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Effect of Milk Protein Concentrate on Lipid Oxidation and Formation of Fishy Volatiles in Herring Mince (*Clupea harengus*) during Frozen Storage

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The effect of milk protein concentrate (MPC) at 0, 2, 4, and 6% on lipid oxidation and volatile formation in frozen stored herring mince (−18 °C) was evaluated by analyzing samples at 0, 2, and 4 months for fatty acid composition, volatiles, and thiobarbituric acid reactive substances (TBARS). Sensory evaluation was also conducted to assess the intensity of fishy odor, and the volatiles were analyzed using static headspace gas chromatography–mass spectrometry (SHGC-MS). The addition of 4 and 6% MPC to herring mince resulted in a 33% and 50% reduction of TBARS, respectively, at month 4 and lessened the intensity of fishy odor throughout storage. However, MPC did not protect fatty acids from enzymatic degradation unless it was added immediately after mincing. Volatile analysis using SHGC-MS showed that 4% MPC was able to reduce headspace volatiles associated with fishy odor. MPC is most effective for reducing 4-heptenal, 3-methyl-1-butanol, 2-hexenal, and 1-penten-3-ol, which are known to be potent odorants associated with lipid oxidation.

KEYWORDS: Herring mince; SHGC-MS; lipid oxidation; fishy volatiles; fatty acids

INTRODUCTION

Because of its abundance and underutilized status, herring mince is considered a good source of raw material for value-added seafood products. However, its high levels of polyunsaturated fatty acids combined with mechanical mincing is believed to make herring mince prone to oxidative rancidity during frozen storage, leading to the development of "fishy odor". Mincing destabilizes the fish tissue due to exposure of tissue lipids to atmospheric oxygen, cellular disruption (*1*), lipoxygenase activity (*2, 3*), and the release of prooxidant, hemoglobin, from erythrocytes (*4*).

The formation of fishy odor can be caused by volatiles of decomposition products derived from lipid oxidation (*5*). Lipoxygenase present in fish tissue can initiate the oxidation of polyunsaturated fatty acids to produce volatiles responsible for oxidative odor, namely, the fishy odor (*6*). Thus, controlling lipid oxidation and inhibiting lipoxygenase can reduce the fishy odor (*7*). Mansur et al. (*8*) identified volatiles responsible for fish odor in both skin and muscle tissues of various marine fish species including Pacific mackerel and sardine. The primary volatiles identified were hexanal, cyclopentanol, 1-penten-3-ol, propanal, and heptanal, and were concentrated more in the skin tissue. The fishy odor is believed to be imparted by the combination of methional and (*Z*)-1,5-octadien-3-one in an aqueous solution (*9*). Compounds (*Z*)-4-heptenal, (*Z*)-1,5-octadien-3-one, and methional imparted an overall fishy odor character to sardines after 2 days of ice storage, whereas compounds (*E*)-2-nonenal and (*E*,*Z*)-2,6-nonadienal appeared to be associated with the slightly rancid aroma (*10*).

Our preliminary study on the cryostabilization of frozen mackerel and herring mince compared antioxidant properties of various nonfish proteins (*11*). Soy protein isolates, soy protein concentrates, milk protein concentrate, milk concentrate, milk protein isolate, whey protein concentrate, whey protein hydrolysate, egg white, potato extract, and cysteine were screened for their ability to control rancidity and fishy odor development. Milk protein concentrate (MPC) at 4% (w/w) came out to be the most effective in terms of antioxidation, texture improvement and cost effectiveness.

Proteins are able to delay lipid oxidation in complex systems (*12*). The antioxidant activity of heated skim milk and milk fractions were first explored in a methyl linoleate emulsion where oxidation was promoted by hemoglobin (*13*). It is reported that casein (*14*), milk whey proteins (*15, 16*), casein calcium peptides (*17*), caseinophosphopeptides (CPP), and casein hydrolysates (*18*) have antioxidant activity.

The high molecular weight (HMW) fraction of whey proteins lowered the formation of lipid peroxides and thiobarbituric acid

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reactive substances (TBARS) when added to salmon oil emulsions stabilized by Tween 20 (*12, 16*). Diaz and Decker (*19*) demonstrated that enriched caseinophosphopeptides, casein hydrolysates, and low molecular weight (LMW) casein hydrolysates (0.5%) all inhibited the formation of TBARS in cooked ground beef. They attributed such inhibition to iron chelation and free-radical scavenging. Gluten, egg albumin, and casein were also found to be effective in the control of lipid oxidation in safflower and sardine oil model systems (*20, 21*).

Proteins have shown the ability to decrease the concentration of headspace volatiles in both aqueous and dry matrices through binding (*22*). Protein's binding of volatiles involves reversible hydrophobic interactions, adsorption or absorption, and chemical bonds of various strengths (*23*). However, the ability of MPC to reduce fishy volatiles in herring mince has never been studied.

The objective of our study was to determine the ability of MPC to control lipid oxidation and to bind LMW reactive compounds responsible for the rancid fishy odor development in herring mince during frozen storage. To introduce fish mince-based seafood products into the current market, the stability of fish mince during frozen storage needs to be improved through the control of lipid oxidation and fishy odor development.

MATERIALS AND METHODS

Materials. Fresh Atlantic herring (*Clupea harengus)* was obtained in May of 2005 from NORPEL in New Bedford, MA. Fish were less than one day old from the time of catch and were kept in ice before being processed on the same day in the laboratory. Milk protein concentrate (MPC 56, formerly Alapro 4560) was supplied by NZMP Fonterra Ltd. (Santa Rosa, CA). Chemicals of analytical grade were purchased from Sigma Chemicals (St. Louis, MO).

Sample Preparation and Storage Conditions. Fresh herrings kept in ice were manually filleted, and fillets with skin on were deboned. A deboning machine (model 694, Baader Machineries, Germany) with a drum hole size of 3 mm was used to remove skins, scales, bones, and some dark meat. The temperature of fish mince was kept below 5 °C during the process to minimize the deteriorative enzymatic reactions.

Fish mince was mixed with 0, 2, 4, and 6% MPC and was formed into 55 × 55 × 35 mm blocks (120 ± 5 g). For 0 month samples, blocks for each type of analysis were vacuum-packed in nylon/polyethylene bags (Market Sale Co., Newton, MA), stored frozen at −80 °C, and analyzed within two weeks, except for the sensory evaluation. For storage study samples, blocks for each MPC level were packed without vacuum in polyethylene bags (Pactiv Corporation, Lake Forest, IL) and stored at −18 °C for 2 and 4-month periods. Two random samples were taken for each type of analysis after each storage period, vacuum-packed in nylon/polyethylene bags (30 × 30 cm), transferred to a −80 °C freezer, and analyzed within two weeks, except for the sensory evaluation in which samples were kept at −80 °C until the end of storage. Prior to analysis, the mince blocks were thawed in 15 °C tap water. Samples for analyses of TBARS and volatiles were taken approximately 1 cm from the sides of the mince block, and ¼ of the mince block (∼30 g) was used for the sensory evaluation.

To verify the time-dependent enzymatic degradation of fish tissue lipids after mincing, in a separate experiment, herring fillets (one day after harvest; May 2007; 5.71% lipid) were mechanically minced using a deboner under the same conditions described previously, and MPC was added at 4% (w/w) at varying intervals (0, 20, and 40 min). The minces were vacuum packed and stored frozen at −80 °C. The 200 g mince for each sample was packed thin (3 mm) in a bag (30 × 30 cm) to allow quick freezing. The TBARS and fatty acid analyses were done within a week.

Determination of Thiobarbituric Acid Reactive Substances (TBARS) Values. The method of Lemon (*24*) was used in measuring TBARS values, with slight modification. Approximately 15 g of tissue was mixed with 7.5 mL of 0.1% (w/v) propyl gallate and 7.5 mL of 0.1% (w/v) EDTA, and the mixture was homogenized for 10 s in a 250 mL Eberbach blending jar (VWR International, Bridgeport, NJ), followed by homogenizing for another 10 s with 15 mL of 7.5% (w/v) trichloroacetic acid solution. Initial homogenization with propyl gallate and EDTA without TCA was to improve antioxidation and chelation. The homogenate was filtered using a 20–25 μm filter paper (Type Q8; Fisher Scientific, Pittsburgh, PA). The 5 mL filtrate was mixed with 5 mL of TBA reagent in a tube capped tightly. The tubes were heated in water bath (100 °C) for 40 min. After they were cooled at ambient temperature, the contents were filtered again using the same type of filter paper. The absorbance of the samples was measured at 530 nm against a blank. TBA values were calculated from a standard curve prepared using 1,1,3,3-tetraethoxypropane (TEP). The analysis was done in triplicate.

Determination of Moisture and Lipid Contents. The moisture content was determined by drying approximately 2 g samples in an oven at 110 °C until constant weight (*25*). The percentage loss of weight was expressed as moisture content.

A single solvent extraction method for total lipid determination in fish tissue (*26*) was used to determine the lipid content using a 2:1 ratio of chloroform and methanol. Both analyses were done in triplicate.

Fatty Acid Analysis. Changes in the fatty acid profile of the mince were analyzed using the gas chromatographic method (*27*) with some modifications. A 5 g thawed sample was weighed and placed into an ice–water jacketed blender. The extraction followed the procedure described previously in the determination of lipid content, except for the mixture of filtrate and 0.5% NaCl being kept on ice for 30 min. A 5 mL extract from the chloroform layer was transferred into a 50 mL test tube and was allowed to evaporate under a stream of nitrogen gas in a 40 °C water bath. The lipid in the tube was weighed and diluted with 2 mL of methylene chloride.

For transmethylation, 1 mL methylene chloride containing 36 μg tricosanoic acid (C23:0) (Sigma Chemicals, St. Louis, MO) was added to 300 μL of lipid extract as an internal standard. After the addition of 3 ml of methanolic HCl, the tubes were capped under nitrogen, mixed gently, and placed in a 70 °C oven for 2 h. They were allowed to cool for 5 min before adding 4 mL of 6% K_2CO_3. The capped tubes were vortexed. The supernatant layer was siphoned off. The remaining layer was dried under nitrogen in a 40 °C water bath, dissolved in 1 mL of hexane, and filtered using a Millex-GN 13 mm syringe filter (0.20 μm pore size, Millipore Corporation, Bedford, MA) into a 2 mL amber crimp top autosampler vial (Fisher Scientific, Pittsburgh, PA).

The sample was capped under N_2 before performing analysis in a Perkin-Elmer AutoSystem XL gas chromatograph (Shelton, CT) equipped with a flame ionization detector (FID). The methyl esters were separated in a DB-Wax column (30 m × 0.25 mm internal diameter × 0.25 mm film thickness, J&W Scientific, Folsom, CA) under the following operation conditions: injection, 2.0 μl; injector temperature, 250 °C; detector temperature, 300 °C; flow rate of carrier gas He, 20 mL/min; oven temperature, 50 °C, held for 2 min following injection; ramp, 40 °C/min to 200 °C, held for 16 min, 210 °C, held for 11 min, and 220 °C, held for 10 min. Polyunsaturated fatty acids (PUFA) No.3 (Sigma Chemicals, St. Louis, MO) was used as reference in the identification of peaks. The relative content of each fatty acid methyl ester is reported as percent peak area of total fatty acid methyl esters using tricosanoic acid (C23:0) as an internal standard. The analysis was done in duplicate.

Static Headspace Gas Chromatography–Mass Spectrometry (SHGC-MS). Minced tissue samples (5.85 ± 0.05 g each) at month 2 were weighed into 20 mL amber glass headspace vials, which were immediately sealed with a crimp top fitted with a Teflon lined septa. An HP 7694 (Agilent Technologies, Wilmington, DE) static headspace autosampler with a 44 sample capacity connected to a volatile interface was used for the analysis. The pressure of the static headspace autosampler was controlled electronically, and the parameters used for the unit were as follows: oven temperature, 80 °C; loop temperature, 140 °C; transfer line temperature, 150 °C; loop capacity, 3 mL; loop fill time, 0.4 min; loop equilibration time, 0.05 min; carrier pressure, 5.1 psi; vial pressurization time, 0.3 min; vial equilibration time, 20 min; injection time, 1.2 min; vial pressure, 10 psi; shaker mode, fast; GC cycle at 60 min.

Volatile separation was accomplished using the volatile interface of a gas chromatograph, model GC6890, fitted with a DB-INNOWAX capillary column, 30 m × 0.25 mm × 0.25 mm film thickness (J&W Scientific, Folsom, CA). Helium was used as carrier gas at 0.9 mL/min at an average velocity of 34 cm/sec in constant flow mode. The volatile interface was operated at 220 °C in splitless mode with a purge flow of 18.3 mL/min and the purge time set to 1.5 min, for a total flow of 21.9 mL/min. The oven temperature was held at 38 °C for 4.5 min, increased to 180 at 4 °C/min, and then followed by a second stepwise increase of 30 °C up to 260 °C. Total run time was 42.67 min.

Chemical identification was achieved with a mass spectrometer MS5973 (Agilent Technologies, Palo Alto, CA). The MS was operated in electron impact mode under the following conditions: temperature of interface, 280 °C; source temperature, 230 °C; quadrupole temperature, 150 °C; solvent delay, 1.8 min; mass range, 15−300 amu; and acquisition rate, 4.94 scans/sec. Tentative identifications were based on matching spectra of unknowns with those found in the National Institute of Standards and Technology (NIST 98) mass spectral data library (Agilent Technologies, Palo Alto, CA). The concentration of volatiles was expressed in terms of abundance of detected ions.

Sensory Evaluation. A panel of five judges assessed the raw samples for the intensity of fishy odor on a 1–9 point scale, with 1 being no fishy odor detected and 9 being extremely fishy. Prior to evaluation, panelists were trained to familiarize fishy odor by allowing them to sniff rancid fish samples and comparing them with fresh ones.

Approximately 30 g of thawed mince were placed in 100 mL plastic cups with lids and were distributed to panelists. The panelists opened the cup and sniffed. The cups were coded with three-digit random numbers.

There were two parts of the evaluation: the first part was comparing the mince of varying MPC levels at each storage period; the second part compared the mince kept at 0, 2, and 4 months of storage at a given MPC level. There were 7 sessions in total, and the evaluation was done in duplicate.

Data Analysis. All analyses were performed using the Statistical Analysis System for Windows, Version 9.1.3, Service Pack 4 XP Pro_Platform (SAS Institute Inc. 2002–2004, Cary, NC). Data on fatty acids and TBARS were analyzed using the general linear model (GLM) procedures. To compare statistical differences between means, least significant difference (LSD) values and Dunnett's test at the 5% level of significance were used. Sensory scores and headspace volatiles were analyzed using the Kruskal–Wallis test, a nonparametric test, followed by the Wilcoxon signed rank test to determine significant difference of treatments from the control. Microsoft Office Excel 2003 SP2 was used to calculate standard deviation, averages, and linear correlation.

RESULTS AND DISCUSSION

Proximate Composition of Mince Samples. The moisture content of herring mince samples ($n = 4$) was 76.08 ± 1.10%, and their lipid content was 4.48 ± 0.30%. At this level of lipid, the fish may be considered medium fatty. Our year-long lipid analysis records show that the lipid content of herring fillets with skin on and caught off the Gulf of Maine ranges from 3.3 to 15.0%, with the lipid content highest in summer and lowest in winter.

Effects of MPC on Lipid Oxidation of Herring Mince during Frozen Storage. MPC was found to significantly reduce the lipid oxidation in fish mince ($p < 0.05$), based on TBARS measurement, even at 0 month (**Table 1**). The whole herring had a TBARS value of 0.94 μmol/100 g tissue prior to processing and storage. However, after mincing, the TBARS value increased to 3.47 μmol/100 g when the sample preparation took around 30 min. The time-dependent changes in TBARS and fatty acids of herring mince during the short period of time are shown in **Figure 1**. These data were obtained from the separate experiment to verify the time-dependent nature of enzymatic lipid oxidation. The TBARS values increased from 0.98 to 1.71 μmol/100 g in 40 min, whereas with addition of MPC at 4% it increased from 0.24 to 0.62 μmol/100 g. The rate of the TBARS increase in the mince without MPC (0.0183/min) was found to be almost twice as fast as that of the mince with MPC (0.0095/min). The difference in changes in TBARS from 0.94 to 3.47 in the first herring mince in 2005 (**Table 1**) and from 0.98 to 1.71 in the second mince in 2007 (**Figure 1**) could be due to the differences in condition and age of fish and the thickness of samples frozen (55 mm vs 3 mm). The former was much thicker and frozen slower than the latter, causing further degradation with increased TBARS. As for fatty acids, EPA and DHA decreased from 36.4 to 10.2 mg/g lipid and 97.9 to 41.6 mg/g lipid, respectively, whereas with addition of MPC at 4% they decreased from 50 to 12.7 mg/g lipid and 105.6 to 61.2 mg/g lipid, respectively. Such time-dependent changes in TBARS and fatty acids in a relatively short period of time clearly suggest that mincing does affect lipid oxidation in fish tissue, probably through cellular disruption and subsequent activation of lipoxygenase. Hsieh and Kinsella (*6*) and Medina et al. (*2*) reported that lipoxygenase present in fish tissues can initiate the oxidation of polyunsaturated fatty acids to produce acyl hydroperoxides. Medina et al. (*2*) and Mansur et al. (*8*) found that lipoxygenase was concentrated in the skin tissue. Mechanical deboning of fillets with skin on will, therefore, activate this enzyme. These results also suggest that the enzymatic degradation of fish tissue lipids is rapid and indicate

Table 1. Effects of Various MPC Concentrations on TBARS (μmol/100 g) in Herring Mince from 0 to 4 Months of Storage (−18 °C)

	MPC (%)[a]			
month	0	2	4	6
0	3.47 ± 0.29 e	3.59 ± 0.02 e	2.24 ± 0.11 f	2.13 ± 0.10 f
2	9.27 ± 1.00 b	8.17 ± 0.19 c	3.49 ± 0.18 e	4.14 ± 0.29 e
4	12.61 ± 0.32 a	12.53 ± 0.85 a	8.48 ± 0.74 bc	6.25 ± 0.23 d

[a] Different letters denote significant differences among samples ($p < 0.05$).

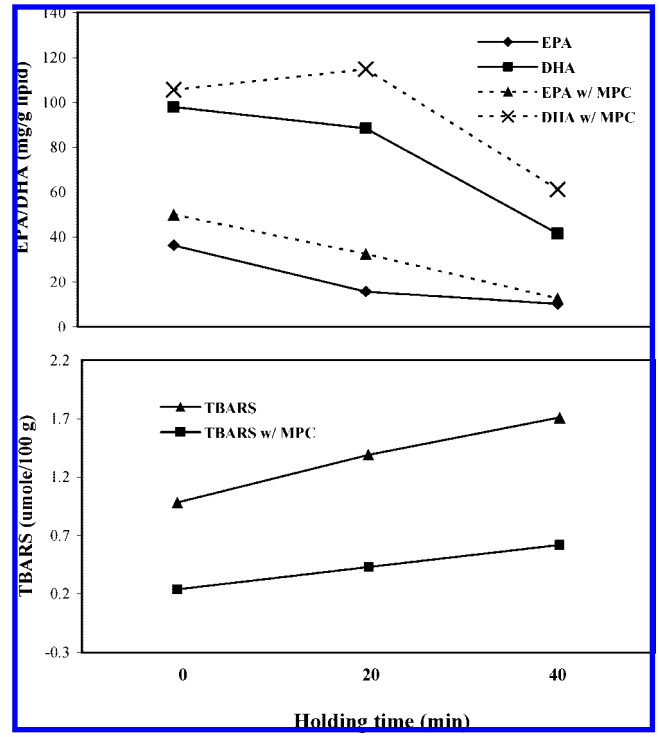

Figure 1. Time-dependent changes in fatty acids (EPA/DHA) and TBARS of herring mince during 40 min holding. The mince was prepared by running skin-on fillets through a mechanical deboner, mixed with MPC at 4%, and kept below 5 °C.

Table 2. Effects of Various MPC Concentrations on Unsaturated Fatty Acids (mg/g Lipid) in Herring Mince from 0 to 4 Months of Storage (−18 °C)[a]

	0% MPC	2% MPC	4% MPC	6% MPC
		0-Month		
18:1	100.4	107.24	118.5	117.6
18:2	9.12	9.78	10.68	10.07
18:3	5.11	5.52	5.84	5.86
18:4	9.12	10.24	10.19	10.19
20:1	138.21	140.38	152.04	142.45
20:4	6.56	7.13	7.45	7.64
20:5	37.14	42.92	45.84	46.12
22:1	217.37	218.97	235.64	226.54
22:5	3.22	3.8	3.73	3.44
22:6	80.83	88.72	99.25	102.82
total	607.08 bc	634.7 b	689.16 a	672.73 a
		2-Month		
18:1	86.02	95.47	104.24	104.06
18:2	8.4	8.38	9.1	9.92
18:3	4.86	4.66	5.29	5.02
18:4	8.51	8.27	8.97	8.79
20:1	128.7	126.9	140.02	139.59
20:4	5.53	5.36	6.15	5.52
20:5	33.5	32.02	35.4	33.77
22:1	197.47	202.69	218.2	219.3
22:5	3.87	3.96	4.55	3.77
22:6	65.46	62.05	77.81	67.41
total	542.32 d	549.76 d	609.73 bc	597.15 c
		4-Month		
18:1	86.99	90.36	89.75	85.44
18:2	7.18	6.78	7.59	7.24
18:3	3.59	3.67	3.73	3.77
18:4	6.49	6.43	6.77	6.52
20:1	107.29	112.14	112.54	110.79
20:4	3.87	3.72	4.14	4.34
20:5	23.61	23.84	27.75	28.24
22:1	171.08	181.54	179.09	177.11
22:5	2.35	2.49	2.62	2.32
22:6	40.87	43.12	54.96	56.77
total	453.32 f	474.09 ef	488.94 e	482.54 ef

[a] Different letters denote significant differences among samples ($p < 0.05$).

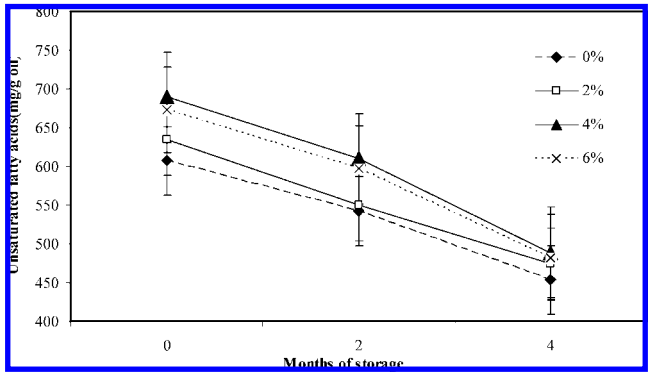

Figure 2. Effect of various MPC levels on total unsaturated fatty acids in herring mince from 0 to 4 months of storage (−18 °C).

the importance of immediate treatment of minced fish tissue with appropriate types of antioxidants. Richards and Hultin (*4*) reported the involvement of the released hemoglobin from erythrocytes during mincing in lipid oxidation based on their observation that bleeding did not delay lipid oxidation in minced tissue, whereas bleeding did delay lipid oxidation in intact muscle when mackerel was studied.

Addition of MPC at 4 and 6% reduced TBARS values by 35.52 and 38.66%, respectively, at month 0 (**Table 1**). Mince

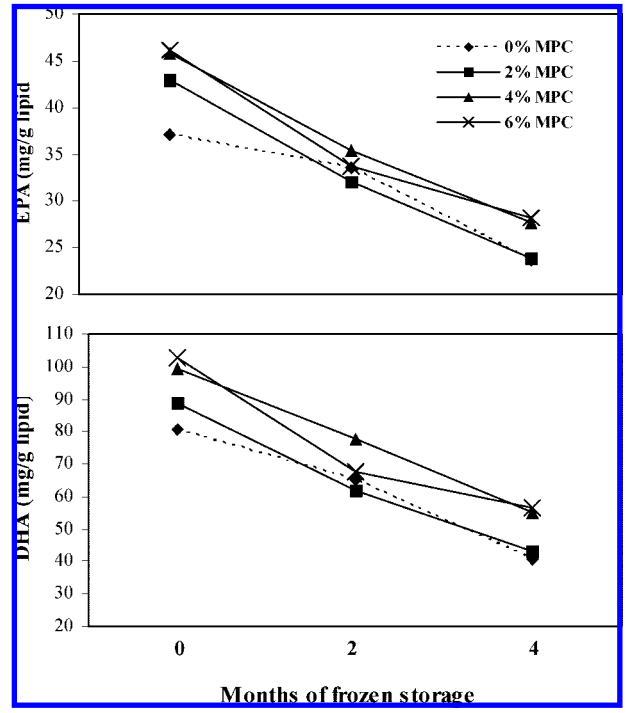

Figure 3. Changes in the concentrations of EPA and DHA in the herring mince with and without MPC during 4-month frozen storage.

treated with 4 and 6% MPC had significantly less TBARS than 2% and the control ($p < 0.05$). Lipid oxidation had already started during mixing and packing, and adding at least 4% MPC was effective in slowing down lipid oxidation, even prior to frozen storage. At month 2, TBARS values of all treatments increased, but the 4% MPC mince had the least, which was not significantly different from 6% MPC.

After four months of storage, fish mince with 4 and 6% MPC had 32.7, and 50.4% average reduction of TBARS, respectively. The TBARS values of mince with 4 and 6% MPC were significantly lower than those of the control and 2% MPC ($p < 0.05$) for all periods of storage.

Effects of MPC on Retention of Fatty Acids in Herring Mince during Frozen Storage. Lipids from herring mince were initially composed of 81.72% unsaturated fatty acids. Changes in the unsaturated fatty acids content in herring mince are shown in **Table 2**. The unsaturated fatty acids were predominantly docosaenoic acid (C22:1), 25.18%; eicosaenoic acid (C20:1), 16.01%; oleic acid (C18:1), 11.63%; docosahexaenoic acid (DHA, C22:6), 9.36%; and eicosahexaenoic acid (EPA, 20:5), 4.25%. Unsaturated fatty acids were significantly reduced ($p < 0.05$) with storage for all treatments (**Figure 2**). Marked decreases in the concentrations of EPA and DHA were observed in minced herring tissue during 4 month frozen storage (**Figure 3**). Because such a decrease is not expected in the intact fish tissue as reported in salmon (*28*), tissue cell destruction and subsequent activation of lipoxygenase are believed to have caused such decreases in fatty acids.

There were also significant differences in fatty acid retention among MPC levels ($p < 0.05$). At month 0, herring mince with 2, 4, and 6% MPC had 4.55, 13.52, and 10.81% more fatty acids than the control (mince without MPC), respectively. After 4 months, mince with 2, 4, and 6% MPC retained 4.58, 7.86, and 6.44% more fatty acids than control. At months 0 and 2, the mince with 4 and 6% MPC retained significantly more fatty acids than those with 0 and 2% MPC. Overall, the highest retention of unsaturated fatty acids was observed at 4 and 6% MPC throughout frozen storage.

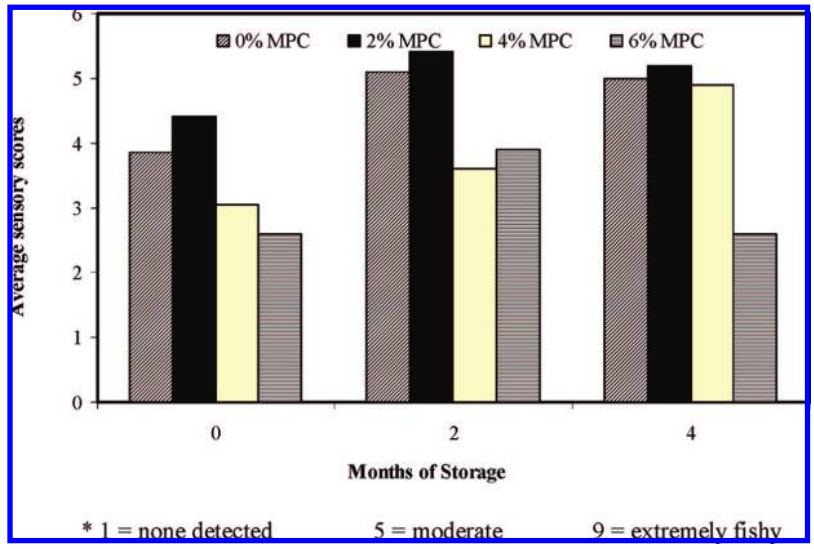

Figure 4. Sensory scores of herring mince comparing various concentrations of MPC for every month of storage (−18 °C).

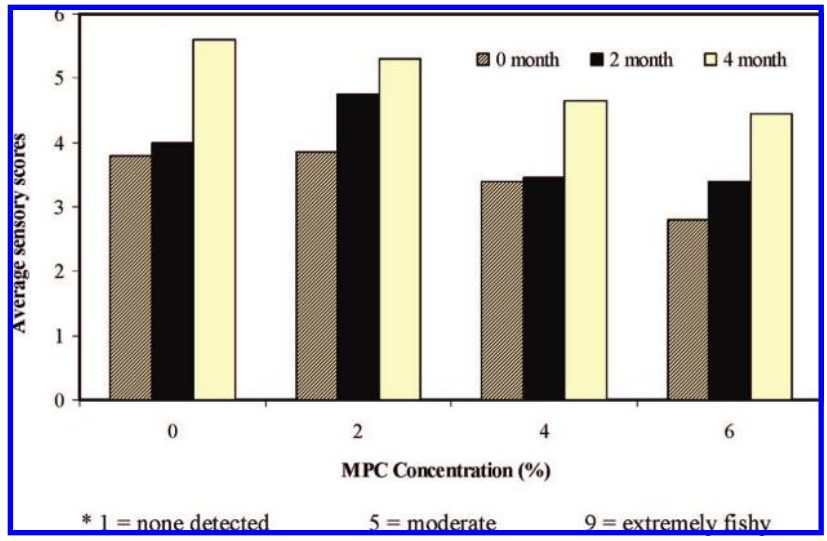

Figure 5. Sensory scores of herring mince comparing varying duration of storage (−18 °C) at a given MPC level.

Table 3. Abundance and Retention Times of Volatile Compounds Identified in Herring Mince at 2 Months of Storage (−18 °C) Using Static Headspace Gas Chromatography–Mass Spectrometry

	MPC (%)		
volatile compounds	0	4	RT (min)
propanal	1,634,930	1,671,609	3.595
1-penten-3-ol	1,040,192	827,838	11.141–11.279
2-hexenal	67,499	43,769	12.294
4-heptenal	14,213	0	12.873
3-methyl-1-butanol	88,828	66,507	13.081
dimethyltrisulfide	648,475	40,419	17.102
pentadecane	107,424	101,665	21.442
benzaldehyde	33,289	33,328	22.089
2,6,10,14-tetramethyl-pentadecane	969,498	995,997	26.835
unknown	307,996	218,724	

The amounts of the total remaining unsaturated fatty acids were inversely correlated with TBARS values ($r = -0.87$). However, even though treated mince appeared to retain more unsaturated fatty acids and lower TBARS values, MPC did not completely protect fatty acids from degradation. There are two possible reasons for the above results. First, it is probable that enzyme-induced lipolytic oxidation had already initiated upon mincing before MPC was added. Second, MPC did not sufficiently prevent radical formation in the initiation stage of oxidation, resulting in the deterioration of fatty acids. MPC was, however, still able to inhibit TBARS formation by interacting with free radical intermediates, thus preventing the formation of secondary products of oxidation.

Sensory Evaluation. The herring mince samples were evaluated for odor intensity. The scores are plotted in **Figures 4** and **5**. The Kruskal–Wallis test showed that fishy odor was progressively reduced with an increase in the MPC level. Panelists could not recognize the differences in the fishy odor intensity between control and 2% MPC throughout storage, whereas 4% MPC was significantly different from control at months 0 and 2.

When odor was assessed with varying durations of storage at a given MPC level, the increase in fishy odor intensity of herring mince for all treatments from month 0 to 4 was significant ($p < 0.05$). The odor intensity of samples at months 0 and 2 were not significantly different, whereas 0 and 2 months compared to 4 months were significantly different ($p < 0.05$). These findings imply that panelists were able to detect that 4-month mince was more fishy than 2- and 0-month mince.

Headspace Volatiles. The volatiles identified in herring mince during 2 months of storage are listed in **Table 3** along with retention times and quality hits. The main volatiles that

correlated with fishy odor were found to be propanal, 4-heptenal, 2-hexenal and 1-penten-3-ol. Propanal formation in fish oil was found to be highly correlated with the content of ω−3 polyunsaturated fatty acids (*29*), and it is believed to be an oxidation product of these fatty acids (*30*).

Like propanal, 1-penten-3-ol also originated from oxidized ω−3 fatty acids but may not be the only cause for the rancid odor in cod liver oil (*31*). Volatiles such as 1-penten-3-ol and 2-hexenal are believed to be the result of lipoxygenase action at C-15 of EPA (*32, 33*). MPC at 4% did reduce the formation of 1-penten-3-ol at month 2. The presence of 4-heptenal in small quantities can be potent when it is combined with other volatiles such as nonadienal, 1-penten-3-one, and 2,4-heptadienal (*34*). Interestingly, in our study, 4-heptenal was not detected in the mince with MPC, suggesting that MPC may have either bound 4-heptenal or inhibited its formation. Mince with 4% MPC contained less 3-methyl-1 butanol, a volatile that was previously detected in dried rancid oat groats (*35*). It was demonstrated that aldehydes, particularly 2-hexenal, were retained to a large extend in skim milk through covalent bonds (*36*).

In the present study, MPC at the 4% level was able to reduce headspace volatiles in herring mince, indicating that MPC either retarded the formation of volatiles or had the ability to bind volatiles.

In summary, milk protein concentrate (MPC) offers a promising alternative to synthetic antioxidants in minimizing rancidity of fish mince containing high amounts of polyunsaturated fatty acids. The mince treated with MPC had less TBARS than control. In most samples, the higher the amount of MPC, the lower the TBARS values, and the more unsaturated fatty acids were retained. However, despite MPC significantly inhibiting lipid oxidation, the rates of fatty acid reduction in all treatments were not different. This finding needs to be further confirmed to understand what mechanism is involved in the fatty acid retention by MPC. The antioxidation mechanisms of MPC could be explained by iron chelation and free radical scavenging as previously reported. The analysis of volatiles using SHGC-MS showed that 4% MPC markedly reduced headspace volatiles. To retard enzymatic degradation of tissue lipids upon mechanical mincing and the subsequent progression of lipid oxidation during frozen storage, especially unsaturated fatty acids, the immediate addition of antioxidants appears to be necessary.

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