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Composition and Chemical Changes during Storage of Fish Meal from Capelin (*Mallotus villosus*)

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The stability of fish meal depends on processing and storage conditions, but habitat and seasonal variations in composition and naturally occurring anti- and pro-oxidants may be equally important. Capelin meal from four different seasons was examined by measuring chemical composition and monitoring lipid oxidation during storage. The results revealed that lipid content was high in the summer but low in the spring. It was further demonstrated that among naturally occurring antioxidants, astaxanthin was high during summer, whereas α-tocopherol was highest in spring. Mineral content varied, with a high copper content in the summer, whereas iron, selenium, and zinc were highest in the winter. Measurements on the stability of capelin meal indicated a decrease in peroxide values, oxygen uptake, and thiobarbituric acid reactive compounds with storage, whereas browning and CO concentration increased with time. Rancidity was highest in autumn, but free fatty acids were highest during spring and summer.

KEYWORDS: Fish meal; capelin (*Mallotus villosus*); composition; lipid oxidation; quality

INTRODUCTION

Capelin (*Mallotus villosus*) is a small, schooling pelagic fish, common in the northern hemisphere, which is mainly utilized for fish meal production. The biology and fishery of the Icelandic capelin have been reviewed extensively (*1*). Capelin is a plankton feeder and spends most of its lifetime in the deep sea, returning to the coast only during feeding and spawning. The main winter migration route of the capelin stock is from the feeding grounds north of Iceland in a clockwise direction along the east coast and then west along the south coast to the main spawning area off the south and west coasts of Iceland. There is a rapid accumulation of body fat during the summer–autumn season, after which time there is a slow decline in fat content for the rest of the year. This energy reserve has become practically depleted at the time of spawning in March. The seasonal changes in chemical composition and quality parameters in capelin have been evaluated (*2*), but the fish meal made from capelin collected at different seasons has not. Fish meal made from capelin contains materials from the whole fish, rendering it highly nutritious. The nutritional benefits are mostly manifested in high protein, mineral, and $n-3$ fatty acids contents, the latter originating from unsaturated fish lipids. It is generally accepted that highly unsaturated fatty acids have nutritional benefits, but they are also more susceptible to oxidation because of the high degree of unsaturation. The consequences of lipid oxidation in fish meal are reduced nutritional value and protein damage of the meal (*3*–*5*). Lipid oxidation can proceed to the extent that it causes heat damage in the meal, which may even lead to combustion (*6*, *7*).

Most problems due to lipid oxidation in fish meal can be avoided by proper cooling of the meal after processing and by the addition of antioxidants (*3*, *5*). Recently, consumers have become concerned with the possible health risks that might be associated with synthetic antioxidants, conventionally added to fish meal. Antioxidants like other lipid soluble constituents can migrate through the food chain and thereby reach consumers as a secondhand product from farmed animals. Ethoxyquin is the antioxidant most frequently added in fish meal because of its effectiveness and low cost. However, it is not permitted as a food additive in the United States and may not exceed 0.5 mg/kg in the muscle of animals or fish that have been fed antioxidant-treated feed (*8*). Manufacturers may therefore soon be faced with firmer regulations on the application of synthetic antioxidants. A trend can also be seen in consumer preferences toward natural foods as well as natural antioxidants. Naturally occurring antioxidants are widespread in nature (*9*, *10*) and are found in fish.

The quality and stability of capelin meal may be influenced by many factors. External factors such as freshness, processing, and storage can be controlled to some extent, but internal factors may be equally important. These are factors such as biology and behavior, which have been found to be highly seasonal in nature (*2*). Capelin meal has earned a reputation, in the fish meal manufacturing industry, of being more stable than meal from other pelagic species. This was originally thought to be due to a relatively low content of polyunsaturated fatty acids in the meal lipids, indicated by the low iodine value of the oil. However, the comparison of fish meal lipids by Opstvedt (*11*) and Barlow and Pike (*12*) showed that capelin meal lipids were more unsaturated than meal lipids from similar North Atlantic species such as herring and mackerel, but meal lipids from South

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American anchovy and sardinella were, on the other hand, more unsaturated than capelin meal lipids. Other factors that may be expected to be of considerable importance for meal stability are the composition and content of naturally occurring anti- and pro-oxidants.

The present investigation was undertaken to provide information on the chemical composition and stability of capelin meal affected by harvest season.

MATERIALS AND METHODS

Raw Material. Capelin (*M. villosus*) was caught in nets at conventional capelin fishing grounds around Iceland in the summer and autumn of 1998 and in the following winter and spring seasons (*2*). After harvesting, the whole, ungutted fish was stored in the hold of the ship at ambient temperature until landing, within 24 h from harvesting. The ocean temperatures at 50 m were 0−6 °C, and the surface temperatures were 4−8 °C.

Capelin Meal. The capelins from summer, autumn, and winter were processed at a fish meal factory located at the eastern coast of Iceland (SR-mjöl hf, Seydisfjördur). The spring capelin was processed at a fish meal factory on the southwest coast of Iceland (SR-mjöl hf, Helguvík). Both factories apply indirect hot air drying, using the same type of dryers (Hetland HLT, Atlas-Stord Norway AS, Bryne, Norway); the temperature of the meal is never higher than 65−70 °C. The selected processing plants were located in the migratory rout of the capelin, and in each case the plants used were the nearest available shore processing facility. One pooled sample (6 kg) of fish meal was collected from each batch. No antioxidants or preservatives were added to the raw material or fish meal during processing and storage.

For shelf-life testing, each fish meal sample was divided into two tightly closed 2 L PE plastic containers for storage in the dark at 10 °C and sampled at 0, 2, and 4 months.

Proximate Analyses. Water content was determined by drying in an oven at 102−104 °C for 4 h (*13*). Fat content was determined according to the AOCS Soxhlet method Ba 3-38 (*14*) using petroleum ether (bp = 30−40 °C) for extraction. Crude protein content was determined with the Kjeldahl method (*15*) and by multiplying the nitrogen content by 6.25. Salt content was determined by using the method of Volhard according to AOAC method 937.09 (*16*). Ash content was determined by carbonization of the sample prior to heating at 550 °C for 3 h (*17*).

Trace Metals. Iron, copper, selenium, and zinc were determined after digestion of the samples in quartz decomposition tubes, applying a temperature program of 80−180 °C (*18*). Iron, copper, and zinc were analyzed by flame atomic absorption (Perkin-Elmer 403 and 1100B) with D_2-background correction. For selenium the ash was heated further with $Mg(NO_3)$, and Se(VI) was reduced to Se(IV) by hydrochloric acid prior to determination by hydride generation atomic absorption.

Lipids. Extraction of lipids was carried out by a chloroform/methanol extraction system based on the method of Bligh and Dyer (*19*) with some modifications (*20*) and with butylated hydroxytoluene (BHT) mixed into all solvents (50−100 mg/L). For determination of the lipid concentration of the extract, a small portion of the chloroform layer was evaporated to dryness at 60 °C for 30 min before weighing. The following analyses were performed on the lipid fraction after evaporation (Büchi, Switzerland) at 37 °C under vacuum.

Fatty Acid Analysis. Saponification, methylation, and gas chromatography of lipids were performed according to the standard AOCS method Ce 1b-89 (*14*). The fatty acid methyl esters were separated and quantified by gas chromatography (Hewlett-Packard 5890 series II gas chromatograph) with an FID and a capillary column [Omegawax 320 (30 m × 0.32 mm × 0.25 μm), Supelco, Sigma-Aldrich, Munich, Germany].

Polyene index (PI) was calculated from fatty acid analysis: PI = (20:5 + 22:6/16:0) (*21*).

Iodine value of the extracted meal lipids was determined according to the method of Hanus (*22*).

Antioxidants. *Astaxanthin* was separated by column chromatography and determined spectrophotometrically at 472 nm in *n*-hexane (*23*). The astaxanthin content was reported as free astaxanthin, diester, and monoester as percentage of (total) astaxanthin.

α-Tocopherol and Retinol. The lipids were saponified, separated, and quantified by high-pressure liquid chromatography (HPLC) based on an assay for vitamin D_3 in cod liver oil (type A) (*24*) with some alterations and scaling down of sample size and reagents (*2*).

Oxidative Status. *Free fatty acids (FFA)* in the lipids were analyzed by dissolving lipid samples in alcohol/diethyl ether (1:1) and titrated with dilute NaOH according to AOCS method Ca 5a-40 (*14*).

Peroxide value (PV) was determined using the official ferric thiocyanate spectrophotometric method (*25*).

Thiobarbituric acid reactive compounds (TBA) were measured by a slightly modified steam distillation method (*26*), where the sample size was reduced to 5 g and antioxidants (5 mL of a 0.5% propyl gallate and 0.5% ethylenediaminetetraacetic acid in water) were added to the sample during blending.

Keeping Quality. The electronic nose "FreshSense", developed by the Icelandic Fisheries Laboratories and Element Sensor Systems (Saudárkrókur, Iceland), was used to monitor the headspace of the meal (*27*, *28*). In each case 200 g of sample, at a temperature of ~10 °C, was placed in the sampling container and the measurement program run for 20 min. The reported sensor values (current; nanoamperes) were calculated from the average response of the last half minute of the program subtracted from the baseline, which was the average response of the last 3 min before measuring.

Oxygen Uptake. The rate of oxygen uptake in the fish meal was measured with a manometric method (*29*) in a Warburg apparatus, series III (Townson and Mercer Ltd., Runcorn, U.K.). Samples (10 g) were weighed into conical flasks (100 mL), and the oxygen uptake was measured at 30 °C. Calculations on oxygen uptake were based on the lipid content determination of the meal (*30*). Results were expressed as oxygen uptake in the lipids (micromoles per gram of oil) during the first day of measurement.

Brown Pigments. Browning of fish meal was measured as acetic acid soluble color (*31*). The absorbance was measured at 400 nm, and the results were expressed as absorbance in 1 mL/g of capelin meal.

Physical Properties. *Color.* The color of the meal was measured by a Minolta CR-300 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) in the Lab* measuring mode (CIE 1976) with CIE Illuminant C. The meal was placed in a Petri disk (60 mm in diameter × 10 mm deep) and measured three times, turning the chromameter head 120° between measurements. Results are reported as L^*, a^*, and b^* values and also as total change in initial color, defined by

$$\Delta E^*_{ab} = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

Water activity (a_w) was measured in a 60 g sample of capelin meal with a water activity meter (aw-Wert-Messer, Durotherm, Germany). The water activity and temperature were read after 3 h. The meter was standardized with a barium chloride solution at 20 °C prior to measurement.

Density of the capelin meal was estimated by both bulk and packing density.

(a) Bulk Density. The fish meal was carefully transferred into a preweighed 100 mL volumetric plastic cylinder. The cylinder was filled to 100 mL and weighed. Bulk density was expressed as the weight of sample divided by volume in grams per milliliter.

(b) Packing Density. The same cylinder with the same sample was packed by hammering the cylinder into the table ~50 times with the hand placed at the upper end of the cylinder. Afterward, the volume in milliliters was read. Packing density was expressed as the weight of the sample divided by the volume of the packed sample in grams per milliliter.

pH was measured with a Ag/AgCl combination electrode connected to a pH meter, model PHM80 (Radiometer, Copenhagen, Denmark). The pH of capelin meal was determined in a water extract from a 5 g sample that was shaken with 100 mL of distilled water at ambient temperature for 3 h. The suspension was then filtered through Whatman 2V filter paper.

Statistical Analysis. In all experiments the experimental design was based on a single-factor analysis correlated with time. Calculations were

Table 1. Composition of Capelin Meal with Season[a]

measurement	summer	autumn	winter	spring
moisture (%)	7.0 ± 0.0 a	6.8 ± 0.1 b	6.6 ± 0.0 b	9.0 ± 0.1 c
protein (%)	70.3 ± 0.1 a	71.1 ± 0.1 b	71.7 ± 0.2 c	69.6 ± 0.3 d
salt (%)	2.6 ± 0.0 a	3.2 ± 0.0 b	2.0 ± 0.0 c	3.4 ± 0.0 d
ash (%)	8.8 ± 0.0 a	9.2 ± 0.1 b	9.4 ± 0.1 c	10.3 ± 0.0 d
lipid[b] (%)	11.9 ± 0.0 a	10.9 ± 0.1 b	11.5 ± 0.1 c	8.4 ± 0.1 d
iron (mg/kg of tissue)	74.6 ± 4.0 a	108 ± 4.9 b	191 ± 11 c	83.5 ± 6.5 a
copper (mg/kg of tissue)	5.10 ± 0.10 a	2.88 ± 0.28 b	2.49 ± 0.20 c	2.22 ± 0.00 c
selenium (mg/kg of tissue)	2.00 ± 0.10 a	1.53 ± 0.06 b	1.60 ± 0.10 b	1.32 ± 0.03 c
zinc (mg/kg of tissue)	63.0 ± 6.0 a	80.4 ± 3.8 b	97.2 ± 2.5 c	96.8 ± 0.9 c
bulk density (g/mL)	0.59 ± 0.00 a	0.54 ± 0.00 b	0.45 ± 0.01 c	0.47 ± 0.00 d
packing density (g/mL)	0.73 ± 0.00 a	0.69 ± 0.00 b	0.64 ± 0.00 c	0.62 ± 0.00 d
water activity (a_w)	0.34 ± 0.02 a	0.34 ± 0.02 a	0.36 ± 0.01 ab	0.43 ± 0.02 b
iodine value	160.5 ± 3.0 a	152.4 ± 1.6 b	147.1 ± 0.3 c	146.9 ± 0.5 c

[a] Each entry represents the mean value ($n = 3$) ± SD of a pooled sample (2 kg) of capelin meal. Means within a row having different letters are significantly different ($P < 0.05$). [b] Petroleum ether soluble lipids.

based on a pooled sample of fish meal. Three sample preparations were made for all samples ($n = 3$), and standard deviation (SD) calculated. Analysis of variance was calculated (Number Cruncher Statistical System, version 2000, NCSS Statistical Software, Kaysville, UT) and a Tukey comparison test used to determine differences between samples ($P < 0.05$).

RESULTS AND DISCUSSION

Chemical Composition. Some seasonal variations were observed in the chemical composition of the capelin meal (**Table 1**). The lipid content was lowest during the spring at 8.4% but 10.9−11.9% during other seasons ($P < 0.05$) with an inverse relationship to moisture content. Previous results (*2*) showed that the lipid content of capelin meal did not vary with season to the same degree as in the whole capelin from which the meal was processed. This was to be expected because the majority of the oil was collected as a separate product during processing of the fish meal; furthermore, the lipid content in capelin declined from ∼14% in the autumn to ∼3% in the spring (*2*).

The iodine value of the meal lipids declined from 160.5 during the summer to ∼147 during the winter and spring ($P < 0.05$). The iodine values of the capelin meal were in agreement with the results of Notevarp and Chahine (*32*). In their study, capelin meal lipids extracted with pentane−hexane had iodine values in the range of 144−168.

The contents of the trace metals copper and selenium were highest in the summer meal, 5.10 mg/kg for copper and 2.0 mg/kg for selenium ($P < 0.05$). The zinc values varied, on the other hand, from 63 mg/kg in the summer to ∼97 mg/kg during the winter/spring ($P < 0.05$). The iron content was highest in the winter at 191 mg/kg but lowest in the summer at 74.6 mg/kg ($P < 0.05$). The levels of metals in the capelin meal were similar to or slightly lower than previously reported results on trace metal content in capelin meal (*32*, *33*). Notevarp and Chahine (*32*) measured two samples of capelin meal and found the meal to contain 256 and 192 mg/kg iron and 4.9 and 4.3 mg/kg copper, respectively. Lunde (*33*) measured iron, copper, zinc, and selenium in capelin meal produced industrially and in the laboratory. The levels of zinc and iron were more than double the concentration in the industrially produced meal compared to the laboratory produced meal, but the levels of copper were similar in both types of meal. Lunde (*33*) suggested that the high values in the factory-produced meal might have been due to contamination during the production process. The reported levels of trace metals were generally in agreement with our results, but the range in the factory-produced meal reported by Lunde (*33*) was greater, especially for the high iron content meal. Similar seasonal variations in the trace metal content of capelin meal, as well as in chemical composition, were found in the fresh capelin from which the meal was produced (*2*). The compositional variation can best be explained by the feeding behavior of capelin during maturation and spawning, which has been found to reflect considerably on seasonal variations in fat content (*1*). Large seasonal variations have also been found in the fat content of Newfoundland capelin (*34*).

The density of the meal was not homogeneous. The summer meal was densest, whether measured as bulk or packing density ($P < 0.05$). It is well-known in the fish meal industry that meal made from capelin harvested during the summer is denser than capelin meal from other seasons, which is believed to be due to a higher content of water soluble materials in the so-called "stick water" in the summer meal. During fish meal processing there are generally three major phases involved, oil, solids, and water phases. Processing conditions were specifically controlled to remove the solids from the water phase and add them back to the solids phase (fish meal). The grinding or milling of the capelin meal was conducted in the same fashion in both processing plants, but minute variability in the industrial process may influence the flow properties and packing of the meal particles, as well as the oxygen diffusion in the meal. Waissbluth and co-workers (*7*) measured the influence of grinding on the rate of oxygen absorption in fish meal. The meal with the largest particle size had an oxygen absorption of 4.1 μmol/h/g of meal, whereas the same meal, with intermediate or small particle size, had oxygen absorptions of 7.3 and 13.7 μmol/h/g of meal, respectively.

The moisture was highest in the spring meal at 9% ($P < 0.05$). The water activity (a_w) of the capelin meal was higher in the spring (0.43) than in the summer and autumn at 0.34−0.36 ($P < 0.05$) but not significantly different from that of the winter meal. The spring meal was relatively stable compared to the other meals, but factors other than water activity, such as low lipid content and high α-tocopherol content, in the lipids of the spring capelin meal may have played an important role in its stability. The results of Waissbluth and co-workers (*7*) indicated that increasing moisture content had a marked pro-oxidant effect in all of the oxidative reactions of whole pilchard and anchovy meal. This was, however, in contrast to other studies on fish meal. Ólafsson (*6*) found that the peroxide values increased rapidly when herring meal was dried to below 10−12%. A more recent study (*35*) indicated that when the moisture content of herring meal was increased from 7 to 12%,

Table 2. Fatty Acid Composition (Percent) of Capelin Meal Lipids with Season Showing Principal Fatty Acids (>90% of Total)[a]

fatty acid	summer	autumn	winter	spring
C14:0	4.6 ± 0.3	4.2 ± 0.1	4.0 ± 0.3	4.3 ± 0.0
C16:0	15.6 ± 0.5 a	15.6 ± 0.2 a	16.0 ± 0.6 a	14.0 ± 0.1 b
C18:0	1.7 ± 0.1 a	1.7 ± 0.0 a	1.6 ± 0.1 a	1.9 ± 0.0 b
total saturated	*21.8 ± 0.8*	*21.5 ± 0.2*	*21.6 ± 0.9*	*20.2 ± 0.1*
Σ C16:1	5.4 ± 0.4	5.0 ± 0.1	5.5 ± 0.3	5.0 ± 0.7
Σ C18:1	12.6 ± 0.5 a	14.0 ± 0.2 b	15.1 ± 0.2 c	15.6 ± 0.9 c
Σ C20:1	8.0 ± 0.6 a	7.5 ± 0.1 a	7.3 ± 0.3 a	9.1 ± 0.8 b
C22:1	10.1 ± 1.1 a	9.6 ± 0.0 a	7.2 ± 0.9 b	10.3 ± 0.1 a
C24:1	0.0 ± 0.0 a	1.0 ± 0.1 b	0.5 ± 0.2 c	1.6 ± 0.0 d
total monoenes	*36.2 ± 0.8 a*	*37.1 ± 0.3 a*	*35.6 ± 0.6 a*	*41.6 ± 0.8 b*
C18:2	1.3 ± 0.2	1.3 ± 0.0	1.3 ± 0.1	1.4 ± 0.0
C18:4	4.5 ± 0.3 a	2.4 ± 0.0 b	2.1 ± 0.1 b	1.3 ± 0.0 c
C20:5	11.2 ± 0.1 a	11.1 ± 0.2 a	11.2 ± 0.3 a	10.5 ± 0.1 b
C22:5	0.7 ± 0.1	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.0
C22:6	15.8 ± 1.6 a	18.1 ± 0.3 ab	18.9 ± 0.2 b	18.2 ± 0.2 ab
total polyenes	*33.5 ± 1.1 ab*	*33.7 ± 0.5 ab*	*34.4 ± 0.4 a*	*32.3 ± 0.4 b*
C20:5 + C22:5 + C22:6	27.8 ± 1.6 a	30.0 ± 0.5 ab	31.0 ± 0.2 b	29.6 ± 0.4 ab
PI[b] = C20:5 + C22:6/C16:0	1.74 ± 0.15 a	1.87 ± 0.05 ab	1.89 ± 0.06 ab	2.05 ± 0.01 b

[a] Each entry represents the mean value ($n = 3$) ± SD of a pooled sample (2 kg) of capelin meal. Means within a row having different letters are significantly different ($P < 0.05$). [b] PI = polyene index.

the oxidation rate decreased by 50%, as measured by oxygen absorption.

Changes in principal fatty acids with season were considerable (**Table 2**). The sums of saturates and polyenes were relatively steady at 22 and 34%, respectively, but showed decreasing tendencies in the spring meal, when the values for the saturates were roughly 20 and 32% for the polyenes. The monoenes, on the other hand, were highest in the spring (41.6%) and relatively steady at other seasons (36−37%) ($P < 0.05$). The polyene index (PI = 20:5 + 22:6/16:0) was significantly higher during the spring (2.05) than in the summer (1.74) ($P < 0.05$). However, the total content of C20:5 + C22:5 + C22:6 was lowest in the summer at 27.8% and highest in the winter at 31% ($P < 0.05$). The spring meal contained less total lipids than the other meals (**Table 1**) and might therefore have been expected to contain a higher proportion of phospholipids than the other meals, where a higher fraction of the lipids constituted membrane lipids. The oil pressed from the capelin press cake during meal processing almost exclusively removes triglycerides (*37*) but leaves the membrane lipids in the meal. Fish lipids are made up of triglycerides and phospholipids; the latter have up to 40% polyunsaturated fatty acids (PUFA) (*37*). However, the results presented here indicate that the PUFA content was not higher in the total lipids of the spring meal than in other meals. The results of the current study were in a fair agreement with the results of Opstvedt for capelin meal (*11*). However, the PI was higher in this study, which can be traced to a somewhat higher content of the most highly unsaturated fatty acids, especially the $n-3$ fatty acids. The reason for this difference may be due to different drying methods. The meal in this study was dried with a gentle drying method that should prevent lipid oxidation better than the older drying methods. Pálmadóttir and co-workers (*36*) found a higher proportion of $n-3$ fatty acids in capelin meal dried with the more recent and gentle steam drying and indirect steam drying methods, compared to the older flame drying methods.

A decrease in the most polyunsaturated fatty acids as a result of lipid oxidation in fish meal is generally accepted and has been reported previously (*11*, *12*, *37*). A marked decrease in C20:5 and C22:6 in menhaden meal has been observed as a result of a lack of antioxidants (*38*).

Unsaturation of fish lipids as measured by the polyene index (PI = 20:5 + 22:6/16:0) can be informative and has been suggested to be a meaningful tool for measuring the oxidative rancidity in fishery products. The PI showed a positive correlation with rancidity scores in dried-salted sardines (*21*). In their study, the PI decreased during storage for 6 weeks, but remained nearly stable after that. It is well-known in the fish meal industry that the initial reactivity of the fish meal lipids is high and the lipid oxidation rate diminishes with time (*35*).

Changes during Storage of Capelin Meal. The content of endogenous antioxidants was highly influenced by season (**Table 3**). Similar seasonal variations were also seen in the whole capelin from which the meal was made (*2*). Total astaxanthin was highest at 47.8 mg/kg in the meal from the summer season and as low as 5−13 mg/kg during other seasons ($P < 0.05$). Astaxanthin was mainly in the diester form except in the summer capelin meal, where free astaxanthin was dominating. The autumn meal had the lowest proportion in the free form, compared with the other seasons. According to Miki and others (*39*) free astaxanthin is the most labile form toward heat, whereas the diester is the most stable form of astaxanthin. Degradation of astaxanthin during storage was most pronounced in the autumn meal, where 60% of the total astaxanthin was lost during 4 months of storage, compared to 40% in the summer meal and ~20% during other seasons ($P < 0.05$). The astaxanthin fractions did not show distinct differences in stability.

α-Tocopherol in capelin meal was found to be highest in the spring at 167 ± 12 mg/kg of lipid and lowest in the autumn at 27 ± 6 mg/kg. The α-tocopherol decreased from 167 ± 12 to 105 ± 21 mg/kg of lipids in the spring ($P < 0.05$). The values from the summer meals varied from 33 ± 15 to 43 ± 6 mg/kg after 4 months of storage and from 27 ± 6 to 50 ± 0 mg/kg in the autumn meal and from 53 ± 6 to 77 ± 6 mg/kg after 2 months and to 60 ± 0 mg/kg of lipid after 4 months of storage. There was no particular trend in the α-tocopherol content except during the spring, when it declined. The most reasonable explanation for no decrease at other seasons may be that the α-tocopherol values were near the detection limit at ~10 mg/kg. The meal lipids generally have a thick consistency, which intensifies with oxidation, and are relatively difficult to measure in the HPLC compared to fresh fish oil. Information on α-tocopherol content in fish meal was not found in the literature, but the α-isomer has been shown to be the principal tocopherol in fish (*40*).

Vitamin A was highest during the spring and winter at 54 and 34 mg/kg, respectively, but much lower during other seasons, or ~13 mg/kg ($P < 0.05$). Although the vitamin A content was also very low, it showed a decrease with time during all seasons ($P < 0.05$), except in the summer.

The summer meal had the highest levels of FFA or roughly 12%, compared to 9 and 10% in the autumn and spring, but 7% in the winter ($P < 0.05$) (**Table 3**). These seasonal variations in the FFA content of the meal were most likely the result of variations in the fresh capelin from which the meal was produced. High autolytic activity was observed in the capelin from the heavy feeding period in the summer (*2*). The values are considerably lower than previously reported FFA content in the lipids of capelin meal, for which the FFA were found to be in the range of 12.6−21.8% based on samples collected

Table 3. Compositional Changes in Capelin Meal from Different Seasons during Storage[a]

meal	storage period (months)	total astaxanthin (mg/kg of lipid)	diester (%)	monoester (%)	free (%)	tocopherol (mg/kg of lipid)	vitamin A (mg/kg of lipid)	lipid[b] (%)	FFA (%)
summer	0	47.8 ± 1.2 a	15.7 ± 2.2 a	39.8 ± 2.7 a	44.5 ± 2.3 a	33 ± 15 a	12 ± 6 a	11.9 ± 0.0 a	11.7 ± 0.1 a
	2	35.1 ± 0.6	5.0 ± 0.2	52.7 ± 0.9	42.4 ± 1.2	40 ± 0	9 ± 0	11.7 ± 0.0	11.4 ± 0.1
	4	28.6 ± 0.1	5.5 ± 0.2	52.9 ± 0.7	41.7 ± 0.8	43 ± 6	14 ± 5	11.8 ± 0.1	11.4 ± 0.2
autumn	0	5.0 ± 0.9 b	50.7 ± 7.1 b	28.8 ± 3.9 b	20.4 ± 3.2 b	27 ± 6 a	14 ± 2 a	10.9 ± 0.1 b	9.2 ± 0.1 b
	2	3.4 ± 0.1	46.9 ± 4.4	30.5 ± 2.6	22.6 ± 2.8	33 ± 6	11 ± 2	10.7 ± 0.2	8.0 ± 0.1
	4	2.0 ± 0.2	47.8 ± 3.8	35.4 ± 1.5	16.8 ± 2.4	50 ± 0	7 ± 2	10.5 ± 0.2	8.5 ± 0.5
winter	0	7.6 ± 1.0 b	43.2 ± 3.5 b	18.7 ± 2.5 c	38.0 ± 5.8 a	53 ± 6 a	34 ± 2 b	11.5 ± 0.1 c	7.4 ± 0.1 c
	2	5.5 ± 1.2	51.2 ± 6.5	21.1 ± 0.7	27.7 ± 6.6	77 ± 6	26 ± 3	11.3 ± 0.0	7.1 ± 0.0
	4	5.9 ± 0.5	53.3 ± 2.3	26.1 ± 2.9	20.6 ± 5.2	60 ± 0	21 ± 0	11.4 ± 0.0	7.3 ± 0.1
spring	0	13.4 ± 1.2 c	64.1 ± 0.0 c	12.9 ± 1.9 c	23.0 ± 1.9 b	167 ± 12 b	54 ± 0 c	8.4 ± 0.1 d	10.3 ± 0.5 d
	2	11.7 ± 0.3	64.6 ± 3.6	12.3 ± 1.9	23.0 ± 2.1	150 ± 10	38 ± 2	8.6 ± 0.0	10.0 ± 0.1
	4	10.6 ± 1.0	66.8 ± 1.1	19.0 ± 1.7	14.2 ± 2.2	105 ± 21	39 ± 4	8.4 ± 0.0	9.9 ± 0.3

[a] Levels of all compounds are given as mean values ($n = 3$) ± SD of a pooled sample (2 kg) of capelin meal. Means at 0 months within a column having different letters are significantly different ($P < 0.05$). [b] Petroleum ether soluble lipids.

Table 4. Changes in Capelin Meal from Different Seasons during Storage[a]

meal	storage period (months)	pH	color[b] Lab L^*	Lab a^*	Lab b^*	ΔE^*_{ab}
summer	0	6.36 ± 0.05 a	50.2 ± 0.4 a	1.14 ± 0.04 a	12.8 ± 0.2 a	
	2	6.35 ± 0.01	52.1 ± 0.3	0.97 ± 0.03	13.3 ± 0.2	2.0 ± 0.1 a
	4	6.37 ± 0.01	52.6 ± 0.1	0.80 ± 0.04	13.5 ± 0.1	2.5 ± 0.4
autumn	0	6.84 ± 0.01 b	51.5 ± 0.3 a	0.38 ± 0.01 b	11.8 ± 0.2 b	
	2	6.83 ± 0.01	50.6 ± 0.6	0.47 ± 0.03	14.5 ± 0.2	2.9 ± 0.2 b
	4	6.76 ± 0.01	51.3 ± 0.2	0.49 ± 0.01	15.1 ± 0.2	3.3 ± 0.2
winter	0	7.00 ± 0.08 b	50.6 ± 0.6 a	0.78 ± 0.17 a	11.6 ± 0.4 b	
	2	6.94 ± 0.08	49.4 ± 1.4	0.62 ± 0.10	13.5 ± 0.4	2.3 ± 0.6 ab
	4	6.71 ± 0.00	50.0 ± 0.5	0.79 ± 0.09	13.7 ± 0.6	2.2 ± 0.5
spring	0	6.53 ± 0.11 ac	46.7 ± 1.0 b	0.17 ± 0.22 b	9.9 ± 0.5 c	
	2	6.60 ± 0.12	46.7 ± 0.6	0.23 ± 0.11	10.6 ± 0.3	0.8 ± 0.4 c
	4	6.74 ± 0.02	46.7 ± 0.1	0.39 ± 0.13	10.7 ± 0.2	1.2 ± 0.3

[a] Each entry represents the mean value ($n = 3$) ± SD of a pooled sample (2 kg) of capelin meal. Means at 0 months within a column having different letters are significantly different ($P < 0.05$). [b] Lab* L^* = lightness, Lab a^* = red, Lab b^* = yellow, ΔE^*_{ab} = change in initial color.

during one year of production (*41*). The difference may be from the handling or the processing of the raw material, which have changed considerably during the 20 years that elapsed between the two studies. According to Barlow and Pike (*12*), the FFA content depends on a number of different factors. FFA can be formed prior to processing, due to the action of hydrolytic enzymes in the fish. Furthermore, they imply that heating in the processing will probably also result in the formation of some FFA by spontaneous hydrolysis, and finally complex interaction during storage of the meal will gradually release FFA. Although it is well-known that the quality of oil may be indicated by the FFA content, it may not necessarily be a useful criterion for evaluating the quality of lipids in fish meal. Opstvedt (*37*) found that the level of FFA in the diet of animals had no effect on their general thriveability and growth. In our study the FFA levels showed tendencies to decrease with storage time, especially in the autumn meal, where the FFA levels fell by 7.7% during 4 months of storage ($P < 0.05$).

The pH value (**Table 4**) of the capelin meal indicated a fair correlation with the FFA measurements ($r = -0.87$). The lowest pH values, indicating the most acidity, were to be found in the seasons with high FFA.

The color of the capelin meal varied with season (**Table 4**). Spring meal was the darkest with $L^* = 46.7$ compared to L^* values of ~50 at other seasons ($P < 0.05$). The spring meal had the lowest values for yellow ($b^* = 9.9$) ($P < 0.05$) and a relatively low value for red ($a^* = 0.17$), but the summer meal had the highest values for yellow ($b^* = 12.8$) ($P < 0.05$) and a relatively high value for red ($a^* = 1.14$). The high red value was probably due to high levels of astaxanthin, which was obvious from visual observation of the red color of the lipid extract. The yellow color increased significantly with time in all of the meal samples ($P < 0.05$) except the spring meal. The total color change (ΔE^*_{ab}) was greatest in the autumn meal during the four months of storage, followed by the summer and winter meal, whereas the spring meal showed only half of the color change observed in the other meal. The smaller the value of ΔE^*_{ab}, the closer the samples were to a perfect match with their initial color. A ΔE^*_{ab} of <0.4 has been considered to be below the threshold of human perception (*42*). First-grade commercial matching may be as high as 0.9 ΔE^*_{ab} and other less critical matching applications can have acceptable limits at 4−5 ΔE^*_{ab}. Total color change during storage between 2 and 3 ΔE^*_{ab} for all meal types except the spring meal was not significant and probably within limits of acceptance.

Lipid Oxidation and Degradation Products. Petroleum ether extractable lipids changed within 1% in all meal samples, except for the autumn meal, for which the decrease was 3.9% ($P < 0.05$) during 4 months of storage (**Table 3**). A decrease in diethyl ether extractable lipid during storage has previously been reported as an indication of advanced oxidation in fish

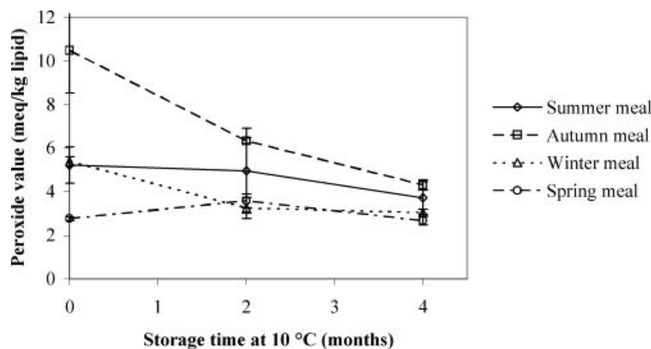

Figure 1. Changes in peroxide value during storage of capelin meal from different seasons. Each point represents a mean value ($n = 3$) ± SD of a pooled sample (2 kg) of meal.

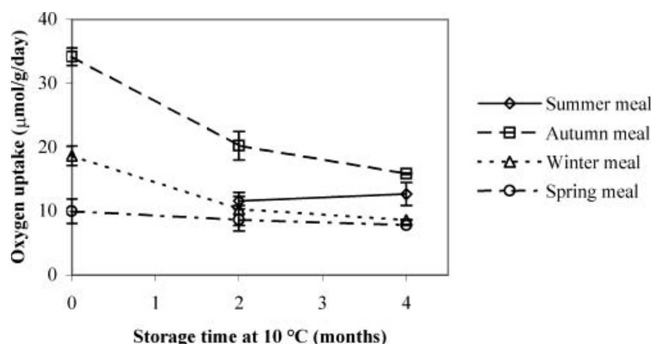

Figure 2. Evaluation of oxygen uptake in capelin meal from different seasons during storage. Each point represents a mean value ($n = 3$) ± SD of a pooled sample (2 kg) of meal.

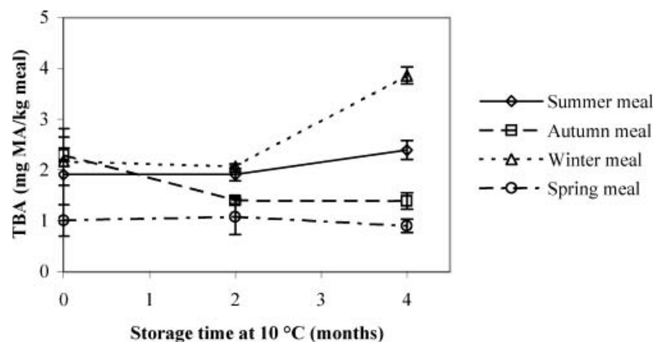

Figure 3. Changes in TBA during storage of capelin meal from different seasons. Each point represents a mean value ($n = 3$) ± SD of a pooled sample (2 kg) of meal.

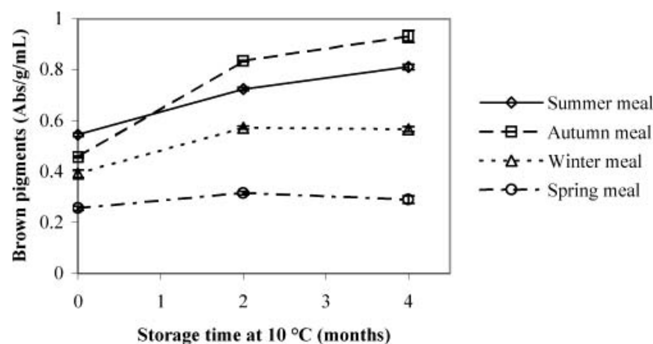

Figure 4. Browning in capelin meal from different seasons during storage. Each point represents a mean value ($n = 3$) ± SD of a pooled sample (2 kg) of meal.

meal (*12*). In this study the Bligh and Dyer chloroform/methanol solvent system was used for the extraction of meal lipids to be used for further analyses of the lipids. This has been shown to be the best available solvent system for extracting both polar and nonpolar lipids, demonstrated by higher yield of total lipids, including oxidized lipids and lipid soluble components (*43*, *44*).

Rancidity as measured by PV was highest in the autumn, 10.5 mequiv/kg of lipids, and lowest in the spring, 2.8 mequiv/kg ($P < 0.05$). The initial PV for summer and winter meals slightly exceeded 5 mequiv/kg but decreased slightly during storage except for the spring meal (**Figure 1**). The fastest decrease in PV was observed during the first months of storage in the autumn and winter meals ($P < 0.05$), but all types of meal seemed to level off to a similar PV, in the range of 3−4 mequiv/kg of lipids. The decrease in PV with storage time was in agreement with the results reported by Barlow and Pike (*38*), although the values were much higher than observed for the capelin meal. In their study with anchovy and pilchard meals without the addition of antioxidants, the PV reached a maximum of 100−125 mequiv/kg of lipids after ∼1 week of storage, which was followed by a subsequent decrease.

A general decrease in oxygen uptake was observed in the capelin meal, although the change was insignificant in the spring meal (**Figure 2**). The oxygen uptake decreased significantly in the autumn meal throughout the storage experiment and for the first 2 months in the winter meal. The spring meal had lower values and did not decrease with time ($P < 0.05$), indicating the greatest stability in the spring meal with respect to oxidation. The oxygen uptake analysis in the fresh summer meal failed, but no significant decrease was observed between 2 and 4 months of storage. According to Astrup and Halvorsen (*35*), a decline in oxidation with time is particularly evident when the initial reactivity is high, which is the case in fish meal with high fat content. Evidently, this phenomenon is well-known in the fish meal industry, because after the manufacture of meal from fatty fish, a "curing" period may be necessary for unstabilized meal when the most reactive PUFA in the meal are allowed to oxidize and then cool off in loosely stacked sacks to avoid spontaneous combustion (*12*). After the initial curing period, the meal is relatively stable and can be stored in compact stacks.

The initial TBA values of the capelin meal from different seasons were similar and in the range of 1−2 mg of malondialdehyde/kg. Despite this, the changes in TBA during storage were slightly inconsistent among the meals (**Figure 3**). Summer and spring meals did not change with time ($P < 0.05$), whereas the TBA in the autumn meal decreased by 40% after 2 months of storage ($P < 0.05$). In contrast, the TBA in the winter meal doubled during the last 2 months of storage ($P < 0.05$). Although often used, the TBA test has not been regarded as a good index of the extent of lipid oxidation in stored dried products (*31*, *45*). TBA is a measure of secondary lipid oxidation products, mainly malondialdehyde. The fact that TBA decreases during capelin meal production and with storage time of the meal implies that the malondialdehyde decomposes with time or reacts further. Gómez-Sánchez and co-workers (*46*) suggested that malondialdehyde reacts with amino sugars, giving rise to pyrroles and other heterocyclic compounds similar to compounds resulting from the Maillard reaction. These heterocyclic compounds are unstable and darken with time. This could be the explanation for the browning of capelin meal during storage. Furthermore, various saturated and unsaturated aldehydes produced in the course of the oxidation of fish lipids have been found to react readily with proteins to form brown products (*47*). Experience has shown that browning of dried fish products is a relevant indicator of the high degree of lipid oxidation occurring in such products (*21*, *31*, *45*, *48*).

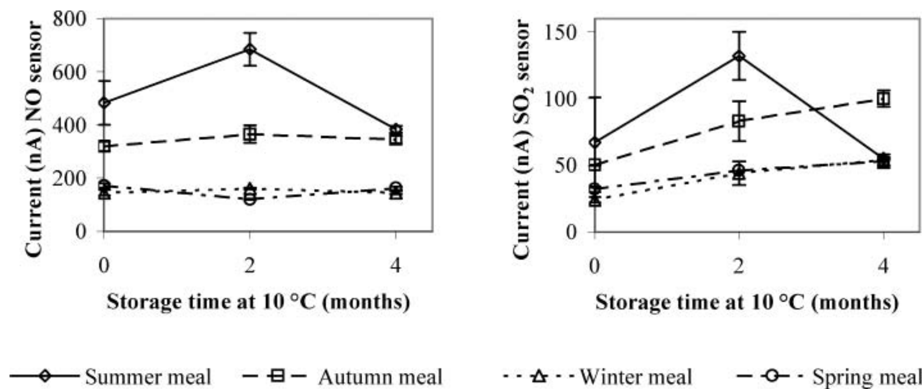

Figure 5. Responses (currents in nanoamperes) of NO and SO_2 sensors to capelin meal headspace during storage. Each point represents a mean value ($n = 3$) ± SD of a pooled sample (2 kg) of meal.

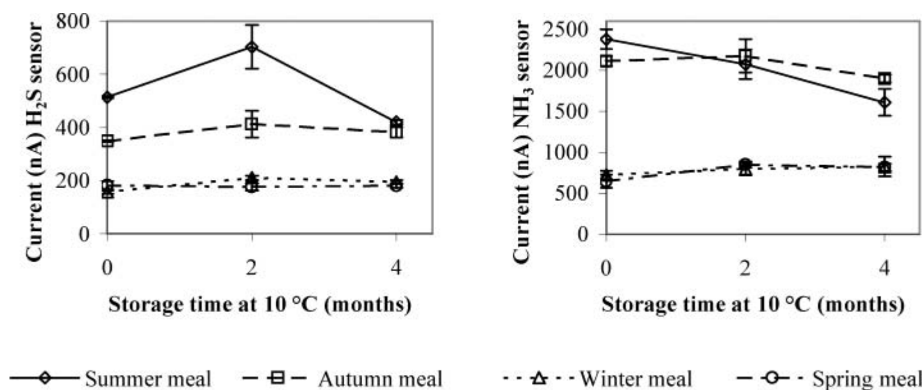

Figure 6. Responses (currents in nanoamperes) of H_2S and NH_3 sensors to capelin meal headspace during storage. Each point represents a mean value ($n = 3$) ± SD of a pooled sample (2 kg) of meal.

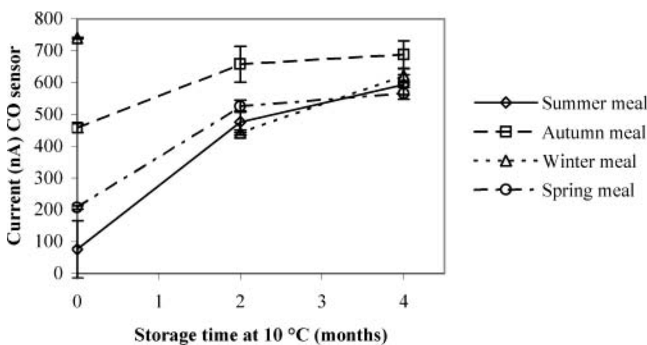

Figure 7. Responses (currents in nanoamperes) of CO sensor to capelin meal headspace during storage. Each point represents a mean value ($n = 3$) ± SD of a pooled sample (2 kg) of meal.

Substantial browning was observed in meal from all seasons during storage ($P < 0.05$) (**Figure 4**). Brown pigments increased by 50% in the summer and winter meals and more than doubled in the autumn meal. Spring meal showed the least, but significant, browning during storage. The browning was faster at the beginning of the storage but declined with time. The measurement of brown pigments was consistent with the Lab b^* and ΔE^*_{ab} color measurements (**Table 4**), with $r = 0.93$ and 0.87, respectively.

Changes in Volatile Components. The electronic nose measurements demonstrated similar patterns for three of the four sensors, or the H_2S, NO, and SO_2 sensors (**Figures 5–7**). Initially, there were lower responses for the spring and winter meals, higher for the autumn meal, and highest for the summer meal ($P < 0.05$), with the exception of the SO_2 sensor. The SO_2 increased significantly with storage except in the summer meal. The summer meal showed a significant increase and then a decrease with storage time for SO_2. This behavior in the summer meal was also observed for H_2S and NO. This might indicate an increase in reactivity with time, followed by a decline during prolonged storage due to lack of substrates.

The NH_3 sensor demonstrated a clear difference ($P < 0.05$) throughout the storage time between the summer and autumn meals and between the winter and spring meals, respectively (**Figure 6**). The summer and autumn meals both showed an overall much higher response in NH_3 than the winter and spring meals ($P < 0.05$). There was a decline in NH_3 in the summer meal ($P < 0.05$), which was not observed in the meals from other seasons. The decline in NH_3 response may be due to amines and other nitrogen compounds reacting with other reactive compounds in capelin meal.

The CO sensor demonstrated probably the most interesting changes with storage time (**Figure 7**). Apart from the high initial value of the winter meal, which cannot be explained and was excluded from the data analysis, all meal samples showed increasing responses with storage time ($P < 0.05$). The autumn meal had the highest CO response ($P < 0.05$) until after 4 months of storage, when only the spring meal was significantly lower. The CO sensor was the only sensor with which the highest response was observed in the autumn meal. The increase in the CO response with time implies the development of short-chain alcohols and aldehydes (*27*), which may be products of a secondary lipid oxidation in the meal.

Conclusions. From a comparison of the overall stability of the capelin meal from different seasons it was clear that the instability of the autumn meal was seen in many parameters. Decomposition of peroxides was more rapid, browning and CO response were more pronounced, and oxygen uptake decreased

more with storage time in the autumn meal than in meal from other seasons, indicating a significant reduction of reactive fatty acids. Petroleum ether extractable lipid changed within 1% in all meal samples with the exception of the autumn meal, for which the extractable lipid decreased significantly by 3.9% during the 4 months of storage. The autumn meal contained the lowest levels of the natural antioxidants α-tocopherol and astaxanthin compared to the meal from other seasons. Degradation of astaxanthin during storage was also most pronounced for the autumn meal. The influence of possible pro-oxidants was, however, not clear in this study, as the iron content was highest in the winter but copper was highest during the summer.

It can be concluded that the stability of capelin meal is strongly influenced by season. The autumn meal was the least stable, with high fat content and PUFA and a low content of lipid soluble antioxidants.

Changes during storage of capelin meal in general were more rapid during the first 2 months than during the following 2 months. The methods most suitable for measuring changes in capelin meal with storage time were methods that measure tertiary lipid oxidation products, which were indicated by color change or brown pigments. Electronic nose measurements with the CO sensor also gave some promising results.

ABBREVIATIONS USED

PI, polyene index; FFA, free fatty acids; L^*, lightness; a^*, red; b^*, yellow; ΔE^*_{ab}, change in initial color.

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