA/kg ($P < 0.05$), but remained low for CALL-T, and increased insignificantly for the GMS sample ($P < 0.05$). The GMS sample was highest in TBARS throughout the storage period ($P < 0.05$).

The ratio of capsules containing vacuoles in the GMS sample was considerably greater than in the CALL sample (Table 2). The GMS sample also had larger particle size than the CALL sample, probably because of the vacuoles expanding the volume of the capsules. The GMS sample was more oxidized in the beginning of the storage time, which may be caused by its larger number of vacuoles compared with the CALL samples. This was in agreement with the results of Keogh and others (2001) who found that vacuole volume (determined by density measurements) was related to the oxidation of the microencapsulated fish oil during storage. The free fat measurement of the CALL samples failed as the method appeared to extract lecithin as well as fish oil from the capsules.

Figure 5 shows the development of the selected oxidatively derived volatiles in the samples. Here, the semi-polar PDMS/DVB fiber, coated with a 65-μm layer, was used instead of the non-polar PDMS fiber used in the 1st experiment. This change was made as new information from literature, for example, Wardencki and others (2004) suggested that this semi-polar fiber was more suitable for this type of volatile compounds. The concentration of the volatiles increased significantly ($P < 0.001$) with storage time. Hexanal was significantly lower ($P < 0.05$) in CALL-T (with tocopherol) than in CALL-O and 2-nonenal and 2,4-decadienal were significantly lower ($P < 0.001$) in CALL-T compared with CALL-O and GMS after 20 wk of storage. This was in agreement with both sensory and TBARS results. The amount of 2-nonenal and 2,4-decadienal were highest in GMS already in the beginning of the storage time, which was in agreement with the TBARS results. This may possibly be an influence from the emulsifier, as flavor release of the volatiles may be reduced depending on the type of bonds between the emulsifier and the volatile compounds (Druaux and Voilley 1997). As expected, the addition of an antioxidant (tocopherol) improved the shelf life of the CALL-T sample.

The different type of fibers used for the SPME, the non-polar PDMS, and the semi-polar PDMS/DBV, are the most commonly used fibers (Wardencki and others 2004). These fibers gave different GC responses whereas the PDMS/DBV fiber gave higher responses (results not shown) and lower detection limits compared with PDMS fiber.

Similar results were seen by Kanavouras and others (2005) who found that the PDMS/DVB gave the overall the best sensitivity for all classes of compounds in virgin olive oil. Brunton and others (2000) also showed that SPME with the PDMS/DVB fiber was a good alternative to other headspace methods for the quantitative determination of hexanal in cooked turkey. Doleschall and others (2001) showed that SPME was efficient to follow the less volatile compounds like *t,t*-2,4 decadienal, which can be a marker component for oxidation.

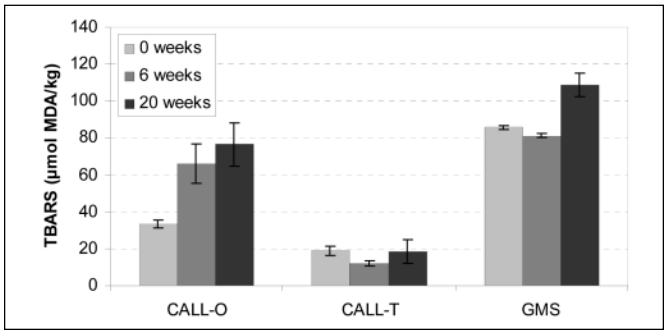

Figure 4—The changes of thiobarbituric acid-reactive substances (TBARS) in microencapsulated cod liver oil samples during storage: gelatin, gum acacia, and maltodextrin (GMS) and caseinate with lactose with (CALL-T) or without tocopherol (CALL-O)

Conclusions

Difference in oxidative stability of microencapsulated fish oil coated with caseinate and carbohydrates coating mixtures could not be traced to difference in capsule size, but the ratio of vacuole containing capsules may explain some of the difference. Greater amount of free fat on the surface of lactose-containing sample did not seem to influence oxidative stability compared with the other samples. The intensity of rancid odor appeared to be least in the lactose-containing sample, although the different between samples was not significant.

The oxidative stability of microencapsulated fish oil coated with fish gelatin blend (GMS) was less than the microencapsulated fish oil

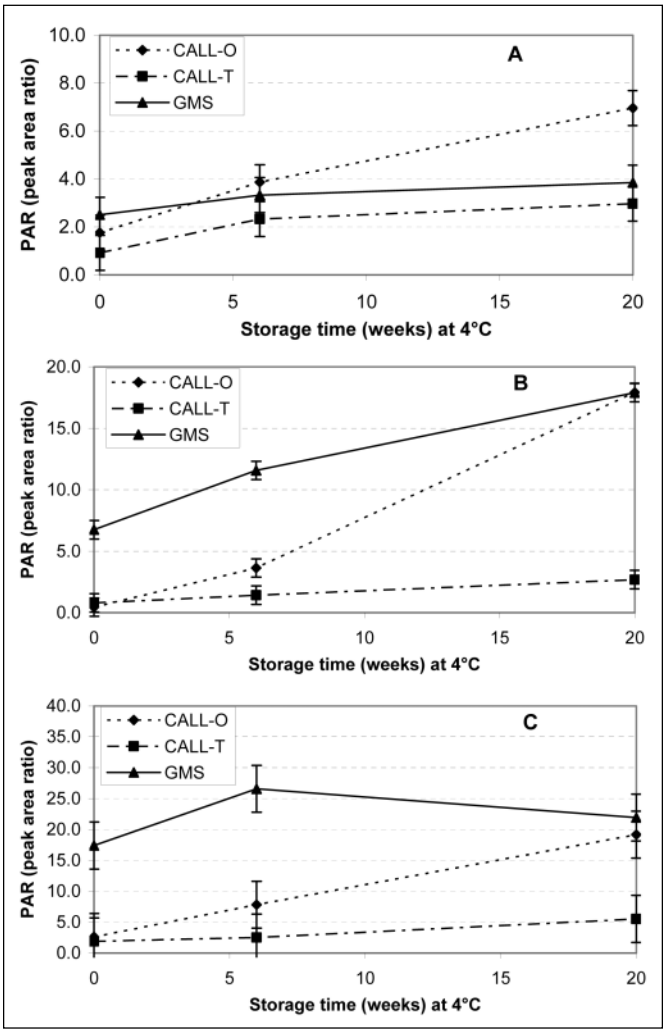

Figure 5—Development of selected volatiles in microencapsulated cod liver oil samples (gelatin, gum acacia, and maltodextrin [GMS]) and caseinate with lactose with (CALL-T) or without tocopherol (CALL-O) analyzed by solid-phase microextraction (SPME)-polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber and gas chromatography–olfactometry (GC-O). (A) hexanal; (B) 2,-nonenal, and (C) 2,4-decadienal.

coated with caseinate, lactose, and lecithin blend (CALL-O). The differences between samples with respect to rancid odor as evaluated by sensory analysis were considerable because the sample containing tocopherol (CALL-T) as an antioxidant had much lower rancidity scores after 20 wk of storage compared with other samples. The oxidative stability test and the TBARS results were in agreement with the sensory analysis. The GC-O and GC-MS results showed that oxidatively derived compounds (for example, hexanal, *cis*-4-heptenal, 2,6-nonadienal, 2-nonenal and 2,4-decadienals) increased with time and can be used to monitor oxidative stability of the microencapsulated fish oil. The SPME proved to be sensitive enough to detect oxidative changes during storage, especially when using a PDMS/DVB fiber. The results indicate that SPME can be a useful tool for rapid evaluation of lipid oxidation in microencapsulated fish oil.

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