A thesis submitted in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in food science
Department of Food Science
University of Iceland

On the Stability of Icelandic Capelin Meal

Margrét Bragadóttir
2001

Supervisors: Heiða Pálmadóttir M.S. and Kristberg Kristbergsson Ph.D.

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ABSTRACT

Seasonal variation of the stability of capelin meal was examined by measuring lipids, chemical composition, and the content of anti- and prooxidants. Lipid oxidation and other deterioration products were monitored in capelin and meal. Changes during storage of capelin meal were measured as antioxidant loss and as the development of primary, secondary and tertiary lipid oxidation products.

Results on changes during landing of whole capelin indicated that the spoilage processes were quite dynamic, starting with decomposition of endogenous antioxidants and gradual onset of rancidity and spoilage.

Considerable seasonal variation was observed in lipids, antioxidants and prooxidants of both capelin and capelin meal. Lipid content of capelin was highest in autumn and lowest in spring. Unsaturation of lipids (polyene index) was highest during spring and lowest in winter. The meal lipids were more unsaturated than the lipids from the raw material, with about 20% higher iodine value and 50% higher polyene index.

As for the natural antioxidants, astaxanthin was extremely high in capelin during summer season, but the respective meal had approximately half the raw material content. At other seasons the astaxanthin content was low, but higher in the meal than in the raw material. α-Tocopherol in capelin was found highest in spring and lowest in autumn, and the meal had generally less than half the raw material content.

The copper content was highest in the summer, whereas iron, selenium and zinc were highest in the winter. The fish meal contained on the average 4-9 times higher levels of these metals.

Rancidity was highest in autumn. Peroxide value was generally 3-4 times higher in the meal than in the raw material, while TBA decreased after fish meal processing, to about half of the raw material values. Free fatty acids were highest during spring and summer and about 3-6 times higher in the fishmeal.

Storage experiments on capelin meal showed generally decreasing peroxide value, oxygen uptake and TBA with storage time, while browning increased and electronic nose measurements showed increasing responses for the CO sensor with storage time.

Capelin and meal from autumn were most unstable, but the material from winter and spring was the most stable. Results indicate that the instability of autumn meal was due to lack of antioxidants together with high unsaturation of lipids.

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1. INTRODUCTION

Capelin (*Mallotus villosus*) is a small pelagic fish that is mainly utilized for fish meal production. Capelin meal contains not only the fish muscle, but also materials from the whole fish, making it highly nutritious. The nutritional benefits consist mainly of high content of protein, minerals and n-3 fatty acids originating from unsaturated fish lipids. It is generally accepted that unsaturated fatty acids have nutritional benefits, but they are also more susceptible to oxidation because of their high content of double bounds. The consequences of lipid oxidation in fish meal are reduced nutritional value and protein damage of the meal (Romoser 1968, Opstvedt 1975, Pike *et al.* 1990). Lipid oxidation can proceed as far as causing heat damage of the meal and even lead to combustion (Ólafsson 1953, Waissbluth *et al.* 1971). Capelin meal on the other hand has after decades of experience the reputation of being more oxidatively stable than other fish meal from pelagic fishes. This stability of capelin meal is generally considered to be due to relatively low content of polyunsaturated fatty acids in the meal lipids, which is reflected in low iodine value. This is not quite right, because comparison of fish meal lipids made by Opstvedt (1971) and Barlow and Pike (1977) has shown that capelin meal lipids are more unsaturated than meal lipids from for example herring and mackerel, were as anchovy and sardinella meal lipids are more unsaturated.

Most problems due to lipid oxidation in fish meal can be avoided by proper cooling of the meal after processing and by addition of antioxidants. But recently consumers have awakened to the possibility of health risk by using synthesized antioxidants such as ethoxyquin that is conventionally added to fish meal. Therefore it is not unlikely that more strict regulations on the application of synthesized antioxidants will be enforced in the near future. This natural trend can be seen in consumer preferences towards natural foods as well as natural antioxidants. Naturally occurring antioxidants are widespread in nature and can be found in most fruits, berries, herbs and vegetables in the form of various antioxidative compounds such as ascorbic acid (Dörnenburg and Davies 1999), flavonoids (Peterson and Dwyer 1998) and polyphenols (Bravo 1998, Kähkönen *et al*. 1999). Semi-fat and fatty fish is no exception and recent research have confirmed several antioxidative systems such as glutathione antioxidant system in mackerel and bluefish muscle (Jia *et al*. 1996), phenolic compound in

shrimp (Seymour *et al.* 1996), ascorbic acid, glutathione, α-tocopherol and ubiquinone-10 in mackerel (Petillo *et al*. 1998), and ascorbic acid and glutathione peroxidase in herring (Undeland 1998).

Several studies on the shelf life of capelin have shown remarkably good organoleptic stability during frozen storage (Jangaard 1974, Shaw and Botta 1977 and Botta *et al.* 1983), but no research on antioxidative systems in capelin or capelin meal can be found in the literature. However, information has been compiled on the biology of capelin and fluctuation in chemical composition that is especially seen in lipid content and fatty acid composition as a result of seasonal variation in feeding and migration behavior of the capelin.

The purpose of this study was to reveal the factors that influence the stability of capelin fish meal. Whether the stability can be connected to seasonal variations in lipid content and fatty acid pattern of the capelin or if there is more complex relationship between other constituents, such as endogenous antioxidants and prooxidants.

2. LITTERATURE REVIEW

The stability of capelin meal is influenced by many factors. External factors such as processing and storage can most often be controlled to some extend, making the internal stability factors of more interest. These are factors such as the biology of the capelin that among other things is reflected in seasonal variation in lipid content and composition. Other things of great importance for the meal stability are the content of naturally occurring anti- and prooxidants.

2.1. Biology of capelin

The biology and fishery of the Icelandic capelin (*Mallotus villosus*) has been reviewed extensively by Vilhjálmsson (1994). Capelin is a small (20 cm) pelagic, schooling fish, native to the northern hemisphere and may in places occur in great numbers. The Icelandic capelin stock inhabits the area around Iceland and between Iceland, East-Greenland and Jan Mayen. Most part of the capelin lifetime is spent at deep sea, for only to return to the coast during feeding and spawning. Capelin feeds on plankton and among the smaller size groups' calanoid copepods is the most important food category. As the capelin grows older and larger this group of food items is replaced by

adult calanus, ephausiids and amphipods. Feeding intensity is highest in summer and generally decreases in autumn. The species has a very short life span, it matures and spawns at 3-5 years of age and has a high spawning mortality. The main winter migration route of the mature part of the capelin stock is from the northern feeding grounds in a clockwise direction along the east coast of Iceland and then west along the south coast to the main spawning area in the south and west coasts of Iceland. Migrations are undertaken in response to seasonal changes in the physical environment and/or in search of food as well as finding or homing in on spawning areas.

2.2. Fishery and processing

In recent decades the capelin became the target of large fisheries in Iceland. After initial experiments with gear and methods for processing, a capelin fishery for meal and oil production was started in 1965. It began with coastal fisheries south and west of Iceland late in the spawning season, evolved into oceanic fishery east of Iceland which continued to the end of the spawning season in March-April and continued with summer and autumn fishery in the deep water area off North and Northwest Iceland. The Icelandic capelin stock is now fished in the period form July to March in the following year whenever stock abundance allows. Annual catches have varied from zero to about 1,3 million tons (Vilhjálmsson 1994).

The capelin is mainly processed for fish meal and oil, but during the winter months when the capelin is at its prime quality, some part of the catch is whole frozen, as well as just the roes for further processing (Gíslason *et al*. 1995). At other seasons the capelin is not of acceptable quality for freezing and further processing because of to high fat content or to high feed content in the stomach. The freshness of the raw material is another crucial factor for product quality. Capelin freshness is routinely measured by TVN (total volatile nitrogen) in the processing plants in Iceland. TVN gives a fairly good estimate on raw material quality, that is the degree of degradation. The shelf life of capelin is highly seasonal and in the summertime it is often only a few days. It is generally accepted that in periods of heavily feeding the capelin is very susceptible to postmortem autolysis due to high intake of redfeed (copepods and ephausiids), although research have shown contradictory results. Icelandic research on capelin caught in February (Guðmundsson and Gunnlaugsson 1974) found that belly bursting due to redfeed autolysis arose before putrid smell in redfeed free capelin.

High roe content in capelin also appeared to enhance degradation. Canadian research on capelin caught in June and July (Botta *et al*. 1992) indicated on the other hand that delay before processing had more effect on capelin quality than the redfeed or roe level. Norwegian finding (Gildberg and Raa 1980) on capelin caught in August and September, showed that despite the fact that belly bursting was most widespread in capelin with high proteolytic activity, it could not be used for predicting belly bursting.

Several methods are practiced on board fishing vessels to reduce this fast decomposition of capelin. Addition of either formic or acetic acid at a concentration of 1 ‰ extends shelf life as well as increasing lipid yield of fresh material during pressing. In recent year's ice chilling is more common and cooling with refrigerated sea water (RSW) in tanks is an example of preservation technique that is practiced for small pelagic fish with good results (Hansen 1996).

2.3. Capelin fish meal production and characterization

The main part of the present world capelin catch is used for the production of meal and oil. Fish meal is valuable in feeds for poultry, pigs, ruminants, fish, crustaceans, pets and fur-bearing animals because it increases productivity and improves feed efficiency (Bimbo and Crowther 1992). Fish meal manufactured from pelagic fish such as capelin, herring, anchovy, pilchard, and menhaden results in fatty fish meal (7-13%) even though a considerable quantity of the fat is removed during processing (Barlow and Pike 1977). The basic process for fish meal production (Bimbo 1989, Bimbo and Crowther 1992) is as follows (Figure 2.1). The raw material (whole capelin) is ground up, cooked and then pressed, for separation of presscake and press liquor. The pressliquor is separated into fish oil and stickwater. The stickwater is evaporated and combined to the presscake, prior to drying. The dried fish meal is then cooled before grinding and packaging. This process of lipid separation and drying alter the chemical composition of the product. Capelin fish meal typically contains 71% protein, 8% water, 11% lipid and 10% ash, whereas raw capelin contains about 13% protein, 2% ash, 3-20% lipid and the rest is water. There is not only large variation in lipid content of the capelin, but also in the chemical composition of the lipids. According to Opstvedt (1985), lipids in fish may be divided into neutral lipids (NL) and phospholipids (PL), in which PL is relatively constant for different fish species and throughout the year, while NL varies depending on lipid content.

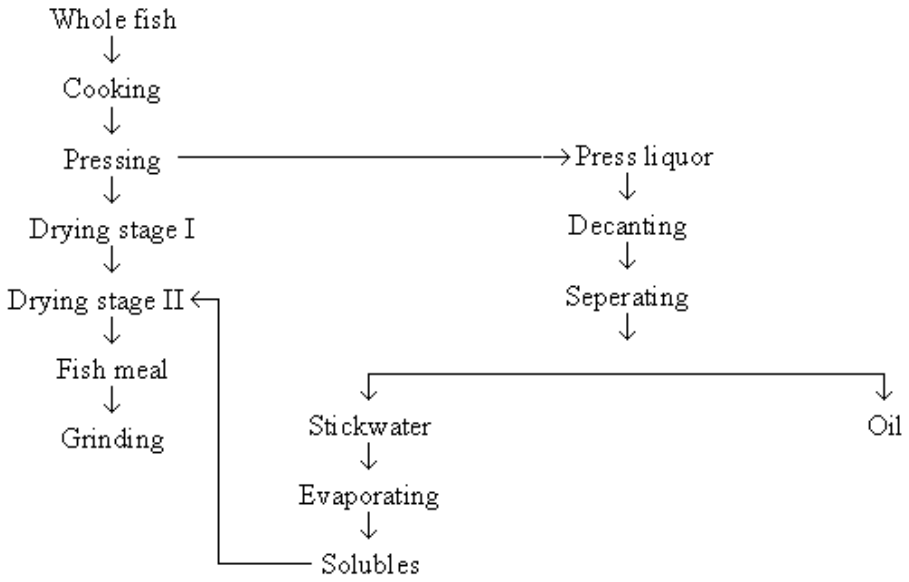

Figure 2.1. Schematic diagram of fish meal and oil processing (Adapted from Bimbo and Crowther 1992).

PL contains more polyunsaturated and less monounsaturated fatty acids than NL. Fish oils are almost exclusively NL and, therefore, contain less polyunsaturated fatty acids than meal lipids (Table 2.1). The lipids of capelin contain high levels of polyunsaturates, but also some unusual fatty acids. The lipids of capelin as well as of herring and mackerel contain odd chain fatty acids, such as C15:0 and C17:0 as well as high content of C22:1. These probably originate from fatty alcohols of North-Atlantic copepod wax esters (Ackman 1980).

The fish meal processing brings about several complex changes in the material. Most processing of fish, such as filleting and mincing, that makes the lipids more accessible to other components in the tissues and to oxygen will affect the oxidation rate (Hardy 1980). Heating or cooking of fish has shown variable effects (Hardy 1980, Undeland *et al*. 1998). which may by due to the use of different conditions. Cooking has been suggested to retard oxidation in fish due to destruction of lipoxygenase, formation of water soluble antioxidants and destruction of heme compounds (Hardy 1980, Flick *et al*. 1992). Undeland *et al*. (1998) observed that precooking inactivated prooxidative enzymes in minced herring as well as influencing the inactivation of antioxidants, activation of hemoproteins and caused general thermal acceleration of lipid oxidation. The exception was temperatures below 60 °C, where it appeared that major part of the aqueous catalyzing enzymes in fish were inactivated without simultaneous denaturation of hemoproteins.

Table 2.1. Partition of lipids in capelin into fish meal and oil (Adapted from Opstvedt 1985).

	Capelin, whole	Meal	Oil
Total lipids (TL), % of dry matter	32	13	100
Percentage of TL in whole fish	-	40	60
Neutral lipids, % of TL	77	60	97
Phospholipids, % of TL	16	24	1
Fatty acid			
C14:0	6.7	4.5	8.1
C16:0	11.3	15.9	9.0
C18:0	1.3	2.0	1.1
Total saturated	*19.3*	*22.4*	*18.2*
C16:1	8.2	7.6	8.7
C18:1	17.3	16.0	17.4
C20:1	20.5	10.0	24.9
C22:1	15.6	7.1	19.5
Total monoene	*61.6*	*40.7*	*70.5*
C20:5	5.3	10.5	2.9
C22:6	7.4	17.9	1.7
Total polyene	*12.7*	*28.4*	*4.6*
Polyene index (C22:6 + C20:5 / C16:0)	1.12	1.79	0.51

Fish meal, however, is not only cooked but also dried, making the chemical reactions even more complex. At high temperatures other chemical products may be formed, such as the Maillard reaction products or nonenzymatic browning between amino- and carbonyl compounds, which have been shown to have antioxidative properties (Alaiz *et al.* 1995, Bedinghaus and Ockerman 1995, Hayase *et al*. 1990). High temperatures are not always needed to form brown Maillard reaction products and they have been found in salted, sun dried fatty fish (Lubis and Buckle 1990, Maruf *et al*. 1990, Smith and Hole 1991). Maillard reaction has been suggested to occur between lipid oxidation products such as malondialdehyde and amino sugars (Gómez-Sánchez *et al*. 1990) or epoxy-heptenal with lysine (Hidalgo and Zamora 1993). The rate of Maillard reaction is influenced by many factors, such as temperature, pH and water activity (Lingnert 1990). Tanaka and co-workers (1990) found highest development of antioxidative effect measured as autoxidation of linoleic acid in a Maillard reaction model system of histidine-glucose, with higher temperature (100 °C in contrast to 120 °C) as well as with lowest water activity (in the region of 0.15 - 0.84). The same Maillard reaction products applied in 1-3% concentration retarded significantly the formation of peroxide value and TBA-number on kamaboko-type sardine.

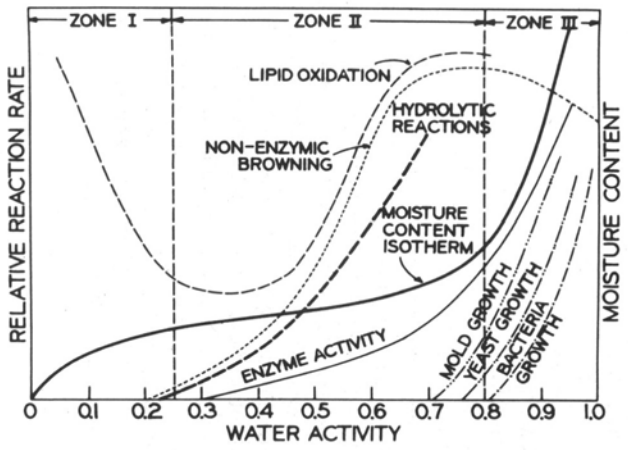

Figure 2.2. Effect of water activity on chemical and microbiological reactivity (from Fennema 1976).

Most chemical reactions as well as microbiological reactivity may be affected by the water activity of the product. The drying process of fish meal production removes most of the available water from the meal. But the remained moisture is very critical and often measured as water activity. Water activity is defined as the partial pressure of water in a sample relative to the vapor pressure of pure water at the same temperature. Specific types of reactions are generally influenced by the water activity of the product (Figure 2.2). The water activity of capelin meal generally lies between 0.35 and 0.45. Consequently, there is no risk of microbiological activity in the water activity range of capelin meal and enzyme activity and hydrolytic reactions are very limited. However, although lipid oxidation is at its lowest at that range, it is the most important reaction in this type of product.

2.4. Lipid oxidation

The problem of oxidative deterioration is of greatest economic importance in the production of fish meal. Oxidation of unsaturated lipids not only produces offensive odors and flavors but can also decrease the nutritional quality and safety by the formation of secondary reaction products in the meal lipids after processing.

Lipid oxidation has been well documented (Frankel 1980, 1991) and proceeds through a free radical chain mechanism involving initiation, propagation and termination steps (Figure 2.3). Under most circumstances, the process referred to as autoxidation involves an initial extraction of a hydrogen (H) from a fatty acid to produce the free radical. It may then proceed through a variety of propagation reactions,

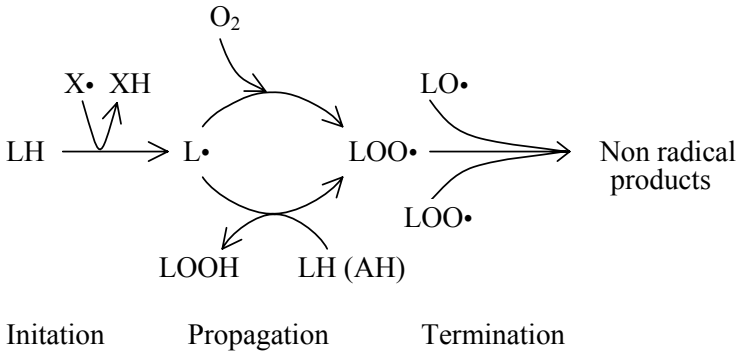

Figure 2.3. Schematic illustration of the lipid oxidation process.
LH = unsaturated lipid, X• = initiator, L• = alkyl radical, LO• = alkoxyl radical, LOO• = peroxy radical, LOOH = hydroperoxide, AH = antioxidant.

which produce further free radicals. When the free radical content becomes great enough, two free radicals may combine to terminate the free radical process. The oxidation sequences are not separated in time. In biological tissues such as fish muscle, other components such as proteins, amino acids, ascorbate, etc., can interact with these free radicals to terminate the reaction. When components other than lipids terminate the reaction, they are often referred to as antioxidants.

Lipid oxidation in fish is influenced by several catalytic systems for oxygen activation. Oxygen in its ground state has two unpaired electrons with parallel spin, giving it a triplet state. In contrast, most organic compounds have singlet ground states. They contain an even number of electrons arranged in pairs with opposite spin signs and no unpaired electrons. The reaction between oxygen and unsaturated lipid is prevented because of this spin restriction. To overcome the spin restriction between ground state oxygen and lipids, the reaction requires initiation (or initiator: X•) which may be the activation of ground state oxygen into singlet oxygen, superoxide radical, hydroxyl radical or peroxides or else the transformation of unsaturated lipids into lipid radicals.

Triplet ground state oxygen can be converted to singlet oxygen via photochemical reaction where a sensitizer (Sen) transfers light energy (hν) to oxygen via Type II pathway (Bradley and Min 1992);

$$^1\text{Sen} \xrightarrow{h\nu} {}^1\text{Sen}^* \xrightarrow{\text{Intersystem crossing}} {}^3\text{Sen}^* + {}^3\text{O}_2 \longrightarrow \text{Sen} + {}^1\text{O}_2 \qquad (1)$$

Natural pigments in foods, such as chlorophyll, riboflavin and protoporphyrin are known photosensitizers due to their conjugated double-bond system that easily absorbs visible light energy.

The superoxide anion ($O_2\bullet^-$) is formed in fish by enzymes and hydrogen peroxide is formed spontaneously or via enzyme-catalyzed dismutation of superoxide anion and microbial metabolism. Superoxide anion and hydrogen peroxide are not able to react directly with unsaturated fatty acids. However, in the presence of transition metal ion (Me) they act as precursors for hydroxyl radical (•OH), which readily reacts with unsaturated fatty acids;

$$O_2\bullet^- \longrightarrow H_2O_2 \xrightarrow{M^{2+}\ M^{3+} + OH^-} \bullet OH \qquad \text{(Haber-Weiss reaction)} \qquad (2)$$

Lipid hydroperoxides are formed in the propagation phase when lipid radicals react with oxygen. Lipid peroxides are taste- and odorless primary products of autoxidation. Pure hydroperoxides are relatively stable and most often rate limiting in the oxidation process, but transition metal ions (Me), such as iron and copper and haem compounds catalyze their decomposition both by oxidation and by reduction;

$$LOOH + Me^{n+} \longrightarrow LO\bullet + OH^- + Me^{n+1} \qquad \text{(Fenton reaction)} \qquad (3)$$

$$LOOH + Me^{n+1} \longrightarrow LOO\bullet + H^+ + Me^{n+} \qquad (4)$$

The decomposition of hydroperoxides involves formation of secondary lipid oxidation products. Secondary products of low-molecular-weight volatile compounds are responsible for rancid flavors, including wide range of carbonyl compounds and hydrocarbons, such as aldehydes, ketones and alcohols (Frankel 1982).

The fatty acid profile of the lipid influences the composition of the compounds produced during lipid oxidation. A wider range of aldehydes and other secondary products are produced from the more unsaturated fatty acids (Frankel 1980). Furthermore, the more unsaturated the fatty acids of the fish lipids, the more easily a hydrogen atom is abstracted and the oxidation process initiated.

Although other enzymes are involved in oxygen activation, the term enzymatic lipid oxidation is normally used to describe the action of lipoxygenases and cyclooxygenases, which catalyze oxidation of unsaturated fatty acids. Lipoxygenase activity has been identified in several tissues of fish, especially on the skin and gills

(Tsukunda 1972, Hsieh *et al*. 1988, Harris and Tall 1994) and they may therefore be involved in the overall lipid oxidation pool of capelin.

2.5. Antioxidants in fish

In biological systems the initiation of oxidation is balanced by the presence of natural antioxidants. Lipid-soluble antioxidants donate a hydrogen atom (H) to a fatty acid based free radical more readily than does an unoxidized fatty acid. Water-soluble antioxidants interacting between the aqueous and lipid phases can then reduce the lipid soluble antioxidant so they can continue to participate in these antioxidative reactions. The ability of an antioxidant to reduce another antioxidant or a lipid derived radical is determined by their reduction potentials (Buettner and Jurkiewicz 1996). In post mortem muscle tissue the ability to keep the antioxidants in the reduced state diminishes with time because of the loss of reducing compounds, the ability to stabilize lipid free radical is lost, and the lipids will eventually oxidize. The time that this process takes is reflected in the lag phase of lipid oxidation.

The inhibitors of lipid oxidation found naturally in fish have been reviewed (Hultin 1992, 1994 and Undeland 1997) and are summarized in Table 2.2. The inhibitors of lipid oxidation may directly or indirectly inhibit the initiation and propagation steps of lipid oxidation. Preventive inhibitors are those which interfere with the lipid oxidation initiation step, for example with various prooxidants. True antioxidants, also called chain breaking antioxidants interfere with the propagation step of lipid oxidation. The action of many inhibitors is highly concentration dependent, making them capable of prooxidant activity under certain conditions (Buettner and Jukiewicz 1996, Marcuse 1962, Porter 1993).

Preventive inhibitors are divided into primary and secondary inhibitors. Primary inhibitors remove active reduction products of oxygen or convert transition metals to inactive forms. Superoxide dismutase removes superoxide anion ($O_2\bullet^-$) while catalase and peroxidases, particularly glutathione peroxidase, remove hydrogen peroxide. Due to high reactivity of the hydroxyl radical (•OH), many components in high enough concentration can function as scavengers of •OH, by keeping it from the fatty acid molecules. Ferrous iron (Fe^{2+}) may be inactivated by chelation or by oxidation to its inactive ferric form (Fe^{3+}).

Table 2.2. Important inhibitors of lipid oxidation (adapted from Hultin 1992).

Preventive inhibitors	True antioxidants
Superoxide dismutase	Tocopherols
Catalase	Ubiquinol
Peroxidases	Carotenoids
Ferroxidases	Ascorbate
•OH scavengers	Glutathione peroxidase
Nucleotides	
Amino Acids	
Peptides	
Organic acids	
Ascorbate	
Phospholipases, proteases	

Ceruloplasmin, a copper-containing enzyme (Symons and Gutteridge 1998) is an example of ferroxidase that is capable of that kind of oxidation:

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{\text{ferroxidase}} 4Fe^{3+} + 2H_2O \quad (5)$$

Important chelators of Fe^{2+} in the fish muscle are nucleotides, amino acids, peptides and organic acids (Hultin 1992).

Secondary initiation inhibitors can affect the production of the primary catalysts of lipid oxidation. Phospholipase-A and proteases are examples of secondary inhibitors that decrease the ability of assorted systems to stimulate oxidation (Hultin 1992).

Propagation inhibitors or true antioxidants (AH) interfere with the propagation step by reacting with the lipid peroxy (LOO•) and lipid alkoxyl radicals (LO•):

$$AH + LOO\bullet \rightarrow A + LOOH \quad (6)$$

$$AH + LO\bullet \rightarrow A + LOH \quad (7)$$

True antioxidants can be divided into lipid soluble and water-soluble antioxidants. Tocopherols are generally considered to be the major antioxidants found in fish tissue. Tocopherols are found in association with the highly unsaturated fatty acids in the interior of membranes with their oxidizable chromanol entity located near the surface. Each tocopherol can donate two electrons as a chain breaking antioxidant before it needs to be recycled.

Ubiquinol is another lipid soluble antioxidant found in fish. Ubiquinol is a reduced form of coenzyme Q and is located in the inner membrane of mitochondria and therefore it becomes especially concentrated in dark muscle tissue (Petillo *et al*. 1998).

Carotenoid pigments such as astaxanthin, canthaxanthin and β-carotene can also serve as antioxidants. Carotenoids are active quenchers of singlet oxygen and as radical scavengers. Carotenoids are most effective at relatively low oxygen pressure and may act either as antioxidants or prooxidants, depending on their concentration (Mortensen and Skibsted 2000).

Ascorbic acid is the most well known water-soluble antioxidant. It can inhibit oxidation as an electron donor, as a metal chelator, as a scavenger of active oxygen species and as a free radical scavenger (Undeland 1997). Its ability to regenerate α-tocopherol as an electron donor is notable. This phenomenon is often called synergism and is known for the reinforcing effect of multi-component stabilizer systems exhibiting a greater combined effect than the sum of their individual effects.

Glutathion peroxidase (GSH-px) is another propagation inhibitor in the aqueous phase of fish muscle that is located in the mitochondria and cytosol of skeletal muscle cells. GSH-px is a selenium-containing enzyme (Symons and Gutteridge 1998) that catalyses the reduction of lipid peroxides (LOOH) with reduced glutathione (GSH):

$$2GSH + LOOH \rightarrow LOH + H_2O + GSSG \quad (8).$$

2.6. Prooxidants in fish

Prooxidants or catalysts of lipid oxidation in fish are listed in Table 2.3. Iron is the principal transition metal in fish (Undeland 1997), however, copper undergoes similar redox cycling, and both metals can therefore initiate lipid oxidation in several ways (Figure 2.3 and equations 2-4).

In fish tissue superoxide and ascorbate are the two reducing systems most important for the action of transition metals (Hultin 1994). As reducing agents they are able to reduce catalytic metals such as Fe^{3+} and Cu^{2+} to the more damaging Fe^{2+} and Cu^{+}. In general low concentrations of ascorbate are required for prooxidant conditions, while high concentrations are needed for antioxidant conditions (Buettner and Jukiewicz 1996). Iron can also be reduced by enzyme systems associated with both mitochondria and microsomes. A large part of iron in fish muscle is bound in various heme proteins such as myoglobin, haemoglobin and cytochromes (Undeland 1997). Haem proteins with iron in the ferric state (Fe^{3+}) can interact with hydrogen peroxide and initiate lipid oxidation.

Table 2.3. Important prooxidants present in fish tissue (Undeland 1997).

Metals	Reducing systems	Heme proteins	Enzymes
Iron	Superoxide	Myoglobin	Lipoxygenase
Copper	Ascorbate	Hemoglobin	Cyclooxygenase
	Mitochondial systems	Cytochromes	Peroxidases
	Microsomal systems		

The enzyme lipoxygenase or cycloxygenase can directly initiate lipid oxidation in fish tissue. Lipoxygenase and cycloxygenase are specific in catalyzing the oxidation of fatty acid containing 1,4-cis-pentadiene and arachidonic acid (C20:6), respectively.

The peroxidase enzymes are also critical for initiation of lipid oxidation in fish (Undeland 1997). The primary, biological function of peroxidase enzymes is to oxidize a variety of hydrogen donors at the expense of peroxide or molecular oxygen in oxidasic reactions.

2.7. Oxidative stability of capelin raw material

Capelin is a very variable raw material in terms of size, redfeed level in the stomach and particularly fat content. The fat content of capelin undergoes a considerable seasonal variation (Figure 2.4), which has been documented for harvested Icelandic capelin (Vilhjálmsson 1994). The Icelandic capelin that spawns in March reaches a minimum fat content at the end of this period. The fish move offshore and feed in summer and autumn. By October the fat content can be as high as 20 per cent. During winter this drops slowly and, as the gonads develop in the spring the fat content declines steadily around 1% per week to almost depletion (2-4%) at the time of spawning.

Large seasonal variations have also been found in Newfoundland capelin fat content (Eaton *et al*. 1975). The fat content of fat-depleted, spawning stocks in the summer was as low as about 1 per cent, whereas the fattening capelin in the fall had over 18 per cent fat. The iodine values of the commercial fish oils was 96-112 in the fat-depleted spawning fish, but the fall fattening fish had an iodine value range of 118-128. Researches have shown remarkably good shelf life of both frozen spawning (Jangaard 1974, Shaw and Botta 1977) and nonspawning (Botta *et al.* 1983) Newfoundland capelin, as judged by organoleptic assessment. Shaw and Botta (1977) found no indication of rancidity by sensory analysis and the characteristic "capelin odor" was present throughout the experiment during 2 years of storage at -23 °C.

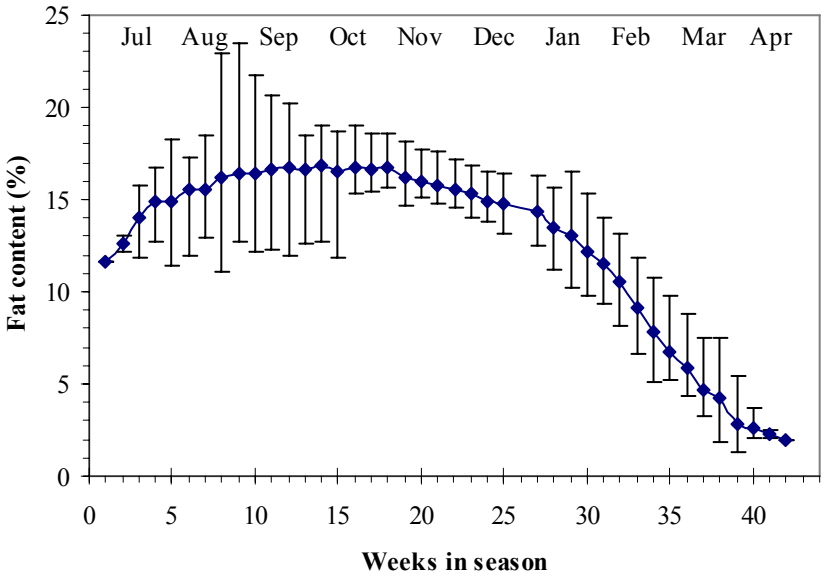

Figure 2.4. Changes in fat content of capelin (Vilhjálmsson 1994).

Same authors concluded that capelin appeared to have a frozen storage shelf life approximately 4 times longer than other small pelagic species.

Jangaard (1974) speculated on the reason for this unexpected good stability of frozen capelin. Good stability could be due partly to the low iodine value of the depot fat, although herring, which is susceptible to rancidity in frozen storage, has in some cases as low iodine value as capelin. The commercial pressing of oil from capelin is more difficult than from herring, which may suggest a different distribution of depot fat globules in the muscle tissue of capelin. Finally, it could also be possible that natural antioxidants are present in capelin.

2.8. Oxidative stability of fish meal

Meal from oily fish may contain over 10% fat with a relatively high content of unsaturated fatty acids. Unsaturated fatty acids are more subject to oxidation because of their high content of double bounds. They react exothermally with oxygen, resulting in the production of varying levels of hydroperoxides, aldehydes, ketones and other low molecular weight compounds as well as high molecular weight polymerized compounds which are insoluble in diethyl ether and hexane. Rancidity in fish meal could formerly be a considerable problem when the rate of oxidation was high enough to cause spontaneous heating of the fish meal during shipping and transportation (Ólafsson 1953, Waissbluth *et al.* 1971). Other effects of lipid

oxidation in fish meal are loss of nutritional value as seen in decreased energy value, protein denaturation and formation of toxic reactants that can result decreased animal growth. This reduction in energy value of fish meal can be avoided by the use of antioxidants, which stabilize the fat fraction (Opstvedt 1975, Barlow and Pike 1977).

Stability of different fish meal is very variable. According to Barlow and Pike (1977), the fat content of fish meal determined by diethyl ether extraction, decreases by 1 to 6 per cent units during prolonged storage due to oxidation of the fatty acids. Experiment on anchovy meal showed that the fat content decreased from 10.4 to 6.4% during four months of storage (Romoser *et al.* 1968). Another experiment showed a 2.2% decease in the fat content of herring meal during eight months of storage (Opstvedt 1969). Icelandic fish meal producers have also noticed some decrease in the lipid content of herring meal during storage (Ólafsson 1953). Measurements on ether extractable lipids in Icelandic capelin meal during a whole fishing season (February to April) showed very little decrease in lipid content with storage (Kristinsson 1975), or less than 0.5% in up to 6 months storage. There are no records of spontaneous heating of fish meal from capelin and it is generally considered very stable, compared to other fish meal types.

Table 2.4. Fatty acid content in freshly manufactured meal made form different species of fish (adapted from Opstvedt 1971).

Fatty acid (%)	Herring	Mackerel	Capelin	Anchovy[a]
C14:0	5.5	6.1	4.8	7.4
C16:0	16.3	15.9	16.6	22.8
C18:0	2.0	4.1	1.6	4.2
Total saturated	23.8	26.1	23.0	34.4
C16:1	4.2	4.9	6.9	7.2
C18:1	14.7	13.8	17.6	13.1
C20:1	12.9	10.1	8.9	1.3
C22:1	16.9	14.7	7.1	0.7
C24:1	1.2	1.1	0.7	0.4
Total monoene	49.9	44.6	41.2	22.7
C18:2	1.2	1.6	1.6	1.0
C18:4	1.2	2.8	1.9	2.4
C20:5	6.3	5.8	10.4	16.3
C22:5	0.5	0.7	0.6	1.7
C22:6	13.4	12.1	16.8	13.5
Total polyene	22.6	23.0	31.3	34.9
C20:5+ C22:5+ C22:6	20.2	18.6	27.8	31.5
Polyene Index[b]	1.21	1.13	1.64	1.31

[a] Commercial stabilized meal. [b] Polyene index (PI): (C20:5 + C22:6 / C16:0)

Table 2.5. Effect of season on the content of polyenoic fatty acid in freshly produced fish meal (Opstvedt 1971).

	C20:5 + C22:5 + C22:6 fatty acids in meal lipids (%)	
	Lean fish	Fat fish
Herring	18.8	21.6
Mackerel	16.2	21.1
Capelin	29.1	26.4

Surprisingly capelin fish meal lipids are highly unsaturated. Opstvedt (1971) has compiled data originated from Nygaard (1970), on the fatty acid composition of the residual lipids in a freshly manufactured meal made from herring, mackerel, capelin and anchovy (Table 2.4). Experimental conditions such as fishing season and sampling were not specified. Comparison of the most unsaturated fatty acids shows that the samples of meal from herring and mackerel contained similar concentrations of the C20:5, C22:5 and C22:6 fatty acids. Somewhat higher levels were found in meal from capelin and the meal made from anchovy had the highest content of these fatty acids. The highest polyene index (PI) was found in meal from capelin.

The content of the C20:5, C22:5 and C22:6 fatty acids in the meal lipids also seems to be dependent on the harvesting season. In samples of fresh meal produced from herring and mackerel with high and low fat content, the level of the C20:5, C22:5 and C22:6 fatty acids was highest in the fish with the highest fat content. This was reverse for capelin where the level of these highly unsaturated fatty acids was found highest in meal made from capelin with low fat content (Table 2.5).

According to Opstvedt (1971) a drastic change in the content of the C20:5, C22:5 and C22:6 fatty acids take place during storage of herring meal. From an original total content of 19.8%, the content was reduced to 1.0% after one year of storage. Still, by retarding the oxidation of the meal lipids by the addition of an antioxidant, the content of the C20:5, C22:5 and C22:6 fatty acids remained relatively unchanged.

This paradox of unsaturated lipids associated with high stability could imply a high content of natural antioxidants in capelin. All the same the capelin is a very variable raw material where spawning and changes in feeding behavior causes a large variation in fat content as well as chemical composition of lipids. Variations in natural antioxidants associated with seasonal variation in lipid content could be expected, and have been reported in fish. In a study with commercial sole fillets, the natural content

of tocopherol showed an inverse relationship to lipid content, being as high as 500 to 600 mg/kg lipid in the lean period of the year, and as low as 100 to 200 mg/kg lipid when lipid was at its highest (Ackman 1974). Rancidity during frozen storage was reported to develop more rapidly in fish caught in the summer months during the post-spawning feeding period with high lipid accumulation. Similar relationship has been postulated in fish meal, although Dreosti (1980) did not find a close relationship between spontaneous heating of fish meal and time of season, the heating rates were generally greater, for example, up to twice as fast during the peak of the season when the fish was in good condition than in the lean season.

3. MATERIALS AND METHODS

3.1. Experimental approach

This study was designed to reveal the factors affecting the stability of capelin meal and clarify seasonal variation in chemical composition of capelin meal. Capelin as raw material for fish meal processing was also included as a criterion for seasonal variation in capelin meal (Figure 3.1).

The study was designed to cover the four seasonal spans of capelin. The summer capelin is found in deep waters, north of Iceland. Summer capelin is rather small and its shelf life is very limited due to heavily feeding on redfeed. Autumn capelin has the highest fat content and it is usually found east of Iceland where the mature capelin starts its spawning route. Winter capelin is usually of prime quality and it is caught on the continental shelf south of Iceland. Spring capelin is the fat depleted spawning capelin caught inshore south-west and west of Iceland.

The quality of the raw material is essential for the production of high quality fish meal. The post mortem autolysis of capelin is very fast, especially during heavy feeding and the time from catching to landing and production should be as short as possible as small pelagic fishes are never bled and gutted. The effect of delayed landing on the stability of the raw material was therefore included in this study.

Compositional data were obtained for each seasonal sample of capelin including proximate analysis (protein, water, lipid, salt and ash), lipids (fatty acid pattern, polyene index and iodine value), antioxidants (tocopherol and astaxanthin).

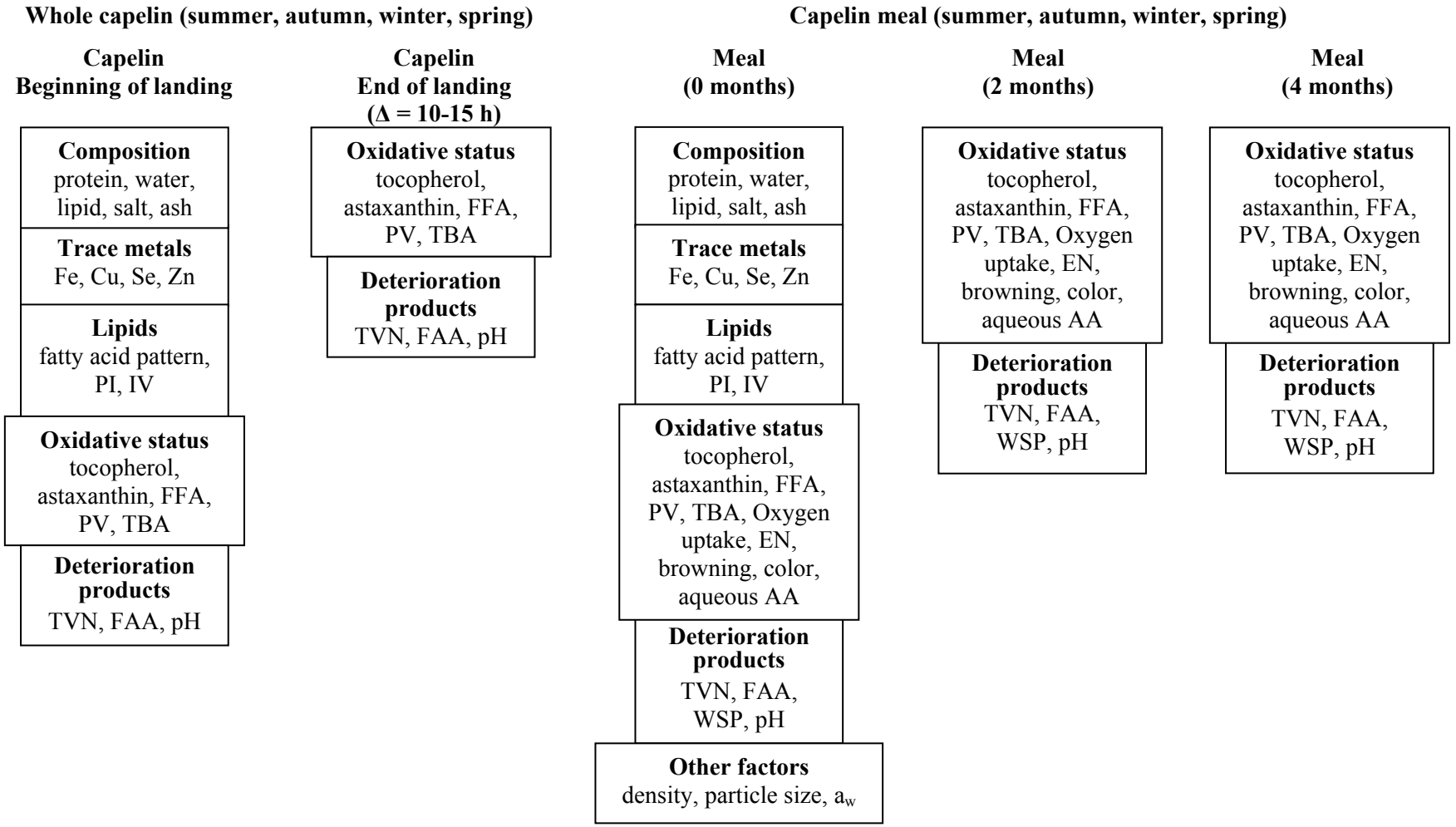

Figure 3.1. Experimental design of the study.
Abbreviations: PI = polyene index, IV = iodine value, FFA = free fatty acids, PV = peroxide value, TBA = thiobarbituric acid value, TVN = total volatile nitrogen, FAA = free amino acids, EN = electronic nose volatiles, WSP = water soluble protein, AA = antioxidant activity.

The trace metals iron and copper were measured as possible prooxidants, but selenium and zinc as possible antioxidants. In order to evaluate the oxidative status of the raw material, measurements of free fatty acids, peroxide value and TBA were performed, as well as measurements on deterioration products such as TVN, free amino acids and pH measurements.

The effect of fish meal processing on chemical composition and stability are numerous and for comparison, the same compositional data were obtained for the capelin meal made from each batch of raw capelin.

The effect of storage on capelin meal was included in the study, to reveal the factors affecting the stability of capelin meal. Changes in endogenous natural antioxidants and the effect of oxidation were monitored by measuring primary oxidation (peroxide value and oxygen uptake), secondary oxidation products (TBA) and volatile spoilage products (electronic nose measurements). Browning, changes in color, TVN and water-soluble nitrogen, as well as free amino acids were measured as possible indicators of tertiary oxidation. Finally antioxidative/prooxidative activity of water solubles in the capelin meal were measured by a coupled oxidation of β-carotene and linoleic acid. Other possible factors affecting the overall stability of capelin meal, such as density and particle size of the meal were also measured, as well as pH and water activity.

3.2. Samples

3.2.1. Season and geography

Capelin (*Mallotus villosus*) from the same migration was caught in net at four different fishing grounds and seasons. Summer capelin was caught on the 11th of July 1998, NNW of Iceland. Autumn capelin was caught on the 13th of November, NNE of Iceland. Winter capelin was caught on the 5th of February 1999, SSE of Iceland. Spring capelin was caught on the 26th of March, SV of Iceland.

3.2.2. Handling and storage of raw materials

After harvesting the whole, ungutted fish was stored without addition of preservatives in the hold of the ship. The raw material was landed and processed within 24 hours from catching. Samples (3 kg) were taken from the raw material two times during landing, at the beginning and at the end, with approximately 10-15 hours interval. The

samples were packed in thick PE plastic bags and then placed into cardboard boxes prior to freezing and storage at -23 ± 2 ° C. The samples were taken from the frozen storage and transported within 1 h to the laboratory, where they were stored at -25 ± 1 °C. The samples were prepared and analyzed within one week from freezing. Samples were thawed for 2-3 hours at room temperature, or just enough to separate the fish. One pooled sample of 120 indiscriminate capelins was made and minced finely, while still half-frozen, in a mixer (Braun electronic type 4265, Germany).

3.2.3. Handling and storage of capelin meal

Summer, autumn and winter meal was processed at the SR-mjöl hf fish meal factory in Seyðisfjörður. The spring meal was processed at the SR-mjöl hf fish meal factory in Helguvík. Both factories apply indirect hot air drying, where the temperature of the meal is never higher than 65-70 °C. One pooled sample (6 kg) of fish meal was taken from each batch described in 3.2.1. 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 2 L, tight shutting bins of robust PE-plastic for storage in the dark at 10 °C.

3.3. Chemicals

n-Hexane, isopropanol, methanol, acetonitrile, petrolieum ether and diethyl ether were HPLC grade (Rathburn Chemicals, Walkerburn, Scotland). The amino acids L-glutamine, L-glycine, alanine, L-arginine HCl, L-aspargine, L-aspartic acid, L-glutamic acid, L-histidine HCl L-isoleucine, L-leucine, L-lysine HCl, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tyrosine and L-valine were from Sigma Kit No. LAA-21 (Sigma Chemical Company St. Louis, MO, USA). Butylated hydroxy toluene (BHT), polyoxyethylene sorbitan monopalmitate (TWEEN 40), ergocalciferol, all trans retinol acetate, dl-α-tocopherol acetate, ethylenediaminetetraacetic acid (EDTA) and and the amino acids L-norvaline and taurine were also obtained from Sigma. β-Carotene, linolic acid, silica gel 60, potassium hydroxide, potassium chloride, iron(II) sulfate heptahydrate GR, iron powder, barium chloride dihydrate, sodium sulfate- anhydrous, acetic acid (100%), hydrochloric acid, 2-thiobarbituric acid, 1.1.3.3-tetraethoxypropane, ascorbic acid, sea sand and titrisol buffer were either garantied reagents for analysis or extra pure (Merck, Darmstadt, Germany). Propyl gallate was obtained from Aldrich (Aldrich

Chemical Co., Inc., Gillingham Dorset, England). Ethanol was (96% V/V) was obtained from Icelandic Pharmaceuticals Ltd. (Reykjavik, Iceland).

3.4. Proximate analysis

3.4.1 Water

Water content was determined by drying the samples (ISO 1983). Fish meal was mixed well and about 5 g weighed accurately in a preweighted metal dish with a lid. Capelin mince was mixed and about 5 g weighed accurately into a preweighted porcelain bowl, containing sea sand and a glass rod. The glass rod was used to mix the sample and the sand to increase the surface prior to drying. Both fish meal and capelin were dried at 102-104 °C for 4 h. After drying the metal dish was closed with the lid and both samples were allowed to cool in a dessicator, prior to weighing. The water content corresponds to the weight loss and was given as percentage of the initial sample weight.

3.4.2. Fat

Fat content was determined by a slightly modified Soxhlet method (AOCS 1998a). The dried samples from the water content determination were quantitatively transferred into cellulose extraction thimbles and placed into a Soxhlet holder. The lipid was extracted from the sample with petroleum ether (Bp. 30-40 °C) for 6 h on a Soxhlet distillation apparatus. Afterwards the ether was evaporated off and the flask dried in a heating oven at 102-105 °C for 15-30 min. The weight increase of the flask corresponds to the fat content in the sample. The fat content was given as percentage of the initial sample weight.

3.4.3. Protein

Crude protein content was determined using a Kjeldahl method (ISO 1979) in an automated distillation apparatus. About 0.5 g of fish meal or 2 g of minced capelin was accurately weighed and mixed with K_2SO_4 and a little of $CuSO_4$ as a catalyst and digested in Kjeldahl bottles with conc. sulphuric acid for approx. 2 h. The liberated ammonia was distilled into boric acid in a Kjeltec Auto sampler 1035/30 system and the acid was simultaneously titrated with diluted H_2SO_4. The crude protein content was calculated by multiplying the nitrogen content by 6.25 and the results given as percentage of initial sample weight.

3.4.4. Salt

Salt content was determined by a modified method of Volhard (AOAC 1990b). The salt content was calculated as percentage of the sample.

3.4.4.1 Capelin. About 5 g of minced capelin was accurately weighed into a centrifuge flask and 200 mL of distilled water added. The flask was shaken for 45 min and an aliquot of the supernatant used for the Volhard titration.

3.4.4.2. Capelin meal. About 0.5 g of meal was weighed accurately into an extraction flask. Excess of $AgNO_3$ solution was added along with 20 mL of HNO_3. The solution was boiled until the meal was dissolved (15 min.), then cooled and 25 mL of distilled water added, prior to Volhard titration.

3.4.5. Ash

Ash content was determined by carbonization and heating of samples (ISO 1978). About 2 g of fish meal or minced capelin was accurately weighed into a preweighted incineration dish and heated until carbonized. The sample was then heated in muffle furnace at 550 °C for 3 h. The weight increase corresponds to the ash content in the sample. The ash content was given as percentage of the initial sample weight.

3.5. Trace metals

The content of the trace metals iron, copper, selenium and zinc were determined by atomic absorption (Auðunsson 1999). The samples were digested in quartz decomposition tubes (MeAna-Konsult, Uppsala, Sweden) with a temperature program of 80-180 °C. Iron, copper and zinc were analyzed by flame atomic absorption (PE 403 and PE 1100B) with D_2-background correction. Selenium was further ashed with $Mg(NO_3)$ and Se(VI) reduced to Se(IV) by hydrochloric acid prior to determination by hydride generation atomic absorption. The metal content was expressed in terms of mg/kg sample.

3.6. Lipids

3.6.1. Extraction of lipids

Lipids were extracted by chloroform/methanol extraction system based on the method of by Bligh and Dyer (1959) as modified by Hanson and Olley (1963), but with some alterations. To hinder oxidation of the lipids, all samples were treated in ice bath,

BHT (butylated hydroxytoluene) (50-100 mg/L) was added to all solvents and care was taken to eliminate as much light as possible. The extract was centrifuged at 1000 x *g* for 20 min at 0-5 °C in a refrigerated centrifuge (Sorvall Superspeed RC5-B, DuPont Instruments, Stockholm, Sweden). The lower layer containing the chloroform with the lipids was filtered by vacuum through a glass filter (Watman GH/D, Springfield Mill, England).

For determination of the lipid concentration in the extract, portion of the chloroform layer was pipetted into a preweighted beaker and the chloroform was evaporated to dryness at 60 °C for 30 min. Afterwards the beaker was cooled inside a dessicator and weighed. The remaining weight was taken as the lipid content in mg/mL.

3.6.2. Fatty acid analysis

Fatty acid analysis was performed on the lipid extracts obtained from 3.6.1. Portion of the chloroform lipid extract (1-5 mL) was evaporated to dryness under a stream of nitrogen gas. Saponification, methylation and gas chromatography were applied for the analysis(AOCS 1998c). The fatty acid methyl esters were separated and quantified by gas chromatography (GC) and FID detection. The GC system consisted of a Hewlett Packard 5890 Series II Gas Chromatograph and Omegawax 320 Capillary Column (30m × 0.32mm × 0.25µm) (Supelco). Injector and detector temperatures were 260° C and 270° C respectively. The oven was programmed as follows; 160° C for 2 min, then raised to 210° C at 3° C/min and held at this temperature for 10 min. Results were expressed as percentage of total fatty acids in the lipid.

3.6.3. Polyene index

The polyene index (PI) was calculated from fatty acid analysis (Lubis and Buckle 1990): PI = (20:5 + 22:6 / 16:0).

3.6.4. Iodine value

Iodine value was measured in the lipids extracts obtained from 3.6.1. Portion of the chloroform lipid extract (10 mL) was taken and the iodine value determined by the method of Hanus (US Pharmacopea 1989). Iodine value is without unit, but it is defined as the concentration of iodine in g, which is bound by 100 g of lipid sample.

3.7. Antioxidants

3.7.1. Astaxanthin

Astaxanthin was measured in the lipids extracts obtained from 3.6.1. Portion of the chloroform lipid extract (25-50 mL) was evaporated to dryness (37 °C, vacuum). Five mL portions of absolute alcohol and two times n-hexane were subsequently added to the sample and evaporated to dryness between solvent additions. The residue was finally dissolved in 5-10 mL n-hexane. In order to prevent oxidation of samples, BHT (butylated hydroxytoluene) (50-100 mg/L) was added to all solvents and care was taken to eliminate light as much as possible. Astaxanthin was separated by column chromatography and determined using spectrophotometer (CE 2292, Series 2 292, Cecil Instruments Ltd., Milton, Cambridge, England) at 472 nm in n-hexane (Lambertsen and Brækkan 1971). The astaxanthin content was given as astaxanthin in terms of mg/kg lipid and as free astaxanthin, diester and monoester in terms of percentage of the (total) astaxanthin.

3.7.2. α-Tocopherol and retinol

The lipid soluble vitamin α-tocopherol and retinol (vitamin A) were determined in the lipids extracts obtained from 3.6.1. Portion of the chloroform lipid extract (50-100 mL) was evaporated on a rotary evaporator (Büchi, Switzerland) at 37 °C under vacuum and finally the residue was evaporated to dryness under a stream of nitrogen gas. The samples were saponified, separated and quantified by high-pressure liquid chromatography (HPLC) based on assay for vitamin-D_3 (European Pharmacopoeia 2000) using 0.5 g sample size and corresponding scaling down of reagents. The isocratic reversed phase HPLC system used asetonitrile/ methanol/ distilled water (100:95:7) at a flow rate of 1.3 mL /min. The UV detection for both vitamins was carried out at 275 nm, using vitamin D as internal standard. The sample was dissolved in 200 µL mobile phase and 20 µL injected. The HPLC system consisted of a Hewlett Packard 1050 series manual injector, isocratic pump and multiple wavelength detector. Data handling was by HP 3365 Chemstation. The column (250 x 4 mm) was packed with 5 µm Spherisorb ODS 2 material. The levels of α-tocopherol were expressed as mg/kg lipid and the levels of retinol as vitamin A in mg/kg lipid.

3.8. Oxidative status

3.8.1. Free fatty acids

Free fatty acids (FFA) were determined in the lipids extracts obtained from 3.6.1. Portion of the chloroform lipid extract (25 -50 mL) was evaporated to dryness (37 °C, vacuum). The dried sample was solubilized in alcohol/diethyl ether (1:1) and titrated with diluted NaOH. FFA was calculated as oleic acid percentage in the lipid (AOCS 1998b).

3.8.2. Peroxide value

Peroxide value was determined in the lipids extracts obtained from 3.6.1. One mL of the chloroform lipid extract was evaporated to dryness under a stream of nitrogen gas. Peroxide value (PV) was determined spectrophotometrically, using ferric thiocyanate method (IDF 1991). The peroxide value was calculated as meq/kg lipid.

3.8.3. TBA

TBA (thiobarbituric acid) is a measure of secondary oxidation products in fats. It is mainly malonaldehyde that reacts with thiobarbituric acid. Unlike many measurements on oxidation of fats, TBA does not require lipid extraction. TBA was measured by slightly modified steam distillation method (Tarladgis *et al*. 1960), in which the sample size was reduced to 5 g and antioxidant (5 mL of a 0.5% propyl gallate and ethylene diamine tetraacetic acid in water) was added to the sample during blending. Results were expressed in terms of mg malonaldehyde (MA)/kg tissue.

3.9. Deterioration products

3.9.2. pH

The pH was measured at room temperature, with an Ag/AgCl combination electrode connected to a pH meter, model PHM80 (Radiometer, Copenhagen). The pH of capelin was determined after mixing even parts of minced capelin and distilled water on a magnetic stirrer. The pH of capelin fish meal was determined in the water phase from the water soluble protein determination (2.9.1).

3.9.2. TVN-total volatile nitrogen

TVN (total volatile nitrogen) was determined by steam distillation (AOAC 1990a). About 10 g of capelin or 5 g of capelin meal were weighed into a distillation flask

along with MgO and distilled in a Struer distillation unit. The distillate was gathered into boric acid and titrated with diluted H_2SO_4. Results were expressed as mg N/100 g sample.

3.9.3. Free amino acids

Free amino acids (FAA) were separated and quantified by high-performance liquid chromatography (HPLC) consisting of Hewlett Packard (HP) 1050 series (gradient) pumping system, HP autosampler, Varian 9070 fluorescence detector, Croco-cil column heater and HP Chemstation data handling system. The column (150 x 4.6 mm) was packed with 3 μm spherisorb ODS-2 material (Hichrom, Reading UK) and suitable guard columns of same packing material were used. FAA were extracted by hydrochloric acid (0.1 M), using norvaline as an internal standard. After homogenization for 1 min, using an Ultra-Turrax homogenizer (type T25, IKA Werke, Staufen, Germany), the samples were centrifuged at 26900 x *g* for 20 minutes. After dilution of the supernatant with distilled water (1:25) the sample (2.5 μL) was derivatized with 5μL of OPA (o-phthaldialdehyde) in the autosampler. The OPA reagent was made of 25 mg OPA in 0.5 mL methanol, with 0.05 mL mercaptoethanol added and then diluted to 5.0 mL with borate-buffer. The borate buffer was made of 24.72 g boric acid and 21 g KOH, diluted to 1.0 L with distilled water and adjusted to pH 10.0 with 6 M KOH. The FAA-derivatives were separated by reversed phase chromatography using binary gradient elution of solvents A and B. Solvent A consisted of 0.45 L methanol, 0.05 L tetrahydrofuran and 4.50 L of 0.1 M acetate-buffer (pH 7.0 with glacial acetic acid). Then 0.35 L of methanol were added to 4.65 L of this mixture (7% dilution). Solvent B consisted of methanol. The flow rate was 1 mL /min. The fluorescence spectrophotometer was set at excitation wavelength 338 nm and emission wavelength 456 nm. Free amino acids (FAA) were expressed in terms of mg/kg sample and as A/B, the proportion of the acidic amino acids (aspartic acid, glutamic acid, serine and threonine) against the basic amino acids (histidine, arginine and lysine).

3.10. Keeping qualities of meal

3.10.1. Electronic nose

The electronic nose "FreshSense", developed by the Icelandic Fisheries Laboratories and Element Sensor Systems (Saudarkrokur, Iceland) was used to monitor the headspace of the meal. The instrument consists of a glass container (5.2 L) closed with a metal sensor box and a PC computer running a measurement program. The sensor box contains five different electrochemical gas sensors (Dräger, Germany: CO, H_2S, NO and SO_2; City Technology, Portsmouth, Britain: NH_3A7AM) and a temperature sensor. Electronics, A/D-converter and microprocessor to read the measurements and send them to the PC are also in the box. A fan is positioned in the container to ensure gas circulation (Ólafsdóttir *et al*. 1997b).

In each case 200 grams of sample, generally at a temperature around 10 °C, were weighed onto a plastic plate and placed in the sampling container. The container was closed with the sensor box and the measurement program run for 20 minutes. The sensor values reported (current; nA) are calculated by subtracting the average response of the last three minutes before measuring (baseline) from the average response of the last half minute of the program.

3.10.2. Oxygen uptake

The rate of oxygen uptake in fish meal was measured manometrically (Thorisson *et al.* 1992) in a Warburg apparatus, series III (Townson and Mercer Ltd., Runcorn, England). Samples (10 g) were weighed into conical flasks (100 mL) and the oxygen uptake was measured at 30 ± 0.1 °C. Calculations on oxygen uptake were according to Umbreit and others (1972). Weight of lipid in the sample was based on the lipid content determination from 3.6.1. Results were expressed as oxygen uptake in the lipid phase (µmol/ g oil) during the first day of measurement.

3.10.3. Brown pigments

Browning of fish meal during storage was measued as acetic acid soluble colour, described by Maruf (1990). Glacial acetic acid (25 mL) was added to about 1.0 g of fish meal and shaken for 15 min in a Burrell Wrist Action Shaker (Burrell Corporation, Pittsburg, PA, USA). After settling, the mixture was filtered and the

absorbance measured at 400 nm against glacial acetic acid. Results were expressed as absorbance in 1 mL /g fish meal.

3.10.4. Water soluble protein

WSP in capelin meal was measured in water extracts of the meal samples (Crooker *et al*. 1978) and protein determination. About 5 g of sample was weighed accurately and shaken with 100 mL of distilled water at ambient temperature for 3 h. The suspension was then filtered through Watman 2^V filter paper and the nitrogen measured in the filtrate according to Kjeldahl method (3.4.3). Water-soluble protein was expressed in terms of percentage of the meal protein content.

3.10.5. β-Carotene bleaching

Evaluation of antioxidant activity of water extracts from fish meal was based on coupled oxidation of β -carotene and linoleic acid as developed by Marco (1968) and modified by Taga and others (1984). Fish meal water extracts were obtained from the water-soluble protein determination (2.8.4). β -Carotene was dissolved in chloroform and diluted to workable absorbance in the region of 0.7 and 0.8 at 470 nm. Portion of the β -carotene solution (~1 mL) was pipetted into a brown boiling flask along with 20 mg of linoleic acid and 200 mg of Tween. The chloroform was removed under a stream of nitrogen gas and the remains dissolved in 50 mL of oxygenated distilled water. Portions (2.5 mL) of that solution were pipetted into disposable cuvettes and 0.1 mL of the sample solution added. A blank sample was prepared using distilled water instead of sample solution. Zero reading of blank and samples was measured immediately at 470 nm. The cuvettes were placed in 50 °C water bath and the absorbance measured at regular intervals (15 to 60 min) for about 6 h, or until the β -carotene was bleached. The aqueous antioxidant activity of sample (AA) was defined as the absorbance of sample subtracted the absorbance of the blank at 45 min, divided by the protein content of the extract. The color difference between sample and blank was corrected for by subtracting the initial absorbance difference from the sample absorbance.

3.11. Physical properties of meal

3.11.1 Color

Color of the fish 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. Equal distances in the Lab* system approximately equal perceived color differences. L* is the lightness variable (L* = 100 is white and L* = 0 is black), a* and b* are the chromaticity coordinates, +a* stands for red and -a* stands for green, +b* stands for yellow and -b* stands for blue. For evaluation of change in color by time, the meal samples were measured fresh and then again after two and four months of storage. The meal was poured into a Petri disk (60 mm in diameter x 10 mm deep) and the surface was made even with a ruler. The chromameter head was placed straight on the sample in the middle of the disk and the color was measured three times, turning the chromameter head 120 ° between measurements. This was repeated twice. Results were given as L*, a* and b* values and also as total change in initial color, defined by:

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

3.11.2. Water activity

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

3.11.3. Density

Density of the capelin meal was measured according to internal analytical method of EWOS AQUA A.S.

3.11.3.1. Bulk density. The fish meal was carefully transferred into a preweighted 100 mL volumetric cylinder of plastic. The cylinder was filled to 100 mL and weighted. Bulk density was expressed as the weight of carefully transferred sample divided by volume in g/mL.

3.11.3.2. Packing density. The same cylinder with same sample was packed by hitting the cylinder in the table about 50 times with the hand on the top of the cylinder. Then

the volume in mL was measured. Packing density was expressed as the weight of sample divided by volume of packed sample in g/mL.

3.11.4. Particle size

The distribution of the capelin meal particle size was determined by a slightly modified sieving method (AOAC 1990c). Well mixed capelin meal sample was placed on top of nested set of sieves, No. 30, 50 and 70 and fixed in a sieve shaker (Fisher_Scientific Co, Pittsburgh) and shaken for 5 min. The weight of sieve fraction was calculated. Particle size was expressed as percentage of each sieve fraction.

3.12. Data handling

3.12.1. Expression of results

Lipid soluble compounds which were analyzed in the lipid extracts were expressed on a lipid basis and not on tissue basis. This is a more conventional way of expressing results and helps to avoid the risk of hiding important differences in the oxidation rate between samples containing different levels of fat. Compounds that were not measured in the lipid extract, but in the tissue (or meal) were expressed on tissue basis.

3.12.2. Statistics

Calculations of statistical variations were based on a pooled sample of fish or meal. To calculate the sample variation, a mean value of each sample preparation was used. Three sample preparations were done on all samples ($n = 3$) and standard deviation (SD) calculated.

Analysis of variance was calculated and Tukey comparison method used to find if significant difference ($p \leq 0.05$) was between sample groups. The software program NCSS (Number Cruncher Statistical System) 2000 (NCSS Statistical Software, Kaysville, Utah, USA) was applied for statistical calculations.

3.12.3. Multivariate data analysis

Number of analytical and instrumental methods were applied in this study since it was assumed that stability of capelin meal can not be determined by any single method. The combined data were analyzed by multivariate data analysis. The advantages of applying multivariate data analysis in experiments with many variables are that it is

possible to extract and display the main relevant and reliable information from large amount data. The data was evaluated by principal component analysis (PCA), where, many parameters can be represented in few principal components (PC) and the covariance between different measurements visualized. Multivariate regression analysis were made by partial least squares regression, PLS1 and PLS2, depending on the number of Y-variables. All variables were standardized by 1/ standard deviation and full cross-validation was employed. The software program Unscrambler 7.5 (Camo A/S, Oslo, Norway) was used for multivariate data analysis.

4. RESULTS AND DISCUSSION

4.1. Capelin

4.1.1 Seasonal variation in chemical composition of capelin

The chemical composition of capelin varied considerably with each season (Table 4.1). The lipid content declined from 13.9% in the autumn to 3.10% in the spring, with a consequent inverse relationship with the moisture contents. Iodine value, reflecting the unsaturation of the lipids, was highest 139 during the summer and declined to 118.4 in the spring.

The copper content of capelin was highest in the summer, 2.10 mg/kg but lowest in the spring, 0.45 mg/kg. Iron content was on the other hand highest in the winter, 14.9 mg/kg but lowest in the spring, 7.8 mg/kg. Selenium and zinc levels were also highest in the winter, 0.39 mg/kg for selenium and 16.6 mg/kg for zinc, but at similar levels at other seasons of about 0.33 mg/kg for the selenium and 14-15 mg/kg for the zinc. Seasonal variation in the metal content of whole capelin was as the lipid content most likely due to changes associated with feeding and spawning of the capelin. Accordingly, all metals, except copper, tended to increase from summer to winter along with decrease in lipid content, but decreased during spawning. This was in agreement with the results of Auðunsson (1999), who found increase in the proportion of selenium, iron and zinc concentrations as the fat content of the liver decreased.

All these metals can play a role as cofactors for enzymes such as SOD, catalase and GSH-peroxidase (Deshpande *et al.* 1996), however, iron and copper are also involved in free-radical chain propagation, especially in the membrane lipids.

Table 4.1. Chemical composition of whole capelin with season.

Compound[a]	Summer	Autumn	Winter	Spring
Moisture (%)	74.9 ± 0.0^{a}	71.3 ± 0.0^{b}	76.1 ± 0.0^{c}	82.2 ± 0.0^{d}
Protein (%)	13.1 ± 0.0^{a}	12.9 ± 0.1^{a}	13.9 ± 0.1^{b}	12.5 ± 0.0^{c}
Salt (%)	0.6 ± 0.0^{a}	0.4 ± 0.0^{b}	0.5 ± 0.0^{c}	0.7 ± 0.0^{d}
Ash (%)	1.6 ± 0.0^{ac}	1.6 ± 0.1^{a}	1.9 ± 0.1^{b}	1.8 ± 0.0^{bc}
Lipid (%)	9.74 ± 0.0^{a}	13.9 ± 0.1^{b}	8.31 ± 0.1^{c}	3.10 ± 0.3^{d}
Iodine value	139.0 ± 0.8^{a}	131.0 ± 0.1^{b}	122.5 ± 1.4^{c}	118.4 ± 0.6^{d}
Iron (mg/kg tissue)	11.6 ± 1.2^{a}	14.0 ± 0.9^{b}	14.9 ± 0.6^{b}	7.80 ± 0.3^{c}
Copper (mg/kg tissue)	2.10 ± 0.60^{a}	0.72 ± 0.04^{b}	0.51 ± 0.08^{b}	0.45 ± 0.03^{b}
Selenium (mg/kg tissue)	0.34 ± 0.02^{a}	0.32 ± 0.10^{a}	0.39 ± 0.03^{b}	0.33 ± 0.10^{a}
Zinc (mg/kg tissue)	13.6 ± 1.5^{a}	14.9 ± 1.3^{b}	16.6 ± 0.1^{b}	15.5 ± 1.2^{b}

[a] The levels of all compounds are given as mean values (n = 3) ± SD of a pooled sample of 120 fish.
[a-d] Means within a row having different superscripts are significantly different ($p \leq 0.05$).

Zinc has also been found to have antioxidant properties because of its ability to compete with iron ions for membrane sites (Madhavi *et al.* 1996) and selenium is necessary for the synthesis and activity of glutathione peroxidase, a primary cellular antioxidant enzyme.

The fatty acids profile changed to a certain extent from summer to spring (Table 4.2). The sum of saturates decreased from 21.2 to 18.4%, the monoenes increased from 46.4 to 57.0% and the polyenes decreased from 24.1 to 18.4% for the period of summer to spring. It may seem strange that the spring capelin with the lowest iodine value, did have the lowest content of saturated fatty acids, but it is a recognized paradox, that the higher the iodine value of a fish oil, the higher the total for the saturated fatty acids (Ackman, 1980). Ackman also found out that the low iodine value (in the range of 100-135) of many fish oils was due to the inclusion of high proportion (20-35%) of C22:1. Comparison of the most unsaturated fatty acids shows that the lipids from autumn capelin contained the highest contents of C20:5+C22:5+C22:6 or 18.2% compared to 12.5% in winter capelin, while the polyene index (PI=20:5+22:6/16:0) was highest during the spring (1.42) but also lowest in the winter (0.89). The results from the fatty acid analysis were in agreement with earlier studies by Sigurgísladóttir and Pálmadóttir (1993) who measured fatty acid profiles of whole capelin caught in Icelandic waters sometime between November and March.

Table 4.2. Fatty acid composition of whole capelin with seasons showing principal fatty acids (> 90% of total).

Compound[a]	Summer	Autumn	Winter	Spring
Fatty acid (%)				
C14:0	6.3 ± 0.3[a]	6.2 ± 0.1[a]	7.5 ± 0.5[b]	6.2 ± 0.2[a]
C16:0	13.6 ± 0.7[a]	14.1 ± 0.5[a]	13.6 ± 1.0[a]	10.8 ± 0.4[b]
C18:0	1.3 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	1.4 ± 0.0
Total saturated	*21.2 ± 1.1*	*21.6 ± 0.6*	*22.5 ± 1.5*[a]	*18.4 ± 0.6*[b]
Σ C16:1	7.3 ± 0.7[a]	7.1 ± 0.2[a]	7.9 ± 0.5[a]	5.3 ± 0.2[b]
Σ C18:1	13.8 ± 0.8[a]	16.4 ± 0.5[b]	17.4 ± 0.9[b]	15.4 ± 0.5
Σ C20:1	11.6 ± 0.7[a]	11.1 ± 0.3[a]	14.2 ± 0.3[b]	16.1 ± 0.1[c]
C22:1	13.7 ± 2.3[a]	14.6 ± 0.7[a]	14.9 ± 1.5	19.0 ± 0.4[b]
C24:1	-	0.7 ± 0.0[a]	0.6 ± 0.2[a]	1.3 ± 0.1[b]
Total monoene	*46.4 ± 1.4*[a]	*50.0 ± 1.0*[b]	*55.0 ± 0.4*[c]	*57.0 ± 0.4*[c]
C18:2	1.6 ± 0.1[a]	1.5 ± 0.1[a]	1.6 ± 0.1[a]	1.3 ± 0.0[b]
C18:4	5.9 ± 0.5[a]	3.5 ± 0.1[b]	2.5 ± 0.1[c]	1.3 ± 0.1[d]
C20:5	8.0 ± 0.5[a]	8.4 ± 0.2[a]	6.2 ± 0.2[b]	6.2 ± 0.1[b]
C22:5	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.6 ± 0.1
C22:6	8.3 ± 0.5[a]	9.3 ± 0.1[b]	5.9 ± 0.4[c]	9.2 ± 0.1[ab]
Total polyene	*24.1 ± 0.4*[a]	*23.2 ± 0.3*[b]	*16.5 ± 0.3*[c]	*18.4 ± 0.1*[d]
C20:5 + C22:5 + C22:6	16.7 ± 0.1[a]	18.2 ± 0.2[b]	12.5 ± 0.4[c]	15.9 ± 0.1[d]
Polyene index	1.19 ± 0.06[a]	1.25 ± 0.03[ac]	0.89 ± 0.08[b]	1.42 ± 0.05[c]

[a] The levels of all compounds are given as mean values (n = 3) ± SD of a pooled sample of 120 fish.
[a-d] Means within a row having different superscripts are significantly different ($p \leq 0.05$).

The results were somewhat different from the results of Opstvedt (1985) (Table 2.1) on Norwegian capelin where the content of saturates, monoenes and polyunsaturates fell between winter and spring capelin in this study but some fatty acids were quite different for example C20:1 which was much lower in this present study.

4.1.2. Seasonal variation in chemical attributes of capelin

Other chemical attributes of capelin measuring proteolytic activity, hydrolytic activity and rancidity did also show substantial seasonal variation (Table 4.3). The summer capelin differed from other raw materials in many ways. Summer capelin had high hydrolytic activity measured as free fatty acids (FFA) of 2.4% as well as low pH (6.84). Summer capelin had the highest proteolytic activity as measured in very high TVN value of 114 mg/kg and high content of FAA (12.9 g/kg). It had also the lowest proportion of acidic against basic amino acids (0.9). The lowest TBA value of 2.5 mg malonaldehyde/kg was found in summer capelin, as well as a rather low peroxide value (1.7 meq/kg), which was also found at other seasons. Astaxanthin was extremely high during the summer (94.1 mg/kg lipid) but the α-tocopherol content was moderate (125 mg/kg lipid).

The autumn capelin had the highest fat content of 13.9% (Table 1) but lowest content of astaxanthin (2.2 mg/kg), α-tocopherol (70 mg/kg) as well as vitamin A (20 mg/kg). Most rancidity, measured as 5.5 mg MA/kg in TBA and 2.3 meq/kg in PV was found in autumn capelin, but it had the lowest content of free fatty acids of 1.4%, as well as highest pH (7.08).

The winter capelin exposed few extremes in other chemical attributes, but it contained the highest proportion of acidic against basic FAA of 1.4 g/kg.

The spring capelin had the highest lipolitic activity when estimated by high content of FFA (3.3%), but low proteolytic activity measured by low TVN value (22.5 mg/100 g) and low content of FAA (3.7 g/kg). The capelin was leanest in the spring with only 3.10% fat (Table 3.1), but the remaining lipid contained the highest quantity of α-tocopherol (390 mg/kg)and relatively high astaxanthin concentration (10.5 mg/kg).

Seasonal variation in astaxanthin content of capelin is in general most obvious in the visual color of the capelin oil. Capelin oil from summer capelin is dark red in color. After the summer season the color of the oil changes to orange and turns to yellow in the spring. The origin of the red color is most certainly due to the eating behavior of capelin. The immature summer capelin eats the smaller size calanoid copepods (redfeed) and as the capelin grows older and larger it is replaced by adult calanus, ephausiids (krill) and amphipods (Vilhjálmsson 1994). Besides that, the feeding intensity of the capelin is highest during summer and autumn. Astaxanthin (3,3-´dihydroxy-4,4-´diketo-β-carotene), is the most commonly occurring pigment in marine organisms (Simpson 1982). The pink to orange-pink coloring of many echinoderms and crustaceans, the skin of several fishes, and the meat of many salmonoid fishes, consists partly or wholly of astaxanthin and its esters (Lambertsen and Brækkan 1971). Lambertsen and Brækkan (1971) measured the astaxanthin content in some crustacean products. The astaxanthin content in krill was in the range of 22.4-77.4 mg/kg and 46.5 mg/kg in redfeed. Same authors measured the astaxanthin content of different fish oils, and found the content in capelin oil in four different samples, 94.3, 39.5, 6.6 and 5.7 mg/kg, mainly as the diester form. The roes of fish may also contain high levels of astaxanthin, thus the fillets of rainbow trout contained astaxanthin form 0.19 to 0.56 mg/kg, whereas the roes contained 0.86 mg/kg (Lambertsen and Brækkan 1971).

Table 4.3. Changes in chemical attributes of whole capelin from different seasons during landing.

Compound[a]	Summer		Autumn		Winter		Spring	
	Beginning of landing	End of landing (Δ= 10-15 h)	Beginning of landing	End of landing (Δ= 10-15 h)	Beginning of landing	End of landing (Δ= 10-15 h)	Beginning of landing	End of landing (Δ= 10-15 h)
TBA (mg MA/kg tissue)	2.5 ± 0.2[a]	3.5 ± 0.2	5.5 ± 0.3[b]	7.1 ± 0.1	3.3 ± 0.2[c]	2.4 ± 0.1	2.8 ± 0.2[ac]	3.1 ± 0.2
FFA (% of lipid)	2.4 ± 0.0[a]	3.1 ± 0.1	1.4 ± 0.0[b]	1.5 ± 0.0	1.9 ± 0.0[c]	1.9 ± 0.0	3.3 ± 0.0[d]	4.1 ± 0.2
PV (meq/kg lipid)	1.7 ± 1.4	1.7 ± 0.6	2.3 ± 0.7	1.5 ± 0.1	1.6 ± 0.2	0.7 ± 0.1	2.1 ± 0.3	2.1 ± 0.5
Astaxanthin (mg/kg lipid)	94.1 ± 2.4[a]	71.3 ± 1.0	2.2 ± 0.9[b]	3.6 ± 0.6	5.1 ± 0.3[bc]	5.1 ± 0.7	10.5 ± 3.3[c]	3.3 8.9 ± 2.7
-diester (%)	52.3 ± 1.4[a]	26.9 ± 1.4	42.9 ± 9.8[a]	32.7 ± 9.1	48.9 ± 5.9[a]	34.1 ± 7.4	74.5 ± 5.3[b]	74.5 ± 8.8
-monoester (%)	10.2 ± 0.9[a]	28.3 ± 2.7	34.1 ± 6.2[b]	36.8 ± 3.6	20.1 ± 0.8[c]	21.9 ± 7.2	12.0 ± 2.9[a]	10.5 ± 2.5
-free (%)	37.5 ± 2.2[a]	44.8 ± 4.2	23.0 ± 3.6[bc]	30.4 ± 5.6	31.0 ± 5.2[ab]	43.5 ± 4.7	13.5 ± 2.4[c]	15.7 ± 5.0
Tocopherol (mg/kg lipid)	125 ± 21[a]	90 ± 28	70 ± 0[b]	60 ± 0	105 ± 7[ab]	90 ± 0	390 ± 0[c]	230 ± 0
A-vitamin (mg/kg lipid)	44 ± 19	23 ± 2	20 ± 2	21 ± 0	32 ± 2	27 ± 0	66 ± 0	38 ± 15
pH	6.84 ± 0.03[a]	7.02 ± 0.02	7.08 ± 0.01[b]	7.16 ± 0.00	6.94 ± 0.01[c]	6.96 ± 0.01	6.93 ± 0.01[c]	7.10 ± 0.01
TVN (mg/100 g tissue)	114 ± 0.7[a]	118 ± 0.5	25.2 ± 0.6[b]	39.5 ± 0.3	22.6 ± 0.8[c]	22.7 ± 0.5	22.5 ± 0.1[c]	44.1 ± 0.5
FAA (g/kg tissue)	12.9 ± 0.2[a]	13.2 ± 0.7	9.1 ± 0.6[b]	9.8 ± 0.7	4.6 ± 0.1[c]	4.7 ± 0.4	3.7 ± 0.2[d]	3.7 ± 0.2
FAA acidic/basic	0.9 ± 0.0[a]	1.1 ± 0.1	1.2 ± 0.1[b]	1.5 ± 0.4	1.4 ± 0.1[c]	1.4 ± 0.1	1.0 ± 0.0[a]	1.6 ± 0.2

[a] The levels of all compounds are given as mean values (n = 3) ± SD of a pooled sample of 120 fish.
[a-d] Means within a row at time (1), having different superscripts are significantly different ($p \leq 0.05$).
Abbreviations: FFA = free fatty acids, TBA = thiobarbituric acid, PV = peroxide value, TVN = total volatile nitrogen, FAA = free amino acids.

This was probably the reason for relatively high content of astaxanthin in the spawning spring capelin, with its bright-yellow roes.

The tocopherol content of capelin was similar to that found in crude capelin oils by Notevarp and Chahine (1972). They reported tocopherol content of fourteen samples of crude capelin oil from five different processing plants ranging from 70 to 440 mg/kg, with an average of 285 mg/kg. They did, however, neither specify the samples processing dates, nor harvest season. Seasonal variations in tocopherol content of other fish species have been reported. Syväoja and Salminen (1985) measured tocopherols in Finnish fish and fish products. Fish caught in the spring, the spawning season of most species, had higher tocopherol content and lower fat content, than when harvested during the autumn. Others have reported the same results. Hardy and Mackie (1969) found a decrease in fat content, followed by an increase in tocopherol content in the period from October to March in sprats. Ackman (1974) found inverse relationship between tocopherol content and fat content in commercial sole fillets. The tocopherol content was as high as 500-600 mg/kg lipid in the lean period of the year, and as low as 100 to 200 mg/kg lipid when lipid was at its highest in September and October. The results of Syväoja and Salminen (1985) showed that the tocopherol content of for example Baltic herring was 360 mg/kg lipid in the spring, but the value for the autumn herring was 200 mg/kg lipid, or about 55% that of the spring herring. The seasonal variation in tocopherol content in this study was much higher, as the autumn capelin had less than 20% of the spring capelin tocopherol content. There are however other differences. The fat content of the Baltic herring was almost unchanged with season (~7%), whereas in this study the fat content of spring capelin was only 22% of the autumn capelin content. Syväoja and Salminen (1985) did, however, observe that in high-fat fish the tocopherol:fat ratio was lower than in low-fat fish. α-Tocopherol was found to be the principal tocopherol homologue and the content of low-fat fish was on the average 1100 mg/kg fat, with 300 mg/kg in the medium-fat and 160 mg/kg in the high-fat species. The reason for this seasonal variation in tocopherol content has been suggested to be a consequence of the sexual maturation of the fish, reaching their peak during the spawning season (Syväoja and Salminen 1985; Hardy and Mackie 1969).

4.1.3. Changes in chemical attributes of capelin during landing

Similar trends in changes in chemical attributes of whole capelin during landing were detected during all seasons (Table 4.3). The temperature of the raw material during landing was kept at or below 7 °C through the landing process. Spoilage products such as TVN and pH, increased during landing in all seasons ($p \leq 0.05$), except winter. Increase in pH during storage of capelin has been reported in Canadian capelin. Botta and co-workers (1978) found that muscle pH of nonspawning capelin increased from 7.12 to 7.28 during 16 days ice storage. The winter capelin was most stable during landing measured in changes in TVN and pH. The changes in free amino acids (FAA) were insignificant but the proportion of acids against basic FAA in spring capelin increased during landing ($p \leq 0.05$).

Rancidity measured as PV and TBA did not increase in the winter capelin during landing nor the hydrolytic activity measured as FFA. In the summer and spring capelin FFA increased significantly, whereas TBA increased in the summer and autumn ($p \leq 0.05$). PV never increased during landing, but showed insignificant decrease in the autumn and winter materials. Decline in PV has though been reported in nonspawning Canadian capelin (Botta *et al*. 1978) during iced storage, although the initial PV was much higher in their experiment, or about 10 meq/kg, compared to approximately 1 to 2 meq/kg in this study.

The lipid soluble antioxidants α-tocopherol and astaxanthin, as well as vitamin A, showed trend to decline with time during landing, but only significantly in the seasons of highest concentrations. Astaxanthin occurred mainly as the diester form at the beginning of landing, but seems to be converted with time to free astaxanthin and in the seasons of higher concentration, to the monoester form. A similar trend was reported by Torrissen and co-workers (1981), who found a slow conversion of astaxanthin diester to the corresponding monoester, when a shrimp waste silage (pH 4) was stored at 4-5 °C for 21 days. Few studies on the stability of endogenous α-tocopherol in fish have been reported. Petillo and co-workers (1998) measured the α-tocopherol loss of both light and dark muscle of mackerel during 11 days storage on ice. At the end of the storage the α-tocopherol content of light muscle had dropped to 40% of the initial level and about three-quarters of the α-tocopherol was lost in the dark muscle. Bandarra and others (1997) measured the α-tocopherol loss of sardine (10% fat) during 6 days ice storage. Fast reduction of the level of α-tocopherol was

registered, and not being detected at the 4th day. Other research has shown remarkably good stability of tocopherols in fish. Erickson (1992) found no decrease in either α- or γ-tocopherols in minced channel catfish during seven days refrigerated storage.

The overall changes during landing of capelin indicate that the spoilage processes were very dynamic, as seen in decomposition of natural antioxidants and gradual onset of rancidity and spoilage. This was most evident in the heavily feeding summer capelin. The determination of the storage life of whole capelin was not the purpose of this study, but research on Icelandic capelin (Ólafsdóttir *et al.*, 1997) caught in February, indicate a storage life during iced storage of at least 5 days, as measured by TVN and by organoleptic assessment. TVN values had then reached 40-50 mg of N/100g, when the first spoilage odors appeared and stale odor was first noticed. Botta and others (1978) suggested from measurements on TMA in offshore capelin stored in ice that substantial degradation began on day 7 or 8. These authors stated that the storage life of offshore, 12% fat capelin in ice was substantially better than that of inshore male capelin (2-3% fat) as well as herring, and mackerel.

4.2. Capelin meal

4.2.1. Chemical composition of capelin meal

The lipid content of capelin meal did not vary with season in the same degree as in the raw material, as major part of the oil is separated from the capelin in the fish meal process. Nevertheless the fat depleted spring capelin resulted in meal with the lowest fat content of 8.4% compared to 10.9-11.9% at other seasons (Table 4.4).

The decline in iodine value from summer to spring was similar in the meal as in the raw material, except from winter to spring meal where the decrease was negligible. The iodine value of the meal lipids declined from 160.5 during summer to around 147 during winter and spring. The iodine values of the capelin meal were in agreement with the results of Notevarp and Chahine (1972). Accordingly, capelin meal oils extracted with pentane-hexane from six dried press cakes and meal from two different processing plants had iodine values in the range of 144-168.

The content of the trace metals copper and selenium was highest in the summer meal, 5.10 mg/kg for copper and 2.0 mg/kg for selenium.

Table 4.4. Composition of capelin meal with season.

Compound[a]	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 (%)	11.9 ± 0.0^{a}	10.9 ± 0.1^{b}	11.5 ± 0.1^{c}	8.4 ± 0.1^{d}
Iodine value	160.5 ± 3.0^{a}	152.4 ± 1.6^{b}	147.1 ± 0.3^{c}	146.9 ± 0.5^{c}
Iron (mg/kg tissue)	74.6 ± 4.0^{a}	108 ± 4.9^{b}	191 ± 11^{c}	83.5 ± 6.5^{a}
Copper (mg/kg tissue)	5.10 ± 0.10^{a}	2.88 ± 0.28^{b}	2.49 ± 0.20^{c}	2.22 ± 0.00^{c}
Selenium (mg/kg tissue)	2.00 ± 0.10^{a}	1.53 ± 0.06^{b}	1.60 ± 0.10^{b}	1.32 ± 0.03^{c}
Zinc (mg/kg 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	0.34 ± 0.02^{a}	0.34 ± 0.02^{a}	0.36 ± 0.01	0.43 ± 0.02^{b}
Particle size, No. 30 (%)	24.3 ± 0.5^{a}	15.9 ± 0.3^{b}	11.8 ± 0.2^{c}	17.6 ± 0.1^{b}
Particle size, No. 50 (%)	27.7 ± 0.7^{a}	23.2 ± 0.3^{b}	23.1 ± 1.0^{b}	28.2 ± 0.1^{a}
Particle size, No. 70 (%)	35.5 ± 6.8^{a}	13.2 ± 0.1^{b}	31.5 ± 4.4^{a}	22.8 ± 0.5
Particle size, >No. 70 (%)	13.4 ± 4.1^{a}	47.8 ± 0.5^{b}	33.7 ± 5.4^{c}	31.1 ± 0.5^{c}

[a] The levels of all compounds are given as mean values (n = 3) ± SD of a pooled sample (6 kg) of capelin meal.

[a-d] Means within a row having different superscripts are significantly different ($p \leq 0.05$).

The levels of zinc were on the other hand lowest in the summer (60 mg/kg) and increased from summer to winter/spring to around 97 mg/kg. Iron content was highest in the winter (191 mg/kg) and lowest in the summer (74.6 mg/kg). The levels of metals in the capelin meal were in agreement with Norwegian research. Notevarp and Chahine (1972) measured fourteen samples of crude capelin oil from five different processing plants and dried capelin meal form two different processing plants. The meal was found to contain 256 and 192 (mg/kg) iron and 4.9 and 4.3 (mg/kg) copper respectively. The capelin meal contained approximately 50-100 times more iron and 40-50 times more copper than the corresponding oils. Lunde (1973) measured the content of several metals in seven samples of capelin meal from different manufactories and seasons. The iron content ranged from 86-757 mg/kg, with an average of 288 mg/kg. The copper content ranged from 2.6-5.7, with an average of 4.3 mg/kg and the zinc content was between 88 and 150, with an average of 118 mg/kg. The average content of two samples of laboratory produced capelin meal was 48 mg/kg for iron, 5.0 mg/kg for copper and 53 mg/kg for zinc. Selenium was only measured in defatted meal and was between 0.5 and 1.6 mg/kg.

The density of the meal was not always the same. The summer meal was most dense, both measured as bulk- and packing density. This was also reflected in the particle-size of the meal, where the summer meal had the highest percentage in the finest sieve

(No. 70). However, autumn meal had even finer particle size, with almost half of its particles smaller than the finest sieve. The milling of the meal may affect the oxygen diffusion in the meal. Waissbluth and co-workers (1971) measured the influence of grinding on the rate of oxygen absorption in fish meal. Meal screened between 18 and 50 mesh had oxygen absorption of 4.1 μmol/hr/g meal, whereas the same meal, milled and screened either between 50 and 100 mesh or 100 and 170 mesh, had oxygen absorption of 7.3 and 13.7 respectively.

The water activity (a_w) was highest in spring meal (0.43) and so was the water content (9%). The results of Waissbluth and co-workers (1971) showed that increasing moisture content had a marked prooxidant effect in all the oxidative reactions of whole pilchard and anchovy meal. This was however in contrast with the findings of other studies. Ólafsson (1953) studied the effect of water content on lipid oxidation in Icelandic herring meal, and found that the peroxide values increased rapidly when the meal was dried to below 10-12%. A more recent study by Astrup and Halvorsen (1985) indicated that when the moisture content of the herring meal increased from 7 to 12%, the oxidation rate decreased by 50%, as measured by oxygen absorption.

Changes in principal fatty acids with season were less pronounced in the meal than in whole capelin. The sum of saturates and polyenes were quite constant of around 21.6% and 33.8%, respectively, until at spring, where the values decreased to 20.2% for the saturates and 32.3% for the polyenes. The monoenes on the other hand were highest in the spring (41.6%) and quite constant at other times (~ 36.3%). The polyene index (PI = 20:5+22:6/16:0) on the other hand was highest during the spring (2.05) and lowest in the summer (1.74). However, the content of C20:5+C22:5+C22:6 was lowest in the summer (27.8%) but rather even at other times (~ 30.2%). These results were in agreement with the results of Opstvedt (1971), for capelin meal (Table 2.4). However, the polyene index was higher in this study, which can be traced to somewhat higher content of the most highly unsaturated fatty acids, especially C22:6. 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. Thus, Pálmadóttir and co-workers (1987) found higher proportion of n-3 fatty acids in capelin meal dried with the more recent and gentle drying methods (steam drying and indirect steam drying), compared to the older flame drying method.

Table 4.5. Fatty acid composition of capelin meal with seasons showing principal fatty acids (> 90% of total).

Compound[a]	Summer	Autumn	Winter	Spring
Fatty acid (%)				
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	1.0 ± 0.1[a]	0.5 ± 0.2[b]	1.6 ± 0.0[c]
Total monoene	*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	18.9 ± 0.2[b]	18.2 ± 0.2
Total polyene	*33.5 ± 1.1*	*33.7 ± 0.5*	*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	31.0 ± 0.2	29.6 ± 0.4[b]
Polyene index	1.74 ± 0.15[a]	1.87 ± 0.05	1.89 ± 0.06	2.05 ± 0.01[b]

[a] The levels of all compounds are given as mean values (n = 3) ± SD of a pooled sample (6 kg) of capelin meal.
[a-d] Means within a row having different superscripts are significantly different ($p \leq 0.05$).

Decrease in the most polyunsaturated fatty acids as a result of lipid oxidation in fish meal is generally accepted and has been reported (Barlow and Pike 1977, Opstvedt 1971, 1985). A clear decrease of C20:5 and C22:6 in menhaden meal has also been noticed as a result of the lack of antioxidant (Ackman and Gunnlaugsdottir 1992).

The polyene index (PI) (20:5+22:6/16:0) of fish lipids can be informative and has been suggested to be a meaningful tool for measuring oxidative rancidity in fishery products. Thus the PI showed good correlation with rancidity scores in dried-salted sardines (Lubis and Buckle 1990). In their study, the PI decreased during storage, but remained nearly stable after 6 weeks storage. This situation is well known in the fish meal industry, that is the initial reactivity of the fish meal lipids is high and the lipid oxidation fades off with time (Astrup and Halvorsen 1985).

4.2.2. Seasonal variations and the effect of fish meal processing

The production of fish meal from capelin changed the nutritional composition of the material greatly (Tables 4.1 and 4.4). The moisture content fell from approximately 70-80% in the raw capelin to 7-9% in the produced capelin meal. The protein content increased from 12.5-14% in the capelin to around 70-72% in the meal. The drying

increased the salt and ash content from 0.4-0.7% to 2-3.4% for the salt and from 1.6-1.9% to 8.8-10.3% for the ash. The fish meal contained on the average 4 times more copper, 9 times more iron, 5 times more selenium and 5.5 times more zinc than the respective raw material. However, calculated from the dry matter content, the average increase with fish meal processing was only 1.4 fold for the zinc and 2.4 fold for the iron, while copper and selenium were almost unchanged. Industrial contamination of iron and zinc may be the case here and probably difficult to avoid. Lunde (1973) measured iron, copper, zinc and selenium in capelin meal produced industrially and in the laboratory. The level of zinc and iron was higher in the industrially produced meal compared to the laboratory produced, but the levels of copper and selenium were similar in both types of meal.

Changes in principal fatty acids with season were less pronounced in the meal than in whole capelin. The differences between fatty acid profiles of the principal fatty acids from lipids of whole capelin and capelin meal were best seen by principal component analysis (PCA) on these samples (Figure 4.1). PCA on the fatty acid profiles was in general such that whole capelin was more associated to monounsaturated fatty acids (16:1, 18:1, 20:1 and 22:1) but capelin meal to polyunsaturated fatty acids (20:5, 22:5 and 22:6).

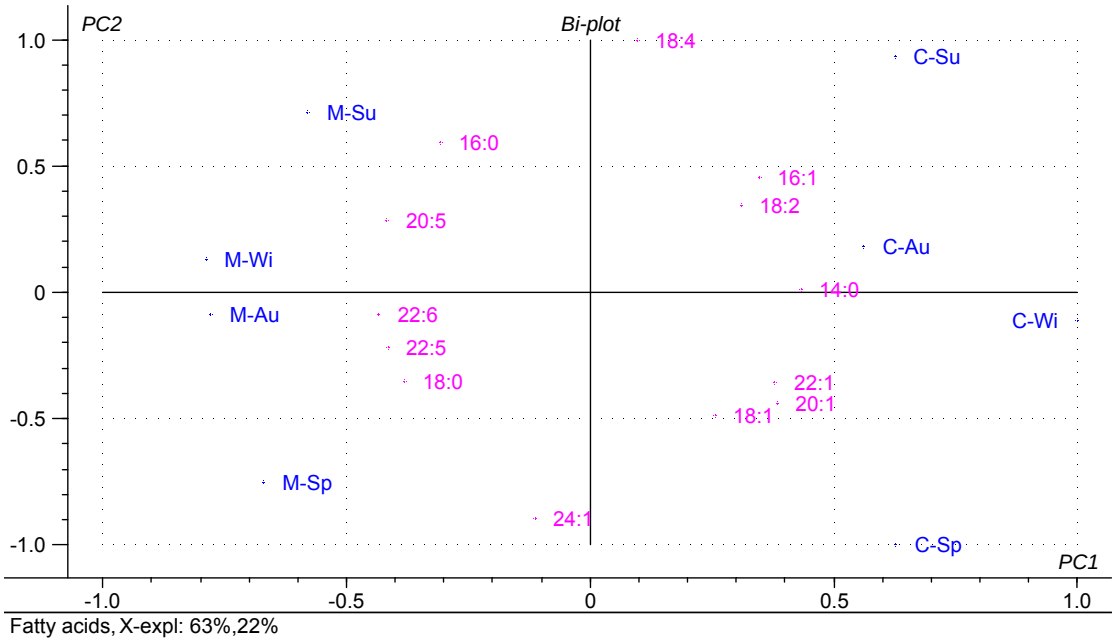

Figure 4.1. PCA biplot of scores and lodings for principal fatty acids (> 90% of total) of whole capelin and capelin meal from different seasons.

Abbreviations for the scores: M = meal, C = whole capelin, Su = summer, Au = autumn, Wi = winter, Sp = spring.

The first two principal components (PC1 and PC2) explained 85% of the variance in the fatty acid pattern of whole capelin and capelin meal. The first principal component (PC1) described the differences between capelin meal and whole capelin, as the whole capelin had positive PC1 values, whereas capelin meal had negative PC1 values. The differences between seasons were best described by PC2, from negative values for the spring samples towards positive values for the summer samples. Similar seasonal trends were observed for both whole capelin and capelin meal. Spring capelin showed extreme behavior, situated far apart from other capelin samples, which may be explained by higher content of monounsaturates, especially the long chained ones.

The content of endogenous antioxidants was highly influenced by season and processing. Astaxanthin was extremely high in the meal during the summer season (47.8 mg/kg), and was found to be approximately half of the content in the raw unprocessed capelin (94.1 mg/kg - Table 4.3). At other seasons the astaxanthin content was low, or 5-13 mg/kg, but higher in the fish meal than in the respective raw material. Astaxanthin was mainly in the diester form in both raw capelin and in the capelin meal, except in the summer capelin meal, where free astaxanthin was dominating. Autumn meal had the lowest proportion in the free form, compared the other seasons. According to Miki and others (1983) free astaxanthin is the most labile form to heat, whereas the diester is the most stable form of astaxanthin. However, astaxanthin in all these forms are powerful antioxidant agents under both hydrophobic and hydrophilic conditions (Kobayashi and Sakamoto 1999). Astaxanthin and carotenoids in general are rather unstable molecules due to their long conjugated double bound systems. They are sensitive to oxygen, light, heat, acid and alkali, particularly combinations of these factors (Britton 1985). According to Simpson (1982), drying of fish or fish meal and subsequent storage are the major factors causing degradation of astaxanthin in the industry. Lambertsen and Brækkan (1971) analyzed shrimp meal, where the total astaxanthin content ranged from 76 mg/kg in vacuum-dried sample to total destruction for some industrial samples. In this study the residual lipids of the meal samples contained higher levels of astaxanthin than the lipids of whole capelin, except for the summer season. The difference between the summer season, apart form higher concentrations was that summer season had much higher proportion of free astaxanthin, compared to other seasons with higher proportions of esterified astaxanthin.

Table 4.6. Changes in capelin meal from different seasons during storage.

	Storage period, Months											
	Summer			Autumn			Winter			Spring		
Compound[a]	0	2	4	0	2	4	0	2	4	0	2	4
Astaxanthin (mg/kg lipid)	47.8 ± 1.2[a]	35.1 ± 0.6	28.6 ± 0.1	5.0 ± 0.9[b]	3.4 ± 0.1	2.0 ± 0.2	7.6 ± 1.0[b]	5.5 ± 1.2	5.9 ± 0.5	13.4 ± 1.2[c]	11.7 ± 0.3	10.6 ± 1.0
-diester (%)	15.7 ± 2.2[a]	5.0 ± 0.2	5.5 ± 0.2	50.7 ± 7.1[b]	46.9 ± 4.4	47.8 ± 3.8	43.2 ± 3.5[b]	51.2 ± 6.5	53.3 ± 2.3	64.1 ± 0.0[c]	64.6 ± 3.6	66.8 ± 1.1
-monoester (%)	39.8 ± 2.7[a]	52.7 ± 0.9	52.9 ± 0.7	28.8 ± 3.9[b]	30.5 ± 2.6	35.4 ± 1.5	18.7 ± 2.5[c]	21.1 ± 0.7	26.1 ± 2.9	12.9 ± 1.9[c]	12.3 ± 1.9	19.0 ± 1.7
-free (%)	44.5 ± 2.3[a]	42.4 ± 1.2	41.7 ± 0.8	20.4 ± 3.2[b]	22.6 ± 2.8	16.8 ± 2.4	38.0 ± 5.8[a]	27.7 ± 6.6	20.6 ± 5.2	23.0 ± 1.9[b]	23.0 ± 2.1	14.2 ± 2.2
Tocopherol (mg/kg lipid)	33 ± 15[a]	40 ± 0	43 ± 6	27 ± 6[a]	33 ± 6	50 ± 0	53 ± 6[a]	77 ± 6	60 ± 0	167 ± 12[b]	150 ± 10	105 ± 21
A-vitamin (mg/kg lipid)	12 ± 6[a]	9 ± 0	14 ± 5	14 ± 2[a]	11 ± 2	7 ± 2	34 ± 2[b]	26 ± 3	21 ± 0	54 ± 0[c]	38 ± 2	39 ± 4
Ether extractable lipid (%)	11.9 ± 0.01[a]	11.7 ± 0.0	11.8 ± 0.1	10.9 ± 0.12[b]	10.7 ± 0.2	10.5 ± 0.2	11.5 ± 0.10[c]	11.3 ± 0.0	11.4 ± 0.0	8.4 ± 0.09[d]	8.6 ± 0.0	8.4 ± 0.0
PV (meq/kg lipid)	5.2 ± 0.8[a]	6.1 ± 3.2	3.7 ± 0.7	10.5 ± 1.9[b]	6.3 ± 0.1	4.3 ± 0.2	5.4 ± 0.2[ad]	3.3 ± 0.5	3.1 ± 0.2	2.8 ± 0.1[c]	3.6 ± 0.3	2.7 ± 0.2
TBA (mg MA/kg tissue)	1.91 ± 0.90	1.91 ± 0.12	2.39 ± 0.19	2.30 ± 0.13	1.40 ± 0.06	1.39 ± 0.16	2.17 ± 0.47	2.07 ± 0.05	3.86 ± 0.16	1.01 ± 0.31	1.08 ± 0.35	0.90 ± 0.13
FFA (%)	11.7 ± 0.1[a]	11.4 ± 0.1	11.4 ± 0.2	9.2 ± 0.1[b]	8.0 ± 0.1	8.5 ± 0.5	7.4 ± 0.1[c]	7.1 ± 0.0	7.3 ± 0.1	10.3 ± 0.5[d]	10.0 ± 0.1	9.9 ± 0.3
pH	6.36 ± 0.05[a]	6.35 ± 0.01	6.37 ± 0.01	6.84 ± 0.01[b]	6.83 ± 0.01	6.76 ± 0.01	7.00 ± 0.08[b]	6.94 ± 0.08	6.71 ± 0.00	6.53 ± 0.11[ac]	6.60 ± 0.12	6.74 ± 0.02
TVN (mg/100 g tissue)	108 ± 0.3[a]	114 ± 1.3	116 ± 1.1	62.3 ± 0.6[b]	79.6 ± 0.7	80.5 ± 1.4	46.0 ± 0.6[c]	49.4 ± 1.2	55.9 ± 1.3	60.4 ± 0.2[d]	75.5 ± 0.6	76.5 ± 0.6
WSP (% of protein)	31.9 ± 0.2[a]	31.9 ± 0.3	31.5 ± 0.1	24.5 ± 0.2[b]	23.5 ± 0.1	23.9 ± 0.1	14.0 ± 0.3[c]	13.6 ± 0.3	13.3 ± 0.2	13.5 ± 0.3[c]	13.9 ± 0.6	12.6 ± 0.2
FAA (g/kg tissue)	54.6 ± 1.0[a]	46.9 ± 1.3	47.1 ± 3.1	24.6 ± 0.3[b]	25.5 ± 0.2	18.1 ± 0.2	11.8 ± 1.9[c]	13.5 ± 0.5	15.4 ± 0.1	11.1 ± 0.2[c]	10.4 ± 2.4	10.2 ± 0.4
FAA, acidic/basic	0.65 ± 0.01[a]	1.12 ± 0.01	0.95 ± 0.03	1.51 ± 0.03[b]	1.13 ± 0.02	1.31 ± 0.01	1.79 ± 0.09[c]	1.36 ± 0.02	1.21 ± 0.04	1.44 ± 0.02[b]	1.25 ± 0.06	1.36 ± 0.11
Lab*-L	50.2 ± 0.4[a]	52.1 ± 0.3	52.6 ± 0.1	51.5 ± 0.3[a]	50.6 ± 0.6	51.3 ± 0.2	50.6 ± 0.6[a]	49.4 ± 1.4	50.0 ± 0.5	46.7 ± 1.0[b]	46.7 ± 0.6	46.7 ± 0.1
Lab*-a	1.14 ± 0.04[a]	0.97 ± 0.03	0.80 ± 0.04	0.38 ± 0.01[b]	0.47 ± 0.03	0.49 ± 0.01	0.78 ± 0.17[a]	0.62 ± 0.10	0.79 ± 0.09	0.17 ± 0.22[b]	0.23 ± 0.11	0.39 ± 0.13
Lab*-b	12.8 ± 0.2[a]	13.3 ± 0.2	13.5 ± 0.1	11.8 ± 0.2[b]	14.5 ± 0.2	15.1 ± 0.2	11.6 ± 0.4[b]	13.5 ± 0.4	13.7 ± 0.6	9.9 ± 0.5[c]	10.6 ± 0.3	10.7 ± 0.2
DE*ab	-	2.0 ± 0.1[a]	2.5 ± 0.4	-	2.9 ± 0.2[b]	3.3 ± 0.2	-	2.3 ± 0.6[ab]	2.2 ± 0.5	-	0.8 ± 0.4[c]	1.2-0.3

[a] The levels of all compounds are given as mean values (n = 3) ± SD of a pooled sample (6 kg at 0 month, 2 kg at months 2 and 4) of capelin meal.
[a-d] Means at 0 months within a row having different superscripts are significantly different ($p \leq 0.05$).
Abbreviations: PV = peroxide value, FFA = free fatty acids, TVN = total volatile nitrogen, WSP = water soluble protein, FAA = free amino acids, L* = lightness, a* = red, b* = yellow, ΔE*ab = change in initial color.

According to Guillou and co-workers (1995) the fatty acids EPA (C20:5) and DHA (C22:6) were the principal fatty acids esterified with astaxanthin in shrimp. This could be the explanation for higher astaxanthin content in capelin meal than in whole capelin, as the capelin meal contained higher proportions of the fatty acid C22:6 than the whole capelin.

α-Tocopherol in capelin meal was found to be highest in the spring (167 mg/kg lipid) and lowest in the autumn (27 mg/kg lipid), and the fish meal contained tocopherol of generally less than half that of the raw material (Tables 4.3 and 4.6). Information on tocopherol content in fish meal was not found in the literature. However, other effect of processing on tocopherol content in fish has been studied. Erickson (1992) studied the effect of cooking on minced channel catfish. The results were that 60% of the α-tocopherol and over 80% of the γ-tocopherol remained after cooking. Processing like freezing also affects the tocopherol content of fish. Syväoja and Salminen (1985) measured α-tocopherol in blast-frozen herring fillets. The tocopherol content fell from 420 mg/kg lipid to 270 mg/kg during six months frozen storage, that is over 60% of the α-tocopherol remained.

Free amino acids (FAA), as well as the water-soluble protein (WSP) were highest in summer meal and decreased from summer to spring. WSP in fish meal has been found to reflect the content of solubles added to the presscake, which may be an indication of the freshness of the raw material. High WSP in summer meal (31.9% of protein) was therefore in accordance with the high TVN-value of the summer capelin. The fish meal contained about 3-4 times more FAA than the respective raw material. However, calculated from the dry matter content, there was a decrease in FAA with fish meal processing, except for the summer meal, where some increase was observed. The proportion of acidic against basic FAA (A/B) was highest in winter meal (1.36) and lowest in summer meal (0.65). The fish meal had in general 30-40% higher A/B than the corresponding raw material, in opposition to the summer meal that had about 30% lower A/B than the raw material.

TVN was highest in summer meal (108 mg/ 100 g) and lowest in winter meal (49.4 mg/100 g). The fish meal contained 2-3 times as much TVN as the raw material, except for the summer meal with similar values as the raw material.

Free fatty acids (FFA) were highest during spring and summer of 10.3% and 11.7% respectively, compared to 9.2% in the autumn and 7.1% in the winter. The levels of FFA in this study were considerably lower than reported by Guðmundsson and Karlsdóttir (1982), where the range of all exported samples of capelin meal during a whole year was from 12.6-21.8% in FFA, with an average of 16.6%. Fish meal processing resulted in an increase in FFA. The FFA values were about 3-6 times higher in the fishmeal compared to the respective raw material. According to Barlow and Pike (1977), FFA content depends on number of factors. FFA can be formed prior to processing, due to the action of hydrolytic enzymes in the fish. Heating in the processing will probably also cause the formation of some FFA spontaneous hydrolysis and finally complex interaction of oxidation during storage of the meal will gradually release free fatty acids. However, according to Opstvedt (1985), FFA content is not a useful criterion of the quality of fish lipids, as the level of FFA in the diet of animals had no effect on their thrive and growth.

Rancidity as measured by TBA and PV was highest in the autumn, 2.30 mg MA/kg tissue for the TBA and 10.5 meq/kg oil for the PV. The PV was 3-4 times higher in fish meal than the respective raw material, except in the spring meal, where only slight increase was detected. TBA on the other hand decreased after fish meal processing, to about half of the raw material level.

The color of the fish meal was somewhat different with season. Spring meal was the darkest (L* = 46.7) compared to L* value around 50 at other seasons. The spring meal was also the least red (a* = 0.17) and yellow (b* = 9.9). The summer meal on the other hand was the most red (a* = 1.14) and yellow (b* = 12.8) in color. This was probably due to high levels of astaxanthin, which was also obvious in red color of the lipid extract.

The results of the PCA on shared measurements from capelin and capelin meal showed that the first principal component (PC1) described the main part of the variance (58%) which reflected the differences between capelin meal and whole capelin, as all the whole capelin samples had negative PC1 values, while all the capelin meal samples had positive PC1 values. The first two principal components (PC1 and PC2) explained 77% of the variance in the data (Figure 4.2).

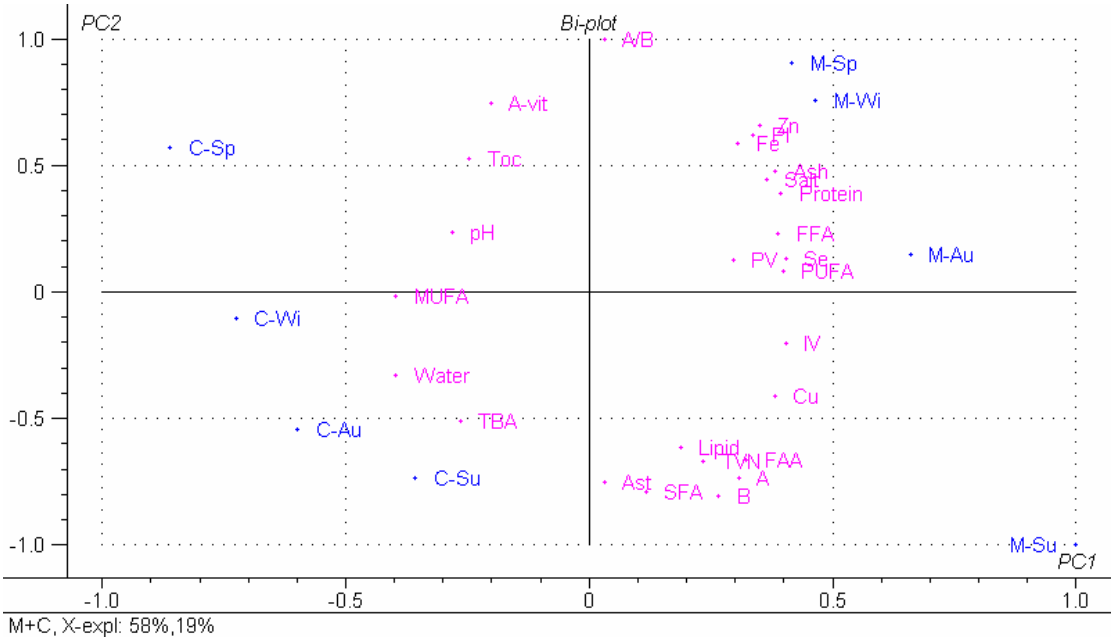

Figure 4.2. PCA biplot of scores and lodings of whole capelin and capelin meal from different seasons.

Abbreviations for the scores: M = meal, C = whole capelin, Su = summer, Au = autumn, Wi = winter, Sp = spring. Abbreviations for the loadings: FFA = free fatty acids, IV = iodine value, TBA = thiobarbituric acid, PV = peroxide value, TVN = total volatile nitrogen, Ast = astaxanthin, A-vit = vitamin A, Toc = α–tocopherol, SFA = saturated fatty acids, MUFA = monoene fatty acids, PUFA = polyene fatty acids, PI = polyene index, FAA = free amino acids (total), A = acidic FAA, B = basic FAA.

The second principal component (PC2) only described 19% of the variance, which reflects the differences between seasons. The variables associated to capelin meal were the metals and the polyunsaturated fatty acids (PUFA), were as variables associated to whole capelin were the monounsaturated fatty acids (MUFA). The rancidity measurements PV and TBA showed that PV was more associated with the meal and the TBA was more associated with the capelin. TBA and PV did not correlate (r^2 = -0.29). Data analysis on separate results of capelin or fresh capelin meal revealed that the data was not suitable for principal component analysis (PCA) as the validated explained variance was very low, implying that the models were no good on new samples. The reason was most probably due to lack of samples.

4.2.3. Changes in capelin meal during storage

Development in lipid oxidation and degradation products in capelin meal with storage at 10 °C showed advanced oxidation of the meal lipids (Table 4.6 and Figures 4.3-4.6). Peroxide value (PV) decreased with storage time, except for the spring meal (Figure 4.3).

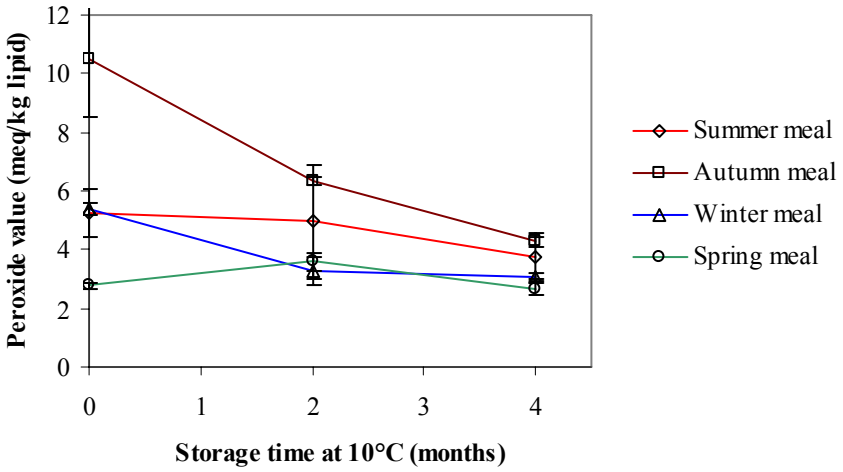

Figure 4.3. Changes in peroxide value during storage of capelin meal from different seasons. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

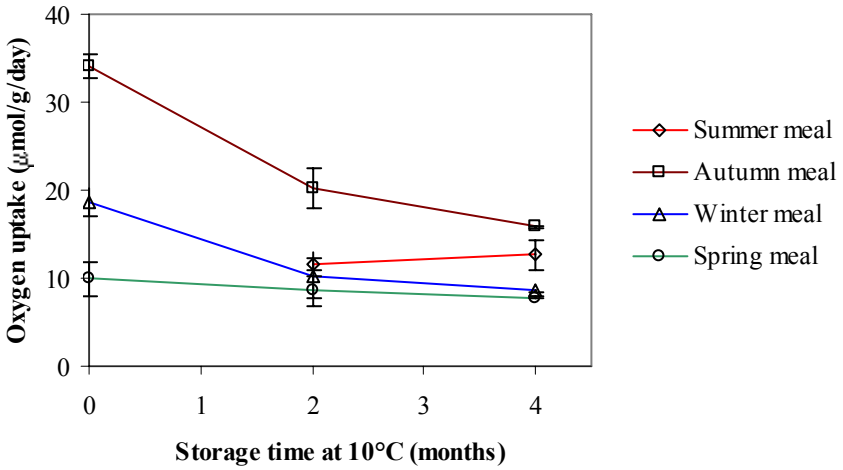

Figure 4.4. Evaluation of oxygen uptake in capelin meal from different seasons during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

Autumn- and winter meal showed the fastest decrease during the first months of storage ($p \leq 0.05$), but all types of meal seemed to end in similar PV, in the range of 3-4 meq/kg oil. This decrease with storage time was in agreement with the results of Dreosti, described by Barlow and Pike (1977). Anchovy and pilchard meal without antioxidant addition reached the maximum PV after about one week's storage. These values were of the order of 100 to 125 meq/kg oil. Thereafter, the PV of the meal decreased. Decrease with time was also seen in the evaluation in oxygen uptake in the capelin meal (Figure 4.4). The oxygen uptake decreased significantly in autumn meal

trough out the storage experiment and for the first two months in winter meal. The spring meal had lower values and did not decrease with time ($p \leq 0.05$), indicating the most stable meal. Measurement on oxygen uptake in fresh summer meal failed, but no significant decrease was observed in the latter part of the storage time. According to Astrup and Halvorsen (1985) 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. This phenomena is well known in the fish meal industry (Barlow and Pike 1977). After the manufacture of meal from fatty fish, a "curing" period may be necessary for unstabilized meal when the most reactive polyunsaturated part of the meal is allowed to oxidize and cool off in loosely stacked sacks. After the initial curing period, the meal is relatively stable and can be stored in compact stacks.

Changes in TBA were somewhat inconsistent (Figure 4.5). Summer and spring meal did not change with time ($p \leq 0.05$), while TBA value in autumn meal decreased by 40% after two months of storage ($p \leq 0.05$). TBA value in winter meal on the other hand doubled during the last two months of storage ($p \leq 0.05$). TBA is a measure of secondary lipid oxidation products, mainly malondialdehyde. The fact that TBA decreases with capelin meal production and with storage time of meal, implies that the malondialdehyde decomposes with time or reacts further. Gómez-Sánchez and coworkers (1990) suggested that malondialdehyde reacts with amino sugars, giving rise to pyrroles and other heterocyclic compounds similar of 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.

Aqueous antioxidant activity (AA) of capelin meal changed to some degree during storage (Figure 4.6). The aqueous AA of the autumn meal was much less than in other meal samples, with the exception of the measurement after two months of storage, that can not be explained. Meal at other seasons showed decline in aqueous AA with storage time, especially during the first two months of storage ($p \leq 0.05$).

The reason for the aqueous AA of capelin meal was not obvious and was sought by data analysis of all meal samples. Variations in the aqueous AA were most likely a result of interactions between water-soluble anti- and pro-oxidants in the meal. Possible factors for consideration were the content of free amino acids, salt and metals, Maillard reaction products (browning) as well as pH.

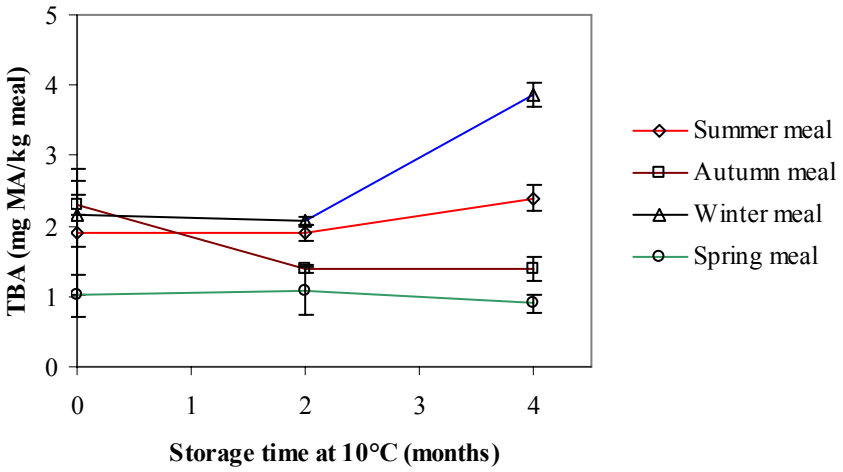

Figure 4.5. Changes in TBA during storage of capelin meal from different seasons. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

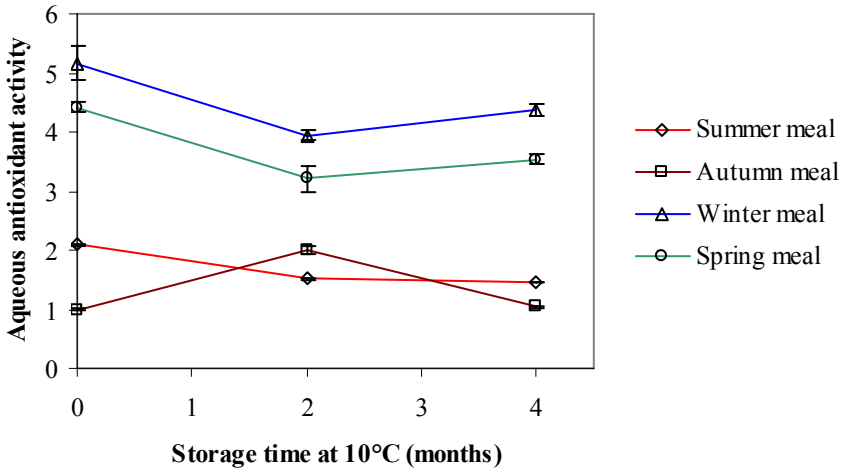

Figure 4.6. Evaluation of aqueous antioxidant activity of capelin meal from different seasons during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

Free amino acids (FAA) (Table 4.6) decreased during storage in summer, autumn and spring meal by 13, 27 and 9% ($p \leq 0.05$) respectively, but increased by 30% in winter meal during four months storage ($p \leq 0.05$) (Figure not shown). Decrease in FAA with time could imply that the free amino acids participate in lipid oxidation reactions. The increase in FAA as observed in the winter meal was on the other hand hard to explain. However, unlike other meal, the pH of winter meal decreased with storage time ($p \leq 0.05$), with possible simultaneous influence on the development of FAA.

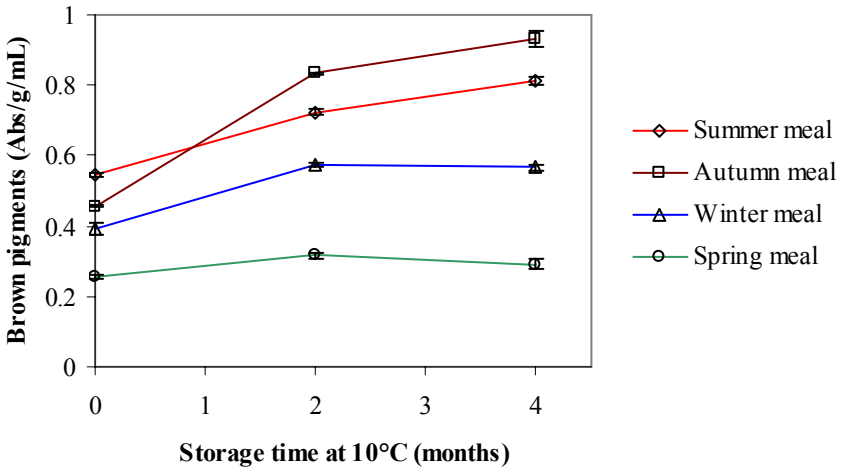

Figure 4.7. Browning in capelin meal from different seasons during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

Substantial browning was observed in meal from all seasons during storage ($p \leq 0.05$), (Figure 4.7). Brown pigments increased by 50% in summer and winter meal and more than doubled in autumn meal. Spring meal showed the least, but significant browning during storage. The browning was faster at the beginning of the storage and declined with time. These results were consistent with the Lab-b* and ΔE*ab color measurements (Table 4.6). Lab-b* representing the yellow color that increased significantly with time in all meal samples, except the spring meal. The total color change was highest for the autumn meal (ΔE*ab = 3.3), followed by summer (ΔE*ab = 2.5) and winter meal (ΔE*ab = 2.2) while the spring meal showed only half of the color change of the other meal (ΔE*ab = 1.2). The smaller the value of ΔE*ab, the closer the samples are to a perfect match to their initial color. According to Parkers (1994), ΔE*ab of less than 0.4 is below the threshold of human perception. Furthermore first grade commercial matching tends to be up to ΔE*ab 0.9 and other less critical matching applications can have acceptable limits as high as ΔE*ab 4.0-5.0. Color change during storage between 2 and 3 ΔE*ab for all meal except the spring meal was not significant, and probably within limits of acceptance.

The results for the electronic nose measurements showed that the H_2S, NO and SO_2 sensors showed similar patterns (Figures 4.8 and 4.9). There were initially lower responses for the spring and winter meal, higher for the autumn meal and highest for the summer meal ($p \leq 0.05$), except SO_2 sensor.

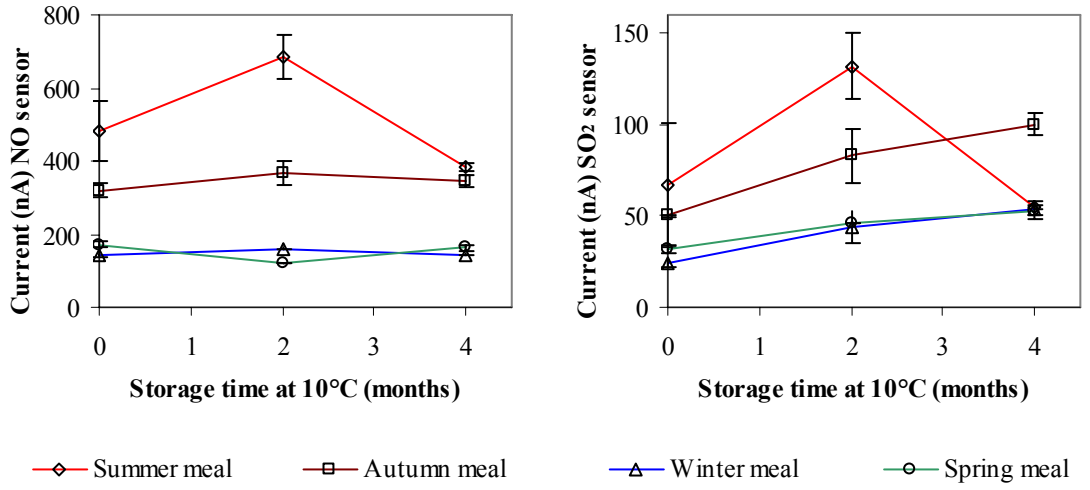

Figure 4.8. Responses (currents in nanoampers) of NO and SO_2 sensors to capelin meal headspace during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

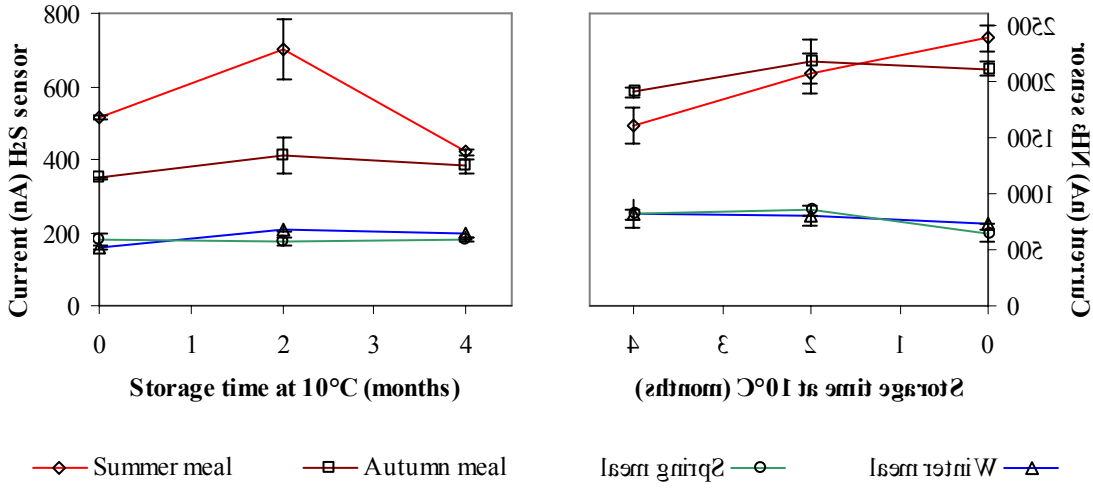

Figure 4.9. Responses (currents in nanoampers) of H_2S and NH_3 sensors to capelin meal headspace during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

There were in general little changes with time, though the SO_2 sensor showed significant rise with storage time with the exception of the summer meal. The summer meal showed significant rise and fall with storage time for H_2S, NO and SO_2. This could mean increasing reactivity, with subsequent decline during prolonged storage due to lack of substrates.

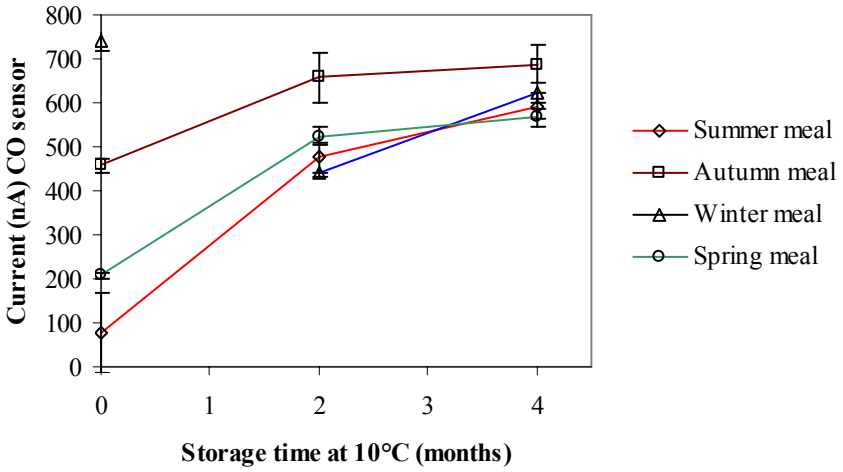

Figure 4.10. Responses (currents in nanoampers) of CO sensor to capelin meal headspace during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

The NH_3 sensor demonstrated a clear difference ($p \leq 0.05$) through out the storage time between summer and autumn meal and winter and spring meal respectively (Figure 4.9). The summer and autumn meal showed both much higher responses in NH_3 and a decline with storage time, whereas winter and spring meal were virtually unchanged. Decline in NH_3 response has been shown to imply that amines and other nitrogen-compounds reacted with other reactive compounds in the meal (Ólafsdóttir *et al.* 1997b). These results were not entirely in agreement with the findings of Ólafsdóttir and co-workers (1997a). Their results indicated that the NH_3 and CO gas sensors could be used as indicators for TVN value of capelin meal, but in this study only the summer meal had high TVN values, while the autumn, winter and spring meal had lower values ($p \leq 0.05$). Hence, the autumn meal with its high NH_3 response and relatively low TVN values, does not fit in.

The CO sensor demonstrated probably the most interesting changes with storage time (Figure 4.10). Apart from the high initial value of the winter meal, which can not be explained and was excluded from data analysis, all meal samples showed increasing responses with storage time ($p \leq 0.05$). The autumn meal had highest CO response ($p \leq 0.05$) until after four months of storage, where only spring meal was significantly lower. The CO sensor was the only sensor that demonstrated highest response for the autumn meal. The increase of CO response with time implies development of short

alcohols and carbonyls (Ólafsdóttir *et al*. 1997b), which could be products of secondary lipid oxidation in the meal.

The results of PCA on the data obtained from measurements of capelin meal with storage resulted in clear grouping of the capelin meal with season (Figures 4.11a and 4.11b). The first two principal components explained 80% of the variance of analyzed variables. The summer meal (M-Su) was situated in the left part of the biplot, the autumn meal (M-Au) in the upper-left part, the winter meal (M-Wi) in the higher-right part and finally the spring meal (M-Sp) in the lower-right corner of the diagram. Changes in the meal with storage were best described by PC2.

Changes with longer storage time, referred to as 0, 2 and 4 (months) were also seen in the samples trend with time towards those variables most associated with autumn meal. The PCA indicated that most of the changes took place form 1-2 months storage and e.g. autumn meal does not change from 2-4 months storage.

The summer meal with its clear grouping from other samples was most associated with FAA, WSP, Cu, FFA and TVN, as well as the volatiles NO, NH_3 and SO_2 (better seen in Figure 4.11b). The variables most associated with autumn meal were the rancidity measurements, TBA, oxygen uptake (overlapping in Figure 4.11a) and PV, but also changes in color, measured as BP and ΔE^*_{ab} as well as the color variables (L*, b*). The color variable a* was located nearer to the summer meal, indicating more red color of the redfeed rich summer meal. The variables most associated to winter meal were the proportion of acidic against basic FAA (A/B), the sum of the fatty acids C20:5+C22:5+C22:6 and the metals Fe and Zn.

The variables most associated with the spring meal were tocopherol and vitamin A, as well as a_w and water content.

The aqueous antioxidant activity (AA) was most associated to winter and spring meal. The variables most associated to aqueous AA with positive correlation were Zn (r^2 = 0.76) and A/B (r^2 = 0.68), but variables with negative correlation with AA were browning (r^2 = -0.64), TVN (r^2 = -0.69) and NH_3 (r^2 = -0.88). Although the proportion of acidic against basic amino acids (A/B) correlated positively with aqueous AA, separate amino acids all correlated negatively and insignificantly with aqueous AA. The PCA indicate that the instability of the autumn meal may be due to the lack of antioxidants and/or high content of PUFA's.

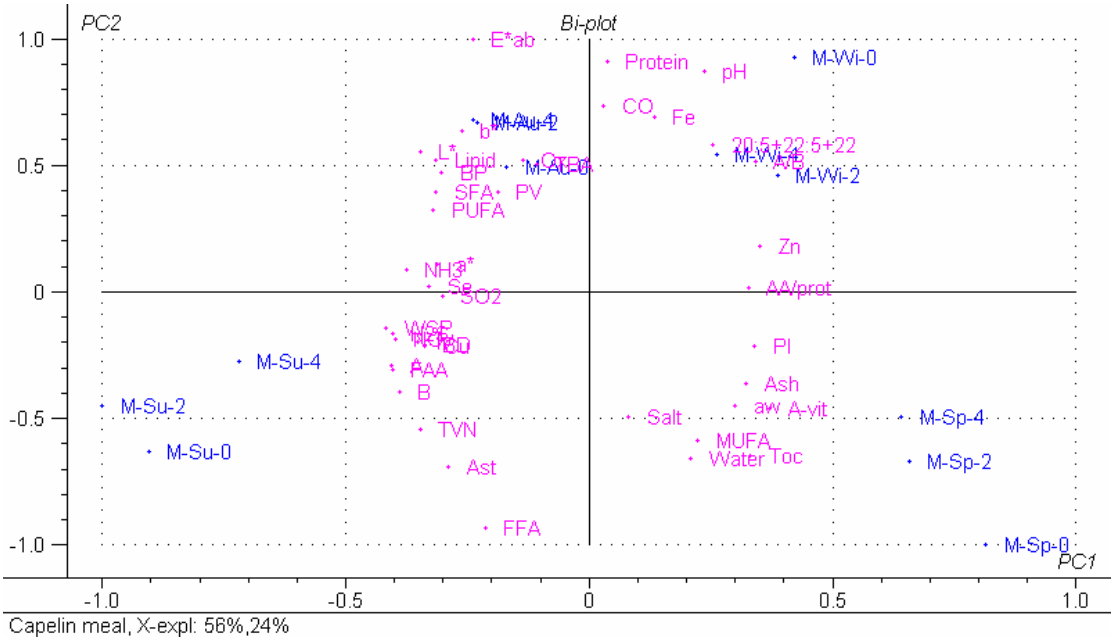

Figure 4.11a. PCA biplot of scores and lodings on data for capelin meal from different seasons during storage.

Abbreviations for the scores: M = meal, Su = summer, Au = autumn, Wi = winter, Sp = spring, 0, 2 and 4 = 0, 2 and 4 months of storage. Abbreviations for the loadings: A = acidic free amino acids, a = red color, AA = aqueous antioxidant activity, Ast = astaxanthin, A-vit = vitamin A, B = basic free amino acids, b* = yellow color, BD = bulk density, BP = brown pigments, E*ab = change in initial color, FAA = free amino acids, FFA = free fatty acids, IV = iodine value, L* = lightness, MUFA = monoene fatty acids, Ox = oxygen uptake, PI = polyene index, PUFA = polyene fatty acids, PV = peroxide value, SFA = saturated fatty acids, TBA = thiobarbituric acid value, Toc = α–tocopherol, TVN = total volatile nitrogen, WSP = water soluble protein.*

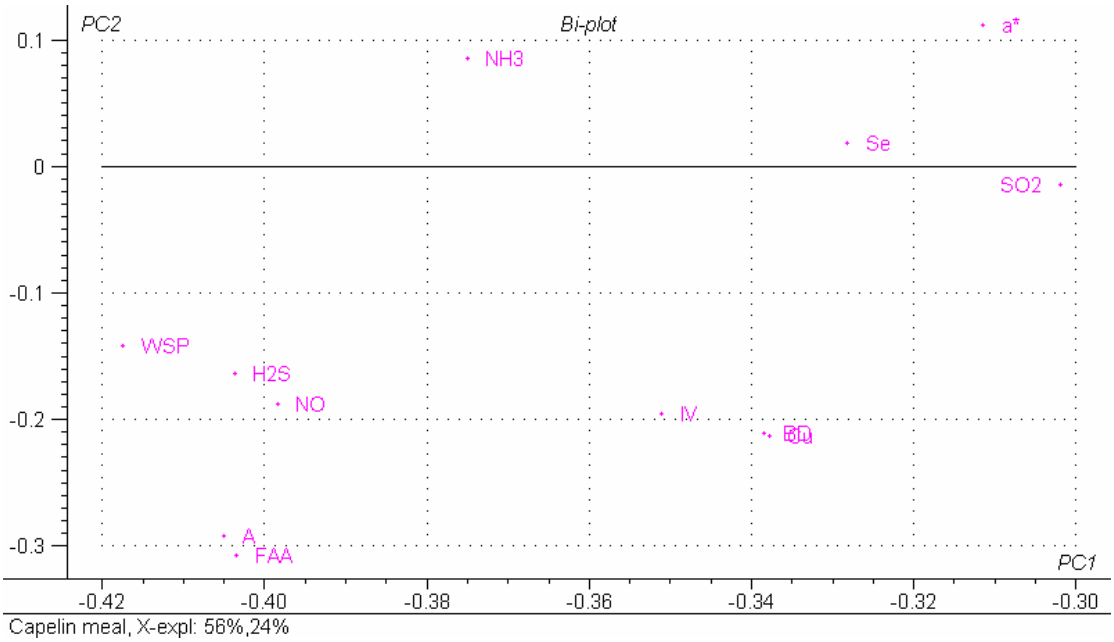

Figure 4.11b. Enlargement of the cluster part of the PCA in Figure 4.10a, on data for capelin meal from different seasons during storage. (BD and Cu overlap).

For abbreviations refer to Figure 4.11a.

The results of this study have confirmed that rancidity in capelin meal is a complex phenomena and inconsistent. PCA on 9 possible rancidity measurements in capelin meal resulted in a model where the first two principal components explained 64% of the variance (Figure 4.12), although the "validated explained" variance was rather low, implying that the model did not hold for new samples. Seasonal variation in the rancidity parameters was obvious with the more stable spring meal situated far apart from the rancidity parameters. Changes with time were most pronounced during the first two months of storage and much slower during the next two months, especially for the autumn meal where M-Au-2 and M-Au-4 were almost unchanged. However some rancidity parameters increased with time, while others decreased. Both oxygen uptake and peroxide value declined with time of storage and showed high correlation ($r^2 = 0.94$). Color changes increased with storage, as measured by ΔE*ab and brown pigments, that also showed high correlation ($r^2 = 0.93$).

Regression models were made in order to find out if any one or several of the rancidity measurements could predict the stability of capelin meal. ΔE*ab gave the best regression model (model not shown). PLS1 regression model with ΔE*ab against the other rancidity parameters resulted in PC1 that only explained 43% of the variance in the X-variables (FFA, TBA, PV, BP, lipid, CO and Ox) and 88% of the variance of the Y-variable (ΔE*ab). PC1 described the difference between sample season and PC2 the difference with storage time. PC2 only explained 19% of the variance of the X variables and 6% of the Y-variable. The residual variance of the model increased after PC1, so taking PC2 into the model, which mainly described the influence of the storage time, only made the model worse. Thus it was not possible to predict changes in other rancidity variables with storage time using ΔE*ab. Multivariate regression analysis was made on the data from fresh capelin and capelin meal, in order to test if the tocopherol or astaxanthin content could be used to predict the stability of capelin meal. No reasonable models were obtained for either tocopherol or astaxanthin, whether made individually (PLS1) or combined (PLS2) (results not shown).

The same can be said about the content of prooxidants. The models failed to show trends that were not explained by the general differences between whole capelin and capelin meal.

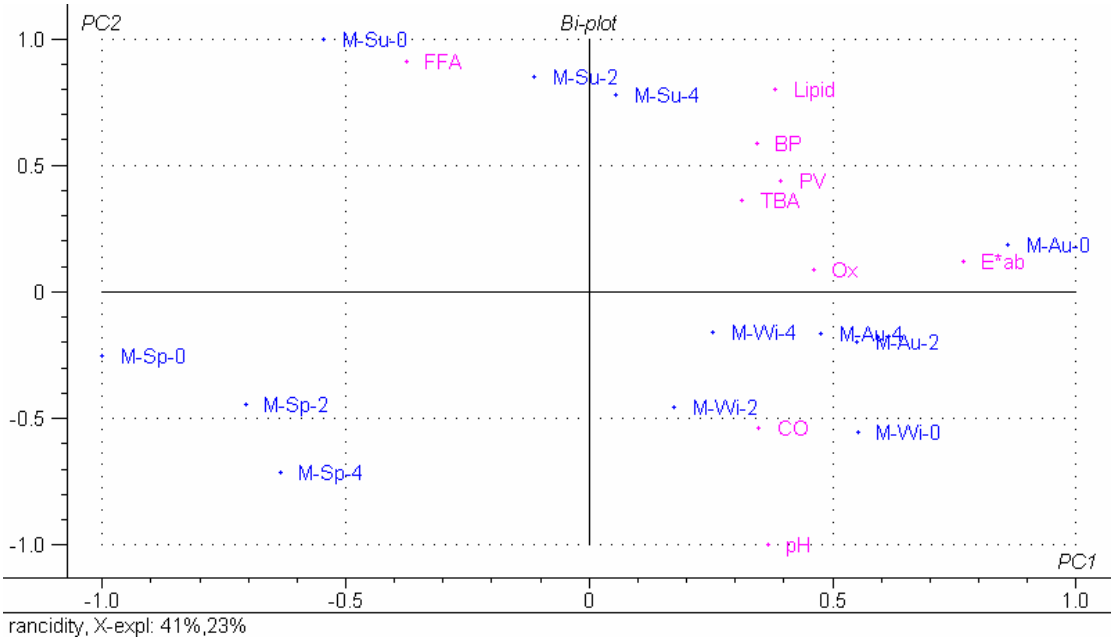

Figure 4.12. PCA biplot of scores and lodings on rancidity parameters of capelin meal from different seasons.

*Abbreviations for the scores: M = meal, Su = summer, Au = autumn, Wi = winter, Sp = spring. 0, 2 and 4 = 0, 2 and 4 months of storage. Abbreviations for the loadings: BP = brown pigments, FFA = free fatty acids, TBA = thiobarbituric acid, PV = peroxide value, E*ab = change in initial color, Ox = oxygen uptake.*

Comparing the overall stability of the capelin meal from different seasons it was clear that the instability of 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 autumn meal than in meal at other seasons. Ether extractable lipid changed within 1% in all meal samples, except for the autumn meal, where the extractable lipid decreased significantly by 3.9% during the four months storage. The aqueous antioxidant activity was also found to be lowest in the autumn meal. Both autumn capelin and its corresponding meal contained the lowest levels of the natural antioxidants α-tocopherol and astaxanthin compared to other seasons. Degradation of astaxanthin during storage was also most pronounced for the autumn meal, were 60% of the total astaxanthin was lost during four months of storage, compared to 20-40% for other seasons. The α-tocopherol did not show such decline with storage time, except for the spring meal. The most reasonable explanation for no decrease at other seasons was that then the content of α-tocopherol was very low, making it difficult to measure precisely, as the values were near the limit of detection (approx. 10 mg/kg). Although the vitamin A content was also very

low it showed decrease with time, where the autumn meal lost more than meal from other seasons.

5. SUMMARY AND CONCLUSIONS

Endogenous anti- and prooxidants, as well as lipid content and composition were highly seasonal in capelin. The overall stability during landing including the stability of endogenous antioxidants was very restricted by dynamic spoilage processes in capelin.

Endogenous lipid soluble antioxidants withstood capelin meal processing and were most often found in up to half the content of the raw material. The total content of prooxidant metals Fe and Cu were considerably higher in capelin meal compared to raw capelin. High peroxide values were associated with high content of prooxidants as well as high iodine value and polyene index and low tocopherol content. TBARS did not show similar pattern.

The stability of capelin meal was influenced by seasonal changes in the capelin. Autumn capelin high in fat and PUFA, as well as low in lipid soluble antioxidants and aqueous antioxidant activity produced the least stable capelin meal. Changes during storage of capelin meal in general were more rapid during the first two months of storage, than during the following two month's.

Methods most suitable for measuring changes of capelin meal with storage were those that measure tertiary lipid oxidation products such as color change and brown pigments. Electronic nose measurements with CO sensor gave also promising results.

6. SUGGESTIONS FOR FURTHER WORK

Future work in the field of stability of capelin meal is needed. It is necessary to find a suitable criterion for the stability of capelin meal and fish meal in general. Satisfactory testing of known rancidity measurements, especially those that measure tertiary lipid oxidation products is needed to reveal if they can be used for quality measurements on fish meal.

Another future aspect for the stability of capelin meal is to examine its aqueous antioxidant activity. It is important to find the cause of this antioxidant activity,

whether is may be influence by processing methods and if it is to be found in fish meal from other fish species as well.

Finally, further work is needed on fish meal from other types of pelagic fish species to compare with the capelin meal. However, in order to compare different types of fish meal it is essential to establish a suitable criterion for the stability of fish meal. Whether suitable criterion of fish meal stability lies in the content of oxidation products, or the content of anti- or prooxidants, is yet to be discovered.

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APPENDICES

Appendix 1. Results of measurements in whole capelin

Table A.1. Analytical results of whole capelin.

Group	Time	repl.	Water (%)	Lipid (%)	Protein (%)	Salt (%)	Ash (%)	IV	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Se (mg/kg)
Su	1	1	74.97	9.73	13.16	0.62	1.67	138.05	11.63	2.27	13.57	0.31
Su	1	2	74.89	9.75	13.14	0.62	1.61	139.58	11.78	1.33	13.42	0.32
Su	1	3	74.89	9.74	13.14	0.62	1.59	139.22	12.01	2.72	14.03	0.36
Su	1	4								1.76		0.35
Au	1	1	71.30	13.90	13.01	0.42	1.67	130.91	15.23	0.67	17.26	0.34
Au	1	2	71.30	13.80	12.99	0.42	1.56	131.66	13.61	0.67	14.52	0.31
Au	1	3	71.30	14.00	12.81	0.39	1.54	130.31	14.63	0.69	15.72	0.32
Au	1	4							14.64	0.72	15.03	0.32
Wi	1	1	76.16	8.34	13.97	0.54	1.82	121.49	14.39	0.51	16.80	0.41
Wi	1	2	76.11	8.37	13.74	0.54	1.94	123.40	14.10	0.61	16.60	0.38
Wi	1	3	76.16	8.22	13.94	0.54	1.84		15.09	0.47	16.63	0.41
Wi	1	4										
Sp	1	1	82.17	3.43	12.51	0.72	1.81	117.92	6.76	0.48	15.50	0.35
Sp	1	2	82.19	2.91	12.43	0.72	1.73	118.14	6.66	0.44	15.69	0.34
Sp	1	3	82.15	2.95	12.44	0.72	1.72	119.04	7.75	0.47	14.60	0.34
Sp	1	4							7.68		16.41	

Table A.1 -continued. Analytical results of whole capelin.

Group	Time	repl.	Count	B&D Lipid (%)	pH	FFA (%)	TBA (mg MA/kg)	PV (meq/kg)	TVN (mg N/100g)	Astaxanthin (mg/kg)	A-diester (mg/kg)	A-diester (%)	A-monoe. (mg/kg)	A-monoe. (%)	Free astax (mg/kg)	Free astax. (%)	A-vit (mg/kg)	Tocopherol (mg/kg)
Su	1	1	50	9.58	6.82	2.42	2.31	1.66	114.50	93.94	49.45	52.65	9.17	9.76	35.31	37.59	57	140
Su	1	2		9.38	6.82	2.36	2.66	2.64	114.90	96.62	48.97	50.68	9.29	9.62	38.36	39.71	30	110
Su	1	3		9.61	6.87	2.41	2.56	0.67	113.50	91.84	49.10	53.46	10.36	11.28	32.38	35.26	44	125
Su	1	4		9.80														
Su	2	1	50	9.07	7.00	3.05	3.76	2.42	116.90	72.51	20.24	27.91	21.94	30.27	30.32	41.82	24	110
Su	2	2		9.28	7.02	3.03	3.34	1.29	117.70	70.69	18.31	25.90	18.67	26.41	33.71	47.69	21	70
Su	2	3		9.34	7.04	3.13	3.37	1.50	117.90	71.60	19.27	26.91	20.31	28.34	32.02	44.76	23	90
Su	2	4		9.41														
Au	1	1	57	13.33	7.08	1.36	4.86	2.10	25.10	3.52	1.75	49.83	1.05	29.77	0.72	20.40	21	70
Au	1	2		13.32	7.07	1.43	5.33	3.25	25.90	2.72	0.98	35.93	1.05	38.53	0.70	25.54	18	70
Au	1	3		13.26	7.09	1.44	5.24	2.41	24.70	3.12	1.37	42.88	1.05	34.15	0.71	22.97	20	70
Au	1	4		13.37				1.45										
Au	2	1	66	12.08	7.16	1.55	6.49	1.35	39.60	4.44	1.88	42.31	1.48	33.33	1.08	24.36	21	60
Au	2	2		12.62	7.16	1.55	6.99	1.46	39.10	3.32	0.81	24.26	1.34	40.44	1.17	35.29	21	60
Au	2	3		12.62	7.16	1.51	6.68	1.64	39.70	3.87	1.23	31.63	1.42	36.75	1.23	31.63	21	60
Au	2	4																
Wi	1	1	55	8.36	6.94	1.95	3.21	1.54	21.73	4.67	2.56	54.83	0.90	19.32	1.21	25.85	30	100
Wi	1	2		8.42	6.95	1.95	3.10	1.50	22.83	5.22	2.55	48.93	1.04	20.00	1.62	31.07	33	110
Wi	1	3		8.60	6.94	1.90	3.50	1.85	23.32	4.88	2.10	42.94	1.02	20.84	1.77	36.22	33	110
Wi	1	4		8.41														
Wi	2	1	50	8.39	6.95	1.95	2.29	0.77	22.06	3.88	1.12	28.92	1.16	29.75	1.60	41.31	27	90
Wi	2	2		8.67	6.96	1.89	2.53	0.67	22.86	5.18	2.04	39.33	1.06	20.43	2.08	40.24	33	110
Wi	2	3		8.46	6.96	1.93	2.36	0.52	23.11	5.03	1.79	35.56	0.78	15.56	2.46	48.90	33	110
Wi	2	4		8.55														
Sp	1	1	78	3.40	6.92	3.36	2.67	1.95	22.48	6.69	5.34	79.79	0.62	9.33	0.73	10.88	66	390
Sp	1	2		3.30	6.94	3.31	2.93	2.51	22.41	11.84	8.83	74.59	1.35	11.43	1.66	13.98	66	390
Sp	1	3		3.20	6.93	3.35	2.66	1.90	22.64	12.93	8.95	69.23	1.95	15.10	2.03	15.67	66	390
Sp	1	4		3.29														
Sp	2	1	81	3.29	7.09	3.90	3.01	2.59	43.54	6.20	5.01	80.70	0.58	9.36	0.62	9.94	48	230
Sp	2	2		3.30	7.11	4.19	3.24	1.75	44.35	9.10	6.21	68.24	1.21	13.33	1.68	18.43	38	230
Sp	2	3		3.41	7.11	4.19	3.13	1.87	44.31	11.54	8.36	72.46	1.00	8.68	2.18	18.86	27	230
Sp	2	4		3.37														

Table A.2. Fatty acids (%) in the lipids of whole capelin.

	Month											
	Su	Su	Su	Au	Au	Au	Wi	Wi	Wi	Sp	Sp	Sp
F.a./repl.	1	2	3	1	2	3	1	2	3	1	2	3
14:0	6.5	6.1	6.3	6.2	6.1	6.3	7.5	8.0	7.0	6.4	6.1	6.2
15:0	0.3	0.3	0.3	-	-	-	-	-	-	-	-	-
16:0	14.1	13.1	13.6	14.4	13.6	14.4	13.6	14.5	12.6	11.0	10.5	10.8
16:1 (n7)	7.3	6.4	6.9	7.1	7.0	7.3	7.9	8.4	7.4	5.4	5.1	5.3
16:1 (n5)	0.5	0.4	0.5	-	-	-	-	-	-	-	-	-
16:2 (n4)	0.7	1.0	0.9	0.4	0.4	0.4	0.6	0.6	0.6	0.7	0.7	0.7
16:3 (n4)	0.4	0.4	0.4	-	-	-	0.2	0.2	0.2	-	-	-
16:4 (n1)	0.8	0.6	0.7	-	-	-	-	-	-	-	-	-
18:0	1.3	1.2	1.3	1.3	1.3	1.3	1.4	1.5	1.3	1.4	1.4	1.4
18:1 (n9)	10.3	9.9	10.1	12.6	12.2	12.8	13.6	13.9	12.6	12.1	11.6	11.9
18:1 (n7)	3.5	2.8	3.2	3.4	3.2	3.4	3.6	3.7	3.3	3.2	3.0	3.1
18:1 (n5)	0.6	0.5	0.6	0.6	0.5	0.6	0.5	0.6	0.5	0.4	0.4	0.4
18:2 (n6)	1.6	1.5	1.6	1.5	1.5	1.6	1.6	1.6	1.5	1.3	1.3	1.3
18:3 (n3)	0.7	0.6	0.7	0.7	0.7	0.7	0.6	0.6	0.5	0.4	0.4	0.4
18:4 (n3)	6.2	5.5	5.9	3.4	3.4	3.6	2.5	2.5	2.4	1.3	1.2	1.3
20:0	0.5	0.1	0.3	0.6	0.6	0.6	-	-	-	-	-	-
20:1 (n9+11)	10.6	11.6	11.1	10.3	10.8	10.7	13.7	13.2	13.6	15.4	15.2	15.3
20:1 (n7)	0.5	0.5	0.5	0.5	0.5	0.4	0.7	0.7	0.7	0.8	0.8	0.8
20:2 (n6)	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2
20:4 (n6)	0.3	0.2	0.3	0.4	0.3	-	0.3	0.3	0.3	0.4	0.4	0.4
20:4 (n3)	0.5	0.3	0.4	0.5	0.4	0.5	0.4	0.4	0.4	0.3	0.3	0.3
20:5 (n3)	8.3	7.6	8.0	8.4	8.2	8.6	6.3	6.2	6.0	6.2	6.1	6.2
21:5 (n3)	0.4	0.4	0.4	-	0.3	-	0.2	0.2	0.2	-	-	-
22:1 (n9,11,13)	12.1	15.3	13.7	13.8	15.1	15.0	14.5	13.6	16.6	18.7	19.3	19.0
22:5 (n3)	0.4	0.6	0.5	0.5	0.5	0.5	0.4	0.4	0.5	0.6	0.5	0.6
22:6 (n3)	7.9	8.6	8.3	9.3	9.3	9.2	5.8	5.5	6.3	9.1	9.2	9.2
24:1	-	-	-	0.7	0.7	0.7	0.5	0.5	0.8	1.2	1.3	-

Table A.3. Free amino acids (g/kg) in whole capelin.

Month	time	repl.	ASP	GLU	ASN	SER	GLN	HIS	GLY	THR	ARG	TAU	ALA	TYR	MET	VAL	PHE	ILE	LEU	LYS	TOTAL	Acidic	Basic	A/B
Su	1	1	593	1129	0	334	592	607	475	706	1050	1538	926	407	389	721	637	496	1277	1277	13152	2762	2934	0.94
Su	1	2	596	1149	0	361	610	594	523	732	1065	1602	962	369	409	754	667	522	1342	1217	13474	2838	2877	0.99
Su	1	3	587	1137	0	373	602	584	524	733	1077	1630	963	385	385	764	683	521	1365	1301	13612	2830	2962	0.96
Su	2	1	216	1361	15	146	688	652	680	893	268	1642	1238	343	500	913	853	628	1693	1185	13912	2616	2104	1.24
Su	2	2	182	1351	7	92	704	642	657	868	112	1600	1235	345	506	875	839	617	1672	1167	13471	2493	1921	1.30
Su	2	3	223	1357	17	241	702	698	724	903	132	1597	1258	379	517	903	870	618	1675	1130	13944	2725	1959	1.39
Au	1	1	293	497	0	149	200	0	313	255	421	1043	590	195	128	271	231	197	462	1007	6253	1194	1428	0.84
Au	1	2	235	529	0	151	199	0	324	267	427	1145	582	200	146	288	256	238	607	903	6495	1182	1330	0.89
Au	1	3	237	533	0	152	206	0	326	268	459	1170	618	213	154	295	286	295	606	1103	6921	1190	1561	0.76
Au	2	1	226	509	0	117	195	0	318	287	313	1447	612	226	176	323	312	224	475	608	6369	1139	921	1.24
Au	2	2	235	528	0	121	202	0	330	297	324	1499	634	234	182	335	323	232	492	630	6598	1180	954	1.24
Au	2	3	236	540	0	118	200	0	333	301	376	1567	634	235	186	345	346	266	708	1281	7673	1196	1657	0.72
Wi	1	1	111	421	0	127	131	0	218	183	293	1680	535	108	105	185	121	107	211	268	4805	843	561	1.50
Wi	1	2	119	431	0	112	135	0	221	181	303	1801	532	112	130	169	125	105	206	293	4976	843	596	1.41
Wi	1	3	115	416	0	112	130	0	215	171	301	1709	517	108	104	182	123	97	200	288	4787	813	588	1.38
Wi	2	1	91	402	0	105	98	0	197	164	224	1576	517	101	94	178	97	92	201	329	4465	763	553	1.38
Wi	2	2	108	472	0	124	113	0	230	187	255	1772	540	107	138	172	146	116	232	372	5084	891	627	1.42
Wi	2	3	107	489	0	128	112	40	233	191	281	1817	550	108	132	175	145	119	237	373	5234	914	694	1.32
Sp	1	1	103	260	0	108	149	0	163	168	291	1260	385	30	119	159	115	114	213	374	4012	640	665	0.96
Sp	1	2	98	252	0	113	144	0	159	160	248	1257	379	93	120	153	121	116	210	368	3992	624	616	1.01
Sp	1	3	94	230	0	107	133	40	147	146	115	1193	348	83	109	151	105	98	192	355	3645	576	510	1.13
Sp	2	1	85	242	0	92	126	0	155	121	0	1561	393	45	97	178	114	101	221	297	3829	540	297	1.82
Sp	2	2	87	251	0	91	135	0	162	127	45	1422	394	46	104	184	119	102	217	329	3814	556	373	1.49
Sp	2	3	79	218	0	78	116	0	146	107	0	1380	361	0	88	161	109	91	190	326	3449	482	326	1.48

Abbreviations: ASP: Aspartic acid, GLU: Glutamic acid, ASN: Aspargine, SER: Serine, GLN: Glutamine, HIS: Histidine, GLY Glycine, THR: Threonine, ARG: Arginine, TAU: Taurine, ALA Alanine, TYR: Tyrosine, MET: Methionine, VAL: Valine, PHE: Phenylalanine, ILE: Isoleucine, LEU: Leucine, LYS: Lysine, A/B: acidic/basic amino acids.

Appendix 2. Results of measurements in capelin meal

Table A.4. Analytical results of capelin meal.

Group	month	repl.	BD (g/mL)	PD (g/mL)	aw	Water (%)	Protein (%)	Salt (%)	Ash (%)	IV	Sieve-30 (%)	Sieve-50 (%)	Sieve-70 (%)	Sieve-mt 70 (%)	Iron (mg/kg)	Copper (mg/kg)	Selen (mg/kg)	Zinc (mg/kg)
Su	0	1	0.597	0.728	0.35	7.00	70.30	2.60	8.80	162.5	24.64	28.16	30.68	16.30	77.7	5.19	2.13	63.00
Su	0	2	0.593	0.732	0.32	7.00	70.35	2.58	8.82	157.0	22.18	27.22	40.30	10.46	74.6	5.20	1.99	54.08
Su	0	3	0.600	0.732	0.33	7.02	70.24	2.56	8.89	162.0					74.5	5.06	1.90	61.15
Su	0	4	0.589	0.727														
Su	0	5	0.592	0.722														
Au	0	1	0.544	0.697	0.35	6.70	71.10	3.15	9.14	153.6	15.64	22.94	13.16	48.20	109.8	3.81	1.81	80.74
Au	0	2	0.545	0.690	0.32	6.84	70.94	3.20	9.06	153.1	16.12	23.38	13.28	47.48	109.5	2.81	1.70	82.40
Au	0	3	0.542	0.695	0.33	6.72	71.17	3.22	9.26	150.6					104.1	2.95	1.61	81.51
Au	0	4	0.539	0.691											110.2	2.98		
Au	0	5	0.544	0.697														
Wi	0	1	0.451	0.635	0.35	6.64	71.71	1.98	9.45	147.0	11.86	22.50	28.32	37.44	190.6	2.34	1.60	96.68
Wi	0	2	0.446	0.646	0.37	6.60	71.87	2.02	9.35	147.4					173.5	2.18	1.77	93.50
Wi	0	3	0.463	0.644	0.36	6.60	71.57	2.02	9.27	146.8	11.88	23.96	34.60	29.88	191.1	2.56	1.57	98.21
Wi	0	4	0.443	0.641														
Wi	0	5	0.443	0.641														
Sp	0	1	0.474	0.616	0.44	9.12	69.26	3.48	10.30	147.2	17.54	28.28	23.18	30.70	85.1	2.20	1.36	94.42
Sp	0	2	0.468	0.616	0.41	8.92	69.78	3.42	10.31	146.4	17.72	28.38	22.46	31.46	83.5	2.21	1.33	96.20
Sp	0	3	0.470	0.611	0.42	9.02	69.62	3.43	10.35	147.2					83.1	2.22	1.34	95.17
Sp	0	4	0.470	0.618											83.9	2.22		97.17
Sp	0	5	0.466	0.621														

Table A.4 -continued. Analytical results of capelin meal.

Group	month	repl.	Lipid (%)	B&D Lipid (%)	WSP (%)	Prot/filt (mg/mL)	AA	BP	pH	FFA (%)	TBA nf MA/kg	PV (meq/kg)	TVN mg N/100 g	Astaxanthin (mg/kg)	A-diester (mg/kg)	A-diester % (%)	A-monoester (mg/kg)	A-monoester % (%)	Free astax (mg/kg)	Free astax % (%)
Su	0	1	11.91	13.71	32.06	1.13	2.075	0.550	6.38	11.64	1.04	4.86	107.80	47.63	8.06	16.92	17.47	36.68	22.10	46.40
Su	0	2	11.88	13.59	32.03	1.13	2.078	0.546	6.31	11.84	1.86	3.18	108.00	46.70	6.13	13.12	19.45	41.64	21.10	45.18
Su	0	3	11.90	13.76	31.64	1.11	2.125	0.537	6.40	11.71	2.84	5.03	107.50	49.10	8.31	16.93	20.15	41.05	20.63	42.03
Su	0	4																		
Su	2	1	11.64	13.53		1.14	1.502	0.716	6.34	11.38	1.79	3.06	112.80	35.30	1.66	4.71	18.22	51.60	15.42	43.69
Su	2	2	11.71	13.38	31.85	1.12	1.528	0.729	6.36	11.50	2.03	4.94	115.40	34.42	1.79	5.21	18.29	53.14	14.34	41.65
Su	2	3	11.70	13.57	31.85	1.12	1.486	0.727	6.36	11.43	1.92	5.94	114.70	35.66	1.77	4.97	19.00	53.29	14.88	41.74
Su	2	4																		
Su	4	1	11.80	13.85	31.61	1.11	1.456	0.819	6.36	11.51	2.40	3.20	115.50	28.51	1.58	5.55	14.95	52.43	11.98	42.03
Su	4	2	11.86	13.68	31.34	1.10	1.445	0.799	6.37	11.17	2.20	4.57	115.10	28.75	1.52	5.29	15.10	52.51	12.13	42.20
Su	4	3	11.68	13.95	31.55	1.10	1.440	0.815	6.38	11.41	2.57	3.41	117.20	28.69	1.61	5.61	15.38	53.63	11.69	40.77
Su	4	4																		
Au	0	1	10.76	13.00	24.43	0.875	1.023	0.453	6.83	9.23	2.38	10.02	61.70	4.06	1.73	42.70	1.35	33.34	0.97	23.96
Au	0	2	11.00	13.14	24.67	0.882	0.993	0.459	6.84	9.13	2.38	8.82	62.25	5.94	3.18	53.53	1.59	26.75	1.17	19.72
Au	0	3	10.86	13.46	24.31	0.869	0.973	0.459	6.83	9.11	2.15	12.63	62.92	5.11	2.86	56.00	1.35	26.41	0.90	17.59
Au	0	4																		
Au	2	1	10.50	13.04	23.41	0.832	2.066	0.833	6.84	8.00	1.34	6.47	79.00	3.29	1.44	43.59	1.01	30.77	0.84	25.64
Au	2	2	10.76	13.04	23.46	0.882	1.938	0.838	6.82	8.00	1.41	6.23	80.45	3.34	1.73	51.90	0.93	27.85	0.68	20.25
Au	2	3	10.76	12.88	23.53	0.841	2.014	0.831	6.83	8.09	1.46	6.32	79.48	3.49	1.58	45.23	1.15	33.00	0.76	21.77
Au	2	4																		
Au	4	1	10.34	12.77	23.96	0.855	1.032	0.916	6.76	8.20	1.21	4.53	79.21	1.90	0.95	50.03	0.65	34.09	0.30	15.94
Au	4	2	10.38	13.16	23.86	0.853	1.040	0.958	6.76	8.16	1.45	4.07	80.16	1.93	0.84	43.48	0.71	36.99	0.38	19.58
Au	4	3	10.64	13.10	23.81	0.846	1.045	0.920	6.77	9.00	1.51	4.37	81.99	2.27	1.14	49.93	0.80	35.15	0.34	14.96
Au	4	4																		
Wi	0	1	11.56	14.22	14.25	0.511	5.151	0.397	6.90	7.33	1.62	5.65	46.66	6.61	2.98	45.02	1.39	21.05	2.24	33.91
Wi	0	2	11.36	14.21	13.98	0.499	4.870	0.404	7.04	7.33	2.47	5.25	45.85	8.51	3.87	45.45	1.62	19.09	3.02	35.46
Wi	0	3	11.50	14.04	13.75	0.496	5.444	0.376	7.05	7.42	2.41	5.31	45.57	7.79	3.05	39.19	1.25	16.08	3.48	44.72
Wi	0	4																		
Wi	2	1	11.28	14.02	13.83	0.498	3.961	0.577	6.95	7.06	2.04	3.64	50.63	4.20	2.43	57.95	0.90	21.50	0.86	20.56
Wi	2	2	11.36	14.23	13.78	0.496	3.921	0.565	6.98	7.14	2.05	3.14	49.41	5.72	2.90	50.68	1.16	20.27	1.66	29.06
Wi	2	3	11.28	14.02	13.47	0.483	4.084	0.575	6.82	7.06	2.13	1.38	48.15	6.55	2.94	44.91	1.41	21.56	2.20	33.53
Wi	2	4			13.30	0.478			7.00											
Wi	4	1	11.34	13.71	13.61	0.490	4.307	0.556	6.71	7.34	3.89	3.20	54.48	5.34	2.97	55.64	1.53	28.57	0.84	15.79
Wi	4	2	11.40	13.88	13.19	0.473	4.327	0.572	6.71	7.24	4.06	2.90	56.28	5.95	3.17	53.33	1.59	26.67	1.19	20.00
Wi	4	3	11.34	13.72	13.23	0.478	4.504	0.572	6.72	7.43	3.67	3.05	56.96	6.38	3.25	50.96	1.46	22.93	1.67	26.11
Wi	4	4			13.22	0.473			6.71		3.82									
Sp	0	1	8.42	10.74	13.43	0.467	4.424	0.250	6.46	9.81	1.36	2.72	60.28	13.40	8.59	64.11	1.72	12.94	3.09	22.95
Sp	0	2	8.40	10.79	13.60	0.474	4.504	0.265	6.49	10.37	0.85	2.73	60.63	12.56	8.05	64.08	1.80	14.29	2.72	21.63
Sp	0	3	8.26	10.74	13.14	0.457	4.343	0.252	6.70	10.79	0.81	2.87	60.22	14.23	9.13	64.13	1.65	11.60	3.45	24.28
Sp	0	4			13.86	0.483			6.47											
Sp	2	1	8.62	10.55	14.02	0.489	3.065	0.324	6.51	10.14	1.43	3.90	75.42	11.48	6.96	60.64	1.66	14.48	2.86	24.89
Sp	2	2	8.60	10.75	13.20	0.462	3.363	0.312	6.70	9.95	1.06	3.26	76.02	12.03	8.11	67.40	1.43	11.87	2.50	20.77
Sp	2	3	8.54	10.75	14.40	0.501		0.312	6.69	9.95	0.73	3.59	74.92	11.45	7.55	65.91	1.22	10.69	2.68	23.39
Sp	2	4							6.48											
Sp	4	1	8.44	10.26	12.79	0.444	3.464	0.31	6.76	10.22	1.07	2.62	76.12	9.49	6.45	67.96	1.83	19.33	1.21	12.70
Sp	4	2	8.41	10.83	12.47	0.433	3.610	0.28	6.73	9.68	0.94	2.51	76.12	10.87	7.20	66.21	2.23	20.54	1.44	13.24
Sp	4	3	8.41	10.72	12.53	0.435	3.561	0.28	6.73	9.76	0.85	2.89	77.11	11.36	7.50	66.08	1.95	17.18	1.90	16.74
Sp	4	4									0.76									

Table A.4 -continued. Analytical results of capelin meal.

Group	month	repl.	A-vit (mg/kg)	Tocopherol (mg/kg)	Lab*-L	Lab*-a	Lab*-b	DE*ab	Oxygen uptake (µ mol O2/g)	EN-CO (nA)	EN-H2S (nA)	EN-NO (nA)	EN-SO2 (nA)	EN-NH3 (nA)
Su	0	1	12	20	49.82	1.19	12.99			13.16	517.02	424.74	42.46	2291.75
Su	0	2	18	50	50.18	1.12	12.69			139.82	511.75	542.11	91.05	2464.74
Su	0	3	6	30	50.60	1.12	12.59							
Su	0	4												
Su	2	1	9	40	51.71	1.00	13.52	1.97	11.95	506.32	794.74	754.74	110.88	1869.30
Su	2	2	9	40	52.18	0.95	13.23	2.08	10.54	482.11	682.81	662.98	143.68	2215.61
Su	2	3	9	40	52.31	0.94	13.17	1.81	11.87	439.12	632.11	637.54	140.70	2131.23
Su	2	4							12.0					
Su	4	1	15	40	52.65	0.85	13.52	2.90	12.13	571.05	414.56	375.26	55.79	1492.46
Su	4	2	18	50	52.53	0.77	13.33	2.46	12.18	614.74	425.79	391.75	54.21	1721.93
Su	4	3	9	40	52.50	0.80	13.62	2.18	11.03					
Su	4	4							15.19					
Au	0	1	15	30	51.35	0.37	11.92		33.67	466.49	345.96	306.49	50.18	2152.63
Au	0	2	15	30	51.38	0.39	11.87		35.46	446.67	350.53	332.28	49.82	2066.67
Au	0	3	12	20	51.88	0.37	11.53		32.47					
Au	0	4							35.07					
Au	2	1	12	30	49.96	0.46	14.69	3.10	19.06	617.78	377.78	341.67	72.78	2031.67
Au	2	2	12	30	50.61	0.45	14.46	2.70	18.78	697.02	448.42	387.89	94.21	2317.72
Au	2	3	9	40	51.09	0.50	14.38	2.96	22.78					
Au	2	4												
Au	4	1	6	50	51.44	0.49	15.22	3.31	15.92	729.12	404.04	359.30	101.05	1846.32
Au	4	2	6	50	51.07	0.50	14.99	3.13	15.70	691.23	366.67	330.53	104.91	1938.07
Au	4	3	9	50	51.44	0.48	14.94	3.44	15.86	644.21	376.32	344.21	93.16	1907.89
Au	4	4												
Wi	0	1	33	50	49.93	0.58	12.01		17.93	516.49	158.07	155.79	26.67	673.68
Wi	0	2	36	50	50.90	0.89	11.37		17.58	558.77	151.23	137.02	21.93	718.60
Wi	0	3	33	60	50.89	0.88	11.38		20.41		163.16	143.51	24.74	774.04
Wi	0	4												
Wi	2	1	24	70	47.94	0.52	13.91	2.75	10.21	448.07	215.96	162.11	37.72	739.82
Wi	2	2	24	80	49.69	0.64	13.46	2.42	7.77	436.84	204.56	158.07	50.00	847.19
Wi	2	3	30	80	50.68	0.71	13.02	1.66	12.90					
Wi	2	4												
Wi	4	1	21	60	49.76	0.70	14.24	2.23	8.34	605.79	190.00	141.58	51.75	737.89
Wi	4	2	21	60	49.79	0.80	13.72	2.60	8.16	636.67	200.00	148.25	57.02	908.77
Wi	4	3	21	60	50.58	0.87	13.02	1.68	8.18					
Wi	4	4							9.62					
Sp	0	1	54	180	45.74	-0.05	10.53		11.48	204.21	172.11	166.67	30.00	554.56
Sp	0	2	54	160	46.65	0.15	9.77		7.67	214.21	175.79	166.14	31.75	674.04
Sp	0	3	54	160	47.70	0.40	9.50		10.23	203.51	199.30	181.93	34.56	702.63
Sp	0	4							10.42					
Sp	2	1	36	160	45.95	0.12	10.85	0.42	8.86	511.23	185.09	121.23	45.96	872.46
Sp	2	2	39	140	47.05	0.24	10.60	0.92	10.58	538.42	166.49	118.60	46.67	820.00
Sp	2	3	39	150	46.99	0.34	10.33	1.10	7.14					
Sp	2	4							7.85					
Sp	4	1	36	120	46.60	0.25	10.80	0.96	7.88	551.93	175.26	156.84	49.12	783.86
Sp	4	2	39	105	46.77	0.41	10.89	1.15	7.70	579.12	186.14	166.32	55.96	846.67
Sp	4	3	42	90	46.65	0.50	10.52	1.47	7.79					
Sp	4	4												

Table A.5. Fatty acids (%) in capelin meal lipids.

	Month											
F.a./repl.	Su 1	Su 2	Su 3	Au 1	Au 2	Au 3	Wi 1	Wi 2	Wi 3	Sp 1	Sp 2	Sp 3
14:0	4.9	4.3	4.5	4.2	4.1	4.2	3.9	3.9	4.4	4.3	4.3	4.3
15:0	0.3	0.2	0.3	-	-	-	-	-	-	-	-	-
16:0	16.1	15.1	15.5	15.5	15.5	15.8	15.6	15.7	16.6	14.0	13.9	14.0
16:1 (n7)	5.5	4.7	4.9	5.0	5.0	5.1	5.4	5.4	5.9	5.8	4.5	5.2
16:1 (n5)	0.4	0.4	0.4	-	-	-	-	-	-	-	-	-
16:2 (n4)	0.7	1.0	0.5	0.3	0.3	0.4	0.3	0.3	0.3	0.5	0.5	0.5
16:3 (n4)	0.3	0.4	0.3	-	-	-	0.1	0.2	0.2	-	0.1	0.1
16:4 (n1)	0.6	0.4	0.4	0.3	0.2	0.2	-	-	-	-	-	-
18:0	1.6	1.7	1.7	1.7	1.7	1.7	1.6	1.6	1.7	1.9	1.9	1.9
18:1 (n9)	9.1	8.9	9.1	10.3	10.4	10.5	11.2	11.2	11.2	11.2	11.1	11.2
18:1 (n7)	3.4	2.8	2.9	3.0	3.1	3.1	3.4	3.3	3.6	3.5	3.5	3.5
18:1 (n5)	0.6	0.5	0.6	0.5	0.5	0.5	0.5	0.4	0.5	0.4	0.5	0.5
18:2 (n6)	1.5	1.2	1.2	1.3	1.3	1.3	1.3	1.3	1.4	1.4	1.4	1.4
18:3 (n3)	0.6	0.5	0.6	0.6	0.6	0.5	0.5	0.5	0.6	0.4	0.4	0.4
18:4 (n3)	4.8	4.2	4.4	2.4	2.4	2.4	2.0	2.0	2.2	1.3	1.3	1.3
20:0	0.4	0.1	0.1	0.4	0.4	0.4	-	-	-	-	-	-
20:1 (n9+11)	7.1	8.1	8.0	7.1	7.2	7.2	7.2	7.2	6.6	9.0	9.0	9.0
20:1 (n7)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.5	0.5
20:2 (n6)	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	-	0.2	0.2	0.2
20:4 (n6)	0.6	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.8	0.7	0.7	0.7
20:4 (n3)	0.5	0.6	0.6	0.5	0.5	0.5	0.4	0.4	0.5	0.4	0.4	0.4
20:5 (n3)	11.3	11.1	11.3	11.2	11.1	10.9	11.0	11.1	11.5	10.6	10.4	10.5
21:5 (n3)	0.4	0.6	0.4	0.4	0.4	0.3	-	-	-	0.3	0.3	0.3
22:1 (n9,11,13)	8.9	11.1	10.2	9.6	9.6	9.6	7.9	7.5	6.2	10.2	10.3	10.3
22:5 (n3)	0.6	0.8	0.7	0.8	0.8	0.8	0.8	0.9	1.0	0.9	0.9	0.9
22:6 (n3)	14.1	17.1	16.3	18.4	18.2	17.8	19.0	19.0	18.7	18.3	18.0	18.2
24:1	-	-	-	1.0	1.0	1.1	0.6	0.3	0.6	1.6	1.6	1.6

Table A.6. Free amino acids (g/kg) in capelin meal.

Group	month	repl.	ASP	GLU	ASN	SER	GLN	HIS	GLY	THR	ARG	TAU	ALA	TYR	MET	VAL	PHE	ILE	LEU	LYS	TOTAL	Acidic	Basic	A/B
Su	0	1	1818	4319	86	1166	343	1166	1881	2810	7289	7375	4047	2640	1336	2985	2581	2081	6120	6376	56419	10112	14832	0.68
Su	0	2	1853	4419	114	1201	349	1198	1916	2898	7480	7595	4149	2712	1334	3063	2640	2132	6257	6464	57775	10371	15142	0.68
Su	0	3	1831	4340	101	1144	329	1172	1839	2800	7251	7410	4032	2599	1282	2996	2543	2059	6012	6016	55755	10115	14439	0.70
Su	2	1	2357	3953	194	1300	288	1028	1770	2620	4788	6602	4271	2604	1636	3328	2933	2328	5315	6382	53698	10230	12199	0.84
Su	2	2	2373	4028	205	1324	291	1057	1790	2632	4895	6724	4336	2682	1672	3358	2974	2362	5366	6517	54585	10357	12469	0.83
Su	2	3	2444	4115	214	1345	298	1079	1876	2794	5051	6925	4443	2691	1727	3455	3040	2419	5509	6893	56319	10698	13024	0.82
Su	4	1	2451	4020	221	1338	288	1027	2064	2744	4979	7752	4325	2871	2033	3278	2740	2265	5084	5299	54780	10553	11305	0.93
Su	4	2	2243	3670	221	1264	276	969	1960	2594	4594	7276	4017	2555	1763	3035	2386	2112	4605	5426	50966	9771	10989	0.89
Su	4	3	2660	4374	247	1540	330	1153	2187	2970	5124	7653	4391	2850	1762	3367	2669	2427	5328	6928	57960	11543	13205	0.87
Au	0	1	812	2206	0	536	0	62	1172	952	1659	8560	2421	830	493	1121	785	698	1557	2043	25907	4506	3764	1.20
Au	0	2	813	2187	0	540	72	0	1193	953	1779	8641	2444	826	508	1146	799	694	1578	2073	26246	4494	3852	1.17
Au	0	3	812	2211	0	553	35	0	1194	968	1819	8782	2488	836	494	1130	797	700	1590	2085	26493	4545	3903	1.16
Au	2	1	786	2185	0	531	96	0	1288	969	1705	7704	2560	717	428	1056	860	748	1591	1709	24935	4471	3414	1.31
Au	2	2	767	2157	0	551	94	0	1298	914	1641	7824	2550	730	420	1039	864	743	1571	1704	24867	4389	3345	1.31
Au	2	3	770	2158	0	575	86	0	1298	902	1530	7833	2553	731	414	1050	860	734	1552	1695	24740	4405	3225	1.37
Au	4	1	598	1646	32	387	70	0	852	571	1461	6157	1869	687	275	802	371	346	773	992	17889	3202	2453	1.31
Au	4	2	657	1782	42	413	49	0	950	631	1584	5584	2063	688	287	828	390	356	803	1041	18149	3483	2626	1.33
Au	4	3	628	1715	42	388	45	0	929	611	1527	5453	2748	674	271	791	371	337	754	1019	18303	3341	2546	1.31
Wi	0	1	265	1318	0	298	85	0	624	273	193	5556	2137	325	250	437	384	252	586	971	13953	2154	1164	1.85
Wi	0	2	198	974	0	203	22	0	485	174	150	4376	1696	257	208	358	254	199	483	770	10806	1548	920	1.68
Wi	0	3	192	981	0	215	41	0	455	188	148	4332	1633	211	191	353	251	188	508	698	10584	1575	846	1.86
Wi	2	1	244	1251	0	268	91	0	593	411	632	5309	1432	295	238	432	338	287	586	964	13372	2175	1595	1.36
Wi	2	2	226	1216	0	270	89	0	577	412	540	5292	1404	289	227	406	344	275	567	1003	13136	2124	1543	1.38
Wi	2	3	249	1298	0	285	93	0	624	442	583	5656	1535	317	246	445	386	229	604	1126	14118	2275	1709	1.33
Wi	4	1	231	1243	0	258	79	0	615	425	670	7070	1565	297	191	406	357	274	573	1072	15326	2157	1742	1.24
Wi	4	2	230	1233	0	256	81	0	606	458	738	7158	1553	291	192	411	356	268	576	1046	15455	2178	1784	1.22
Wi	4	3	223	1217	0	263	79	0	652	408	734	7185	1572	294	191	406	351	266	572	1077	15491	2111	1811	1.17
Sp	0	1	221	836	0	266	0	0	487	333	581	4594	1242	318	261	527	211	205	433	546	11060	1657	1127	1.47
Sp	0	2	212	813	0	264	0	0	487	335	559	4646	1269	391	258	517	218	196	425	580	11169	1624	1139	1.43
Sp	0	3	206	805	0	255	0	0	469	327	528	4564	1246	317	252	510	211	194	429	584	10898	1594	1112	1.43
Sp	2	1	153	590	0	181	0	0	384	255	401	4400	926	200	137	375	141	154	315	501	9113	1179	902	1.31
Sp	2	2	152	587	0	183	0	0	386	236	386	4395	915	191	147	373	141	156	315	537	9101	1159	923	1.25
Sp	2	3	145	565	0	176	0	0	390	222	391	4345	904	195	140	364	137	150	314	541	8978	1108	932	1.19
Sp	4	1	163	670	0	185	0	0	419	279	432	5318	1108	225	142	413	161	172	345	556	10587	1297	987	1.31
Sp	4	2	159	629	0	187	0	0	384	250	435	5058	1071	218	135	395	151	168	336	518	10093	1225	953	1.29
Sp	4	3	160	630	0	217	0	0	370	252	361	4901	1048	203	134	394	149	168	336	485	9808	1259	846	1.49

Appendix 3. Oxygen uptake graphs

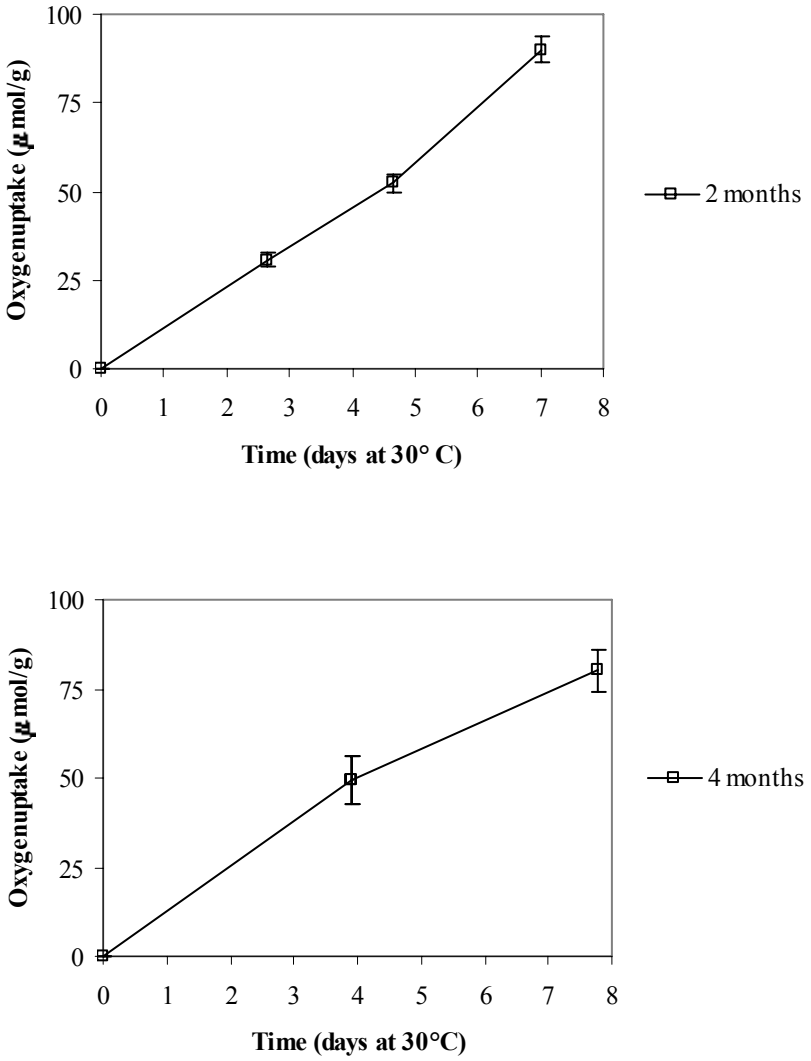


Figure A.1. Evaluation in oxygen uptake of summer meal during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

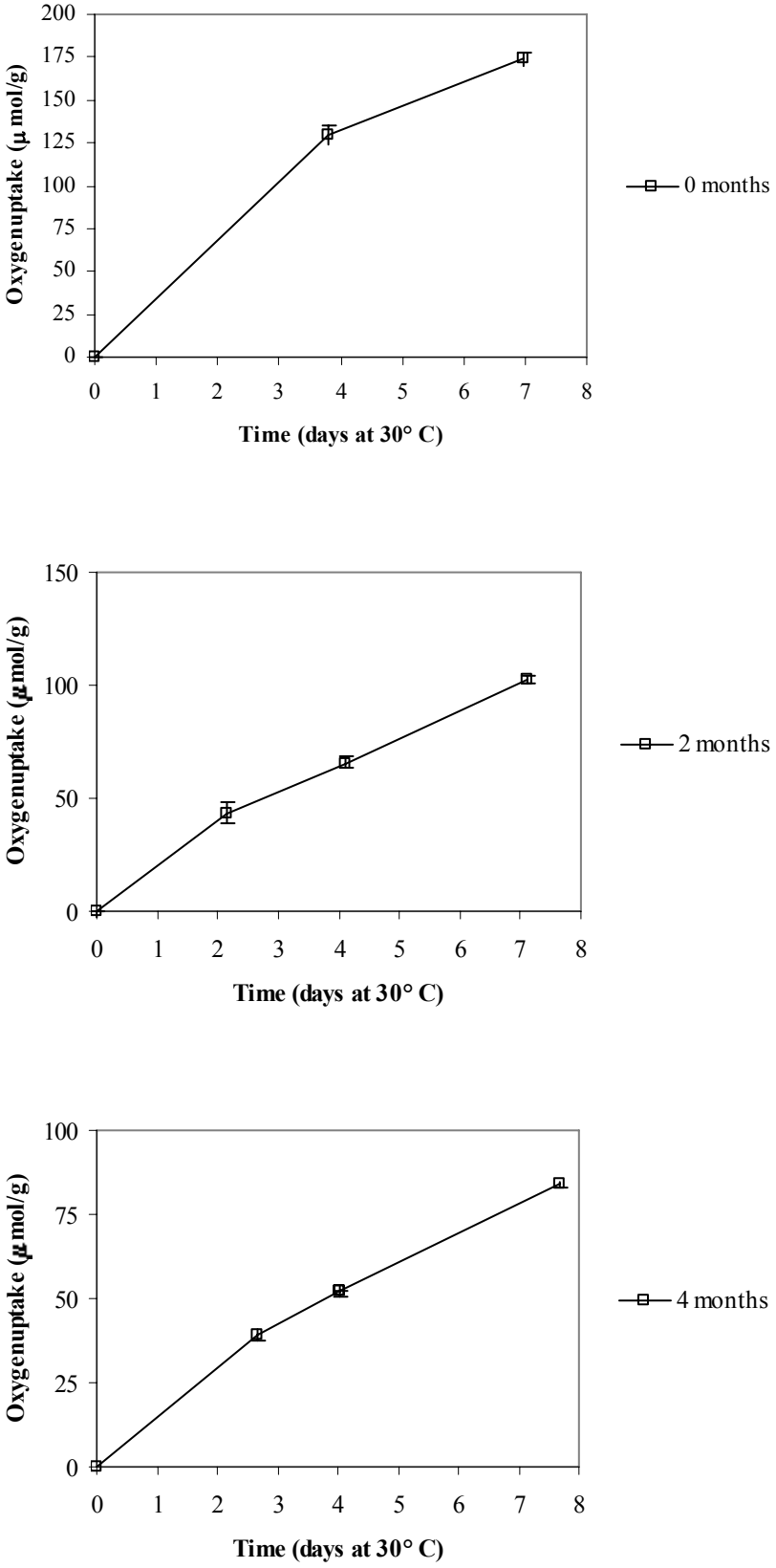


Figure A.2. Evaluation in oxygen uptake of autumn meal during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

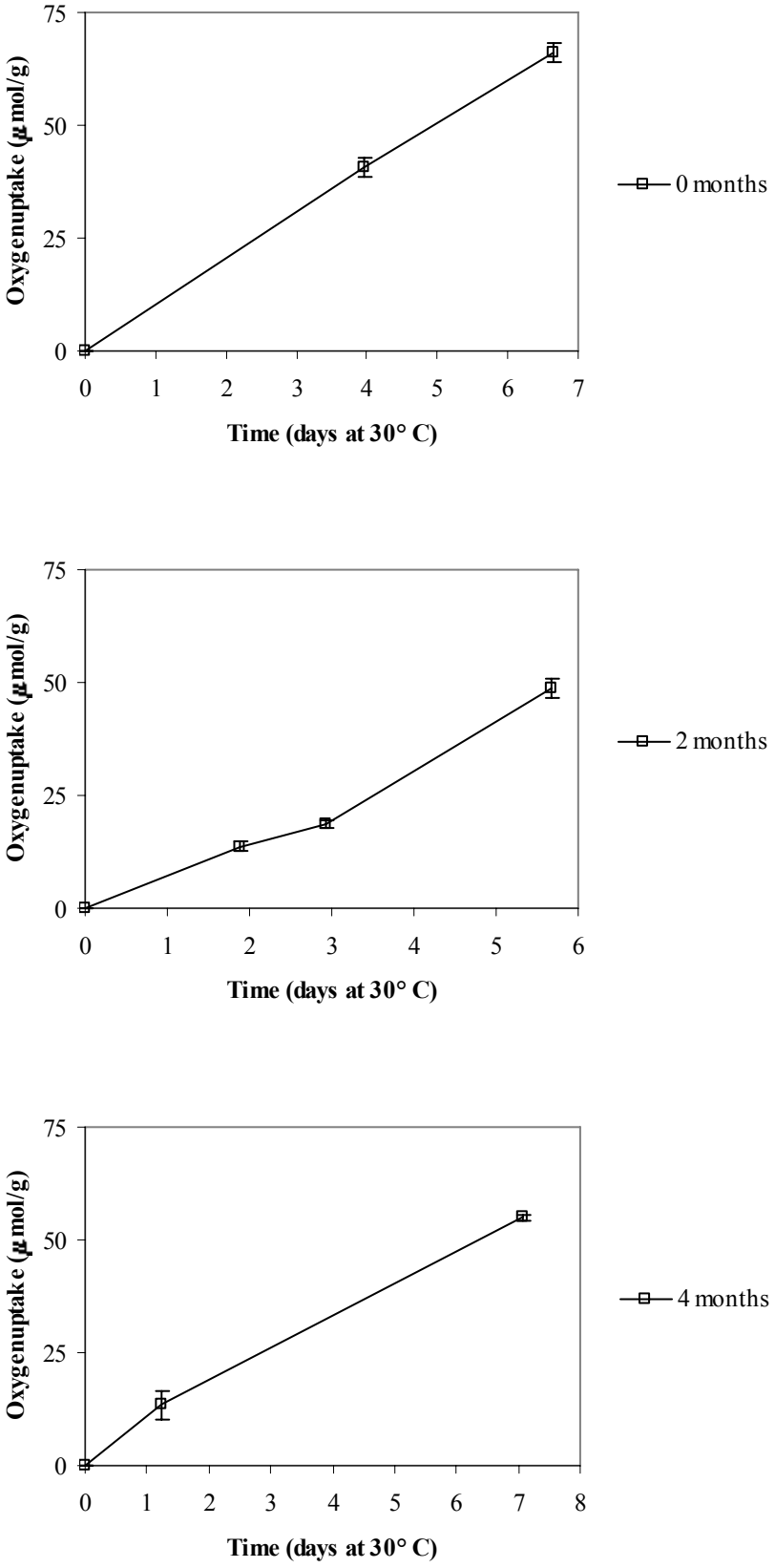


Figure A.3. Evaluation in oxygen uptake of autumn meal during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

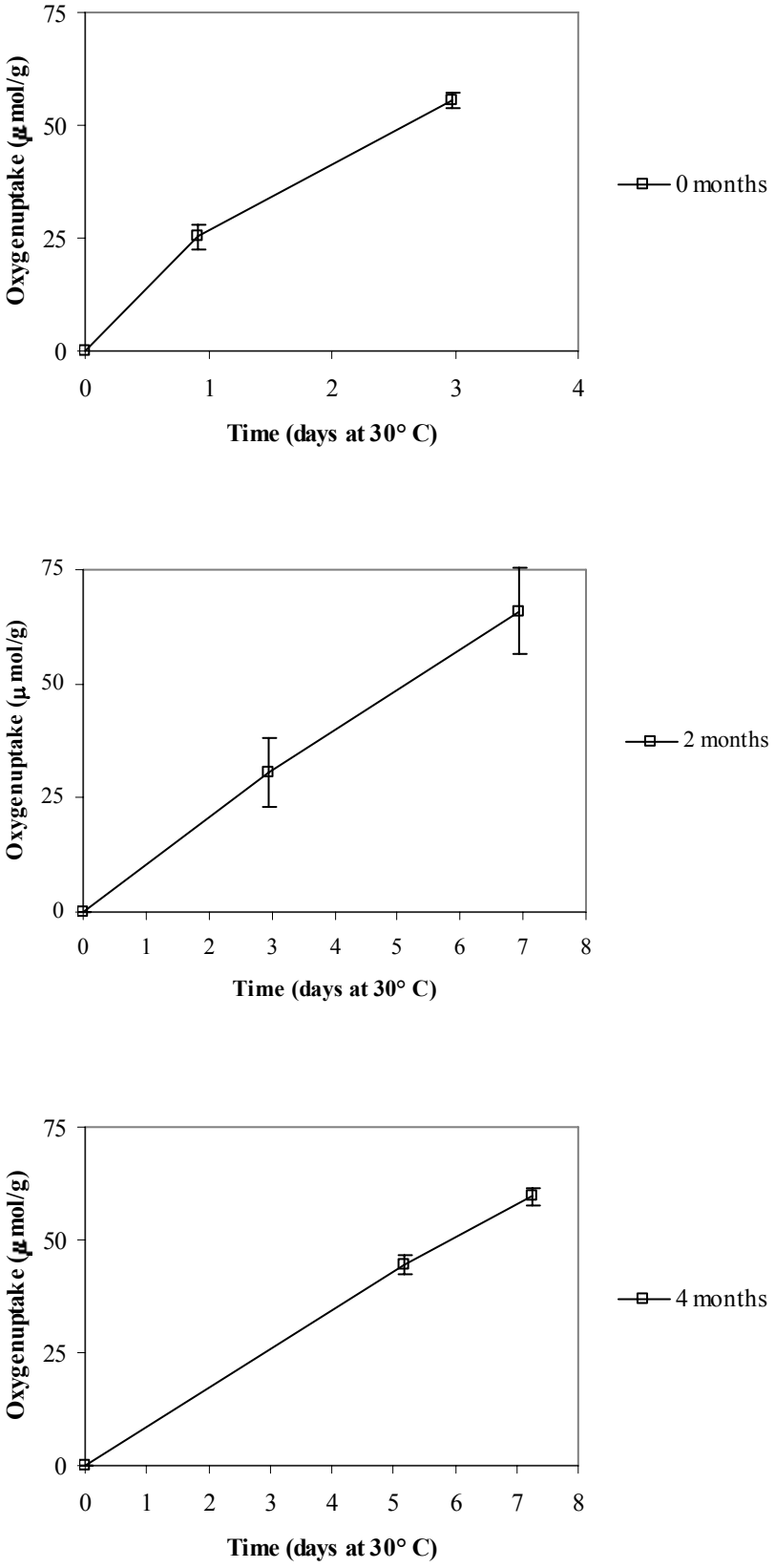
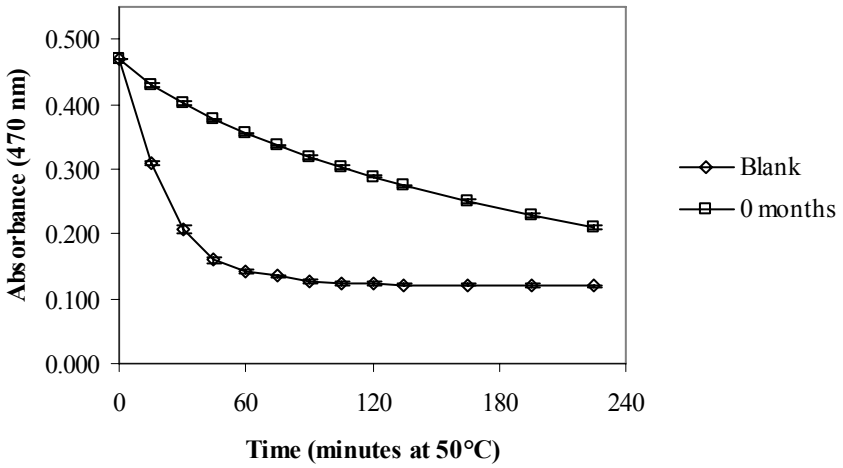
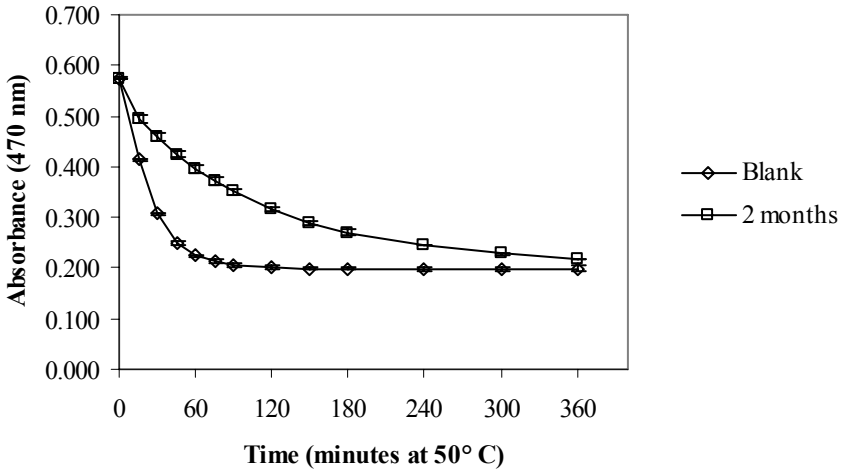


Figure A.4. Evaluation in oxygen uptake of winter meal during storage. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

Appendix 4. Aquous antioxidant activity graphs





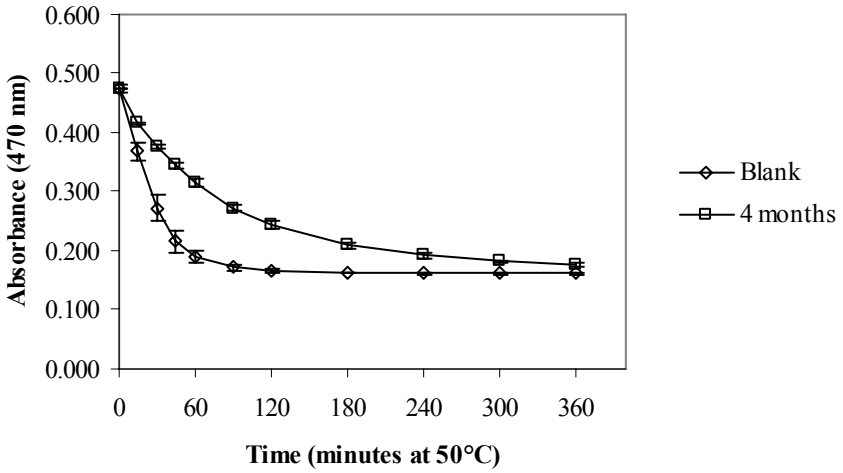
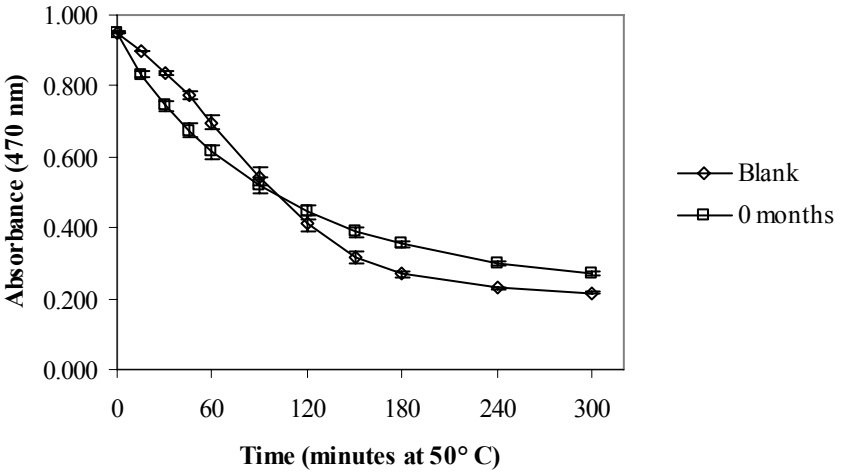
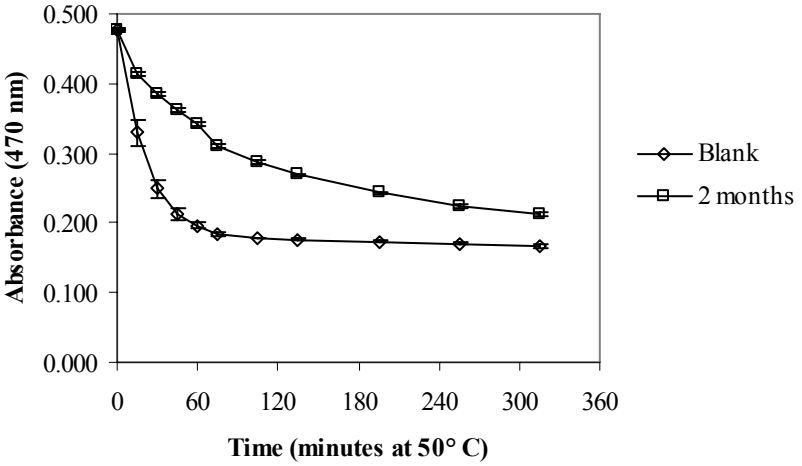


Figure A.5. Evaluation in β-Carotene bleaching of summer meal water soluble fraction during storage, representing aqueous antioxidant activity. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.





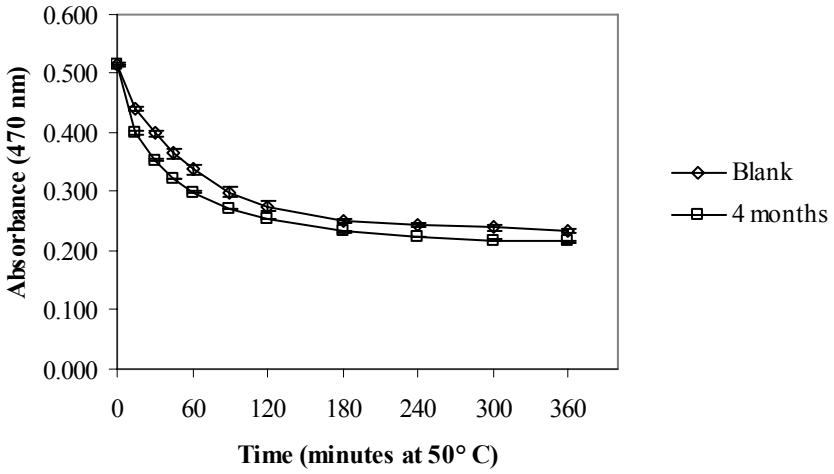
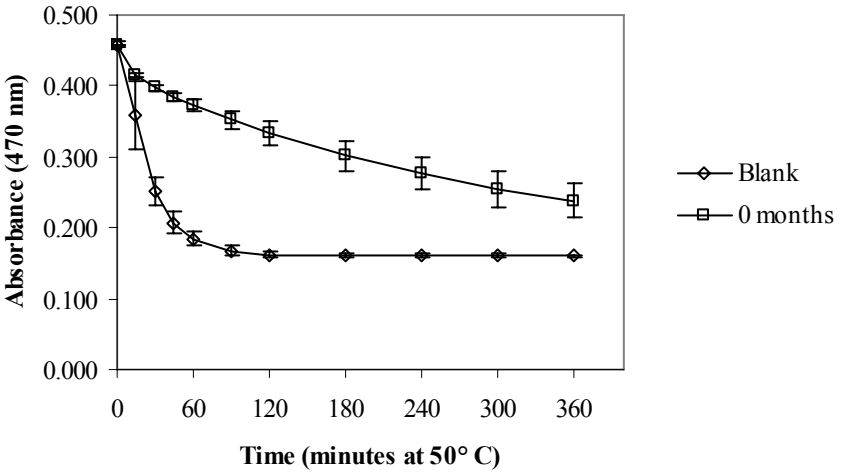
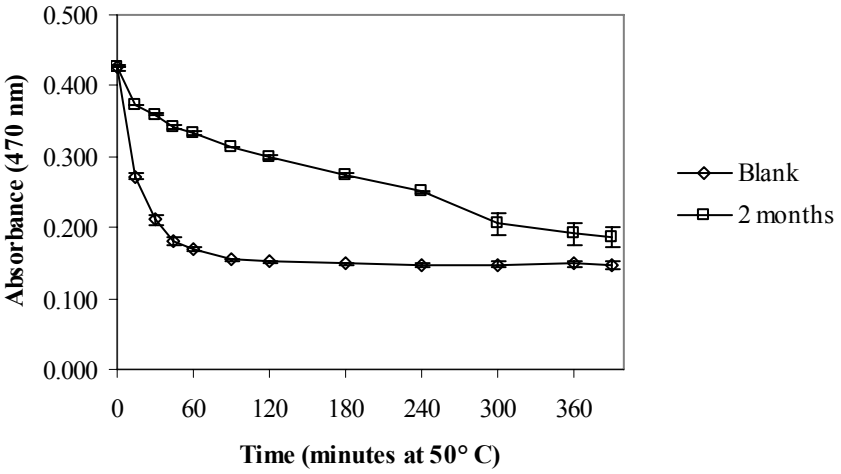


Figure A.6. Evaluation in β-Carotene bleaching of autumn meal water soluble fraction during storage, representing aqueous antioxidant activity. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.





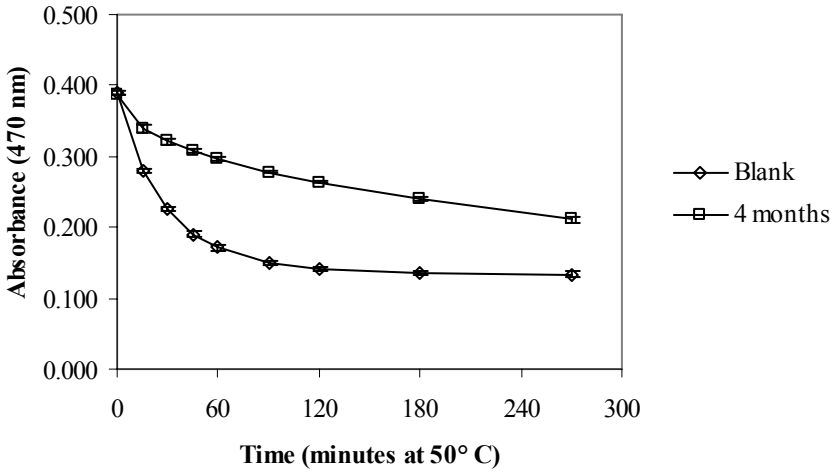
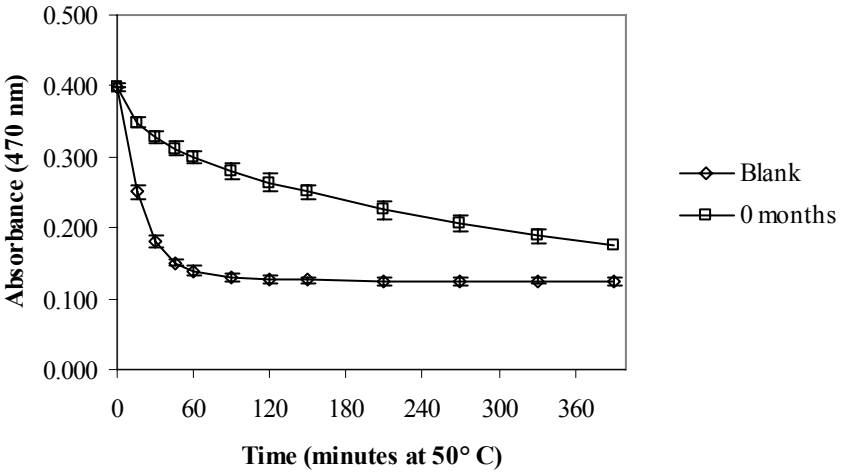
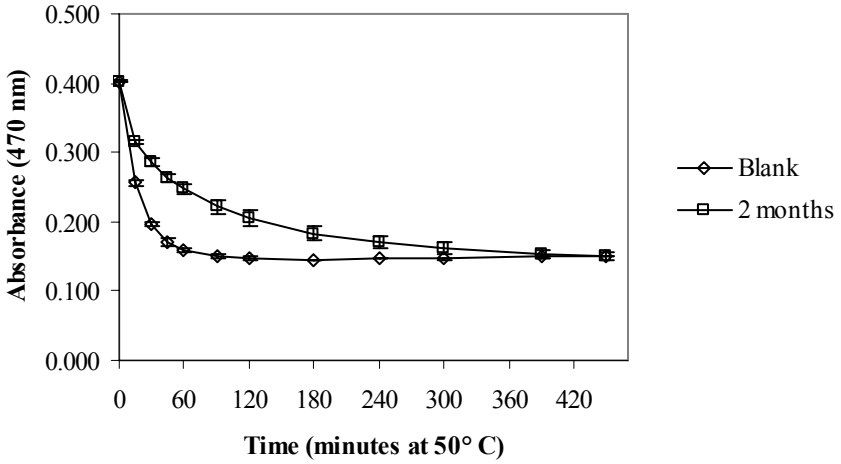


Figure A.7. Evaluation in β-Carotene bleaching of winter meal water soluble fraction during storage, representing aqueous antioxidant activity. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.





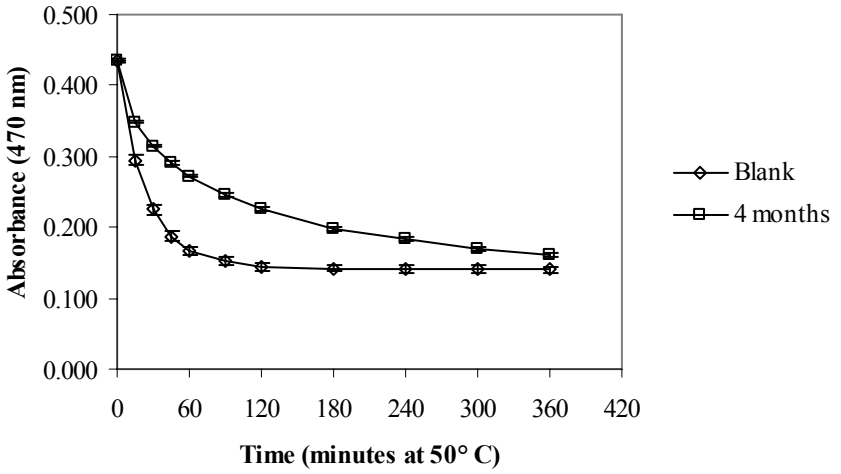


Figure A.8. Evaluation in β-Carotene bleaching of spring meal water soluble fraction during storage, representing aqueous antioxidant activity. Each point represents a mean value from a pooled sample of meal (6 kg at 0 month, 2 kg at months 2 and 4) (n = 3) ± SD.

Appendix 5. Example of chromatograms on fatty acid methyl esters

Autumn capelin meal lipids	Autumn capelin meal lipids	Control sample, Menhaden oil from United States Department of Commerce, National Marine Fisheries Service.

Area Percent Report

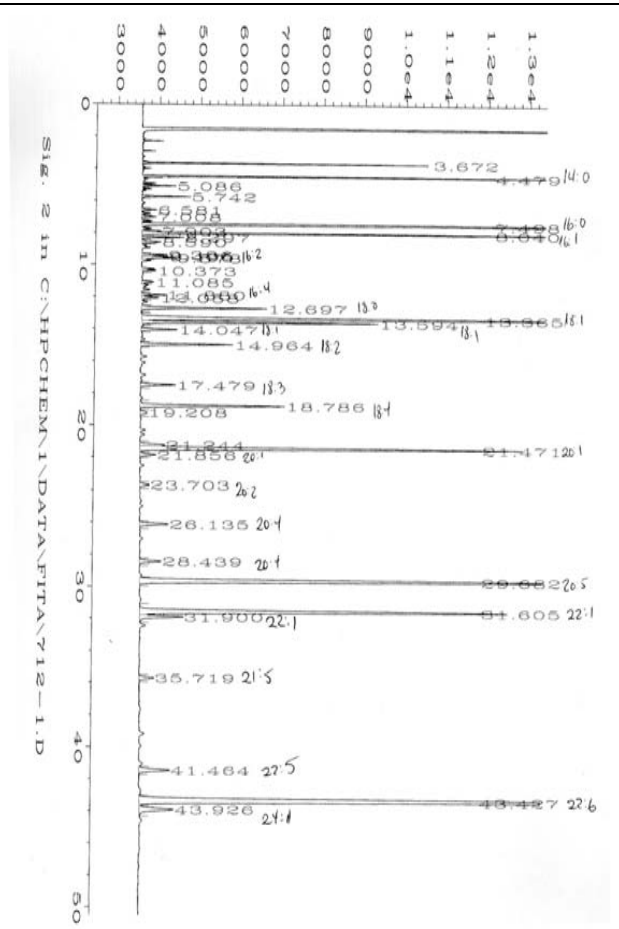
Data File Name : C:\HPCHEM\1\DATA\FITA\712-1.D
Operator : heida
Acquired on : 11 Dec 98 01:21 PM
Sample Name : 712-1
Run Time Bar Code:
Instrument Method: FITA.MTH
Analysis Method : FITA.MTH
Sample Info : 712-1

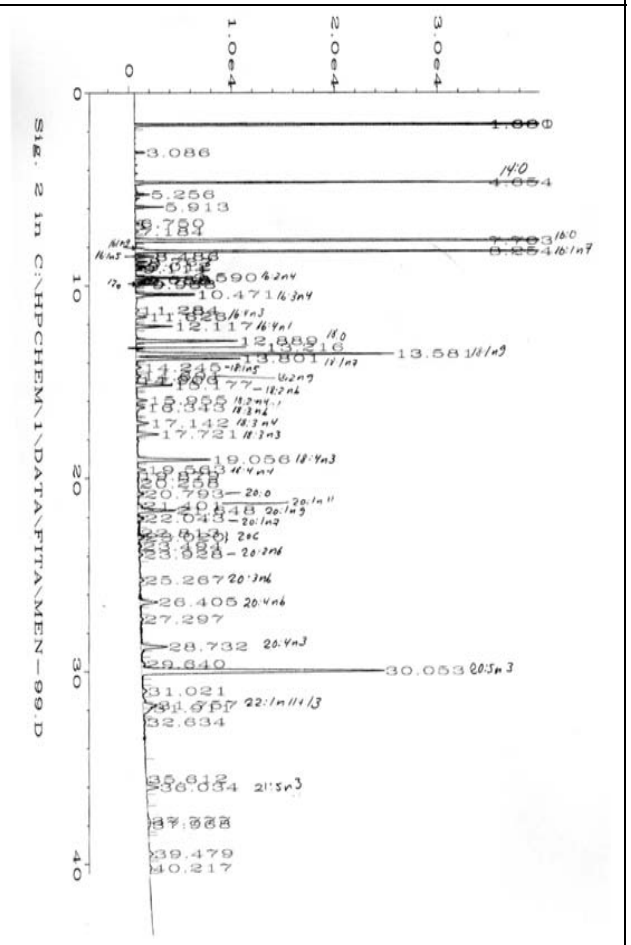
Instrument : ANALYZER1
Vial Number :
Injection Number:
Sequence Line :

Sig. 2 in C:\HPCHEM\1\DATA\FITA\712-1.D

Pk#	Ret Time	Area	Height	Type	Width	Area %
1	3.672	12853	7053	PB	0.028	1.2670
2	4.479	42395	19497	BV	0.033	4.1790
3	5.086	2244	824	PB	0.041	0.2212
4	5.742	3398	1186	BB	0.044	0.3349
5	6.581	1534	371	BV	0.059	0.1512
6	7.008	1642	350	VB	0.068	0.1619
7	7.498	157131	43240	VV	0.055	15.4890
8	7.903	1772	466	VV	0.057	0.1746
9	8.040	50646	13290	VV	0.058	4.9924
10	8.297	3776	1020	VV	0.059	0.3722
11	8.590	2384	465	VV	0.072	0.2350
12	9.386	2589	613	BV	0.063	0.2552
13	9.495	2695	644	VV	0.061	0.2656
14	9.578	4524	829	VV	0.079	0.4460
15	10.373	1508	337	VB	0.060	0.1486
16	11.085	1767	284	BV	0.092	0.1742
17	11.880	2876	573	BV	0.079	0.2835
18	12.058	2569	420	VV	0.092	0.2532
19	12.697	16913	3046	BB	0.087	1.6672
20	13.365	104866	17264	BV	0.094	10.3371
21	13.594	30917	5734	VV	0.083	3.0476
22	14.047	5091	882	BB	0.090	0.5019
23	14.964	13237	2237	BB	0.092	1.3048
24	17.479	5588	847	BB	0.100	0.5508
25	18.786	24772	3513	BB	0.110	2.4418
26	19.208	1938	165	BB	0.163	0.1910
27	21.244	4218	601	BV	0.106	0.4158
28	21.471	72415	9272	VV	0.126	7.1382
29	21.856	3166	390	VB	0.119	0.3121
30	23.703	1940	233	BB	0.119	0.1912
31	26.135	6103	702	BB	0.137	0.6016
32	28.439	4691	529	BB	0.139	0.4624
33	29.682	113699	12429	VB	0.142	11.2078
34	31.605	88334	8909	BV	0.156	8.7075
35	31.900	9661	1053	VV	0.145	0.9523
36	35.719	3621	368	BB	0.159	0.3569
37	41.464	8276	746	VB	0.173	0.8158
38	43.427	186372	15316	VV	0.184	18.3715
39	43.926	10343	836	VB	0.196	1.0196

Total area = 1014463





Appendix 6. Example of statistical calculations

Analysis of Variance Report
Page/Date/Time 1 8.2.2001 13:30:09
Database M:\MARGRET\1290\NYTT\SKRIF\LOKASKYRSLA\NCSS\CAPELIN.S0
Response Lipid

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: Group	3	Yes	S	S+sA
S	8	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

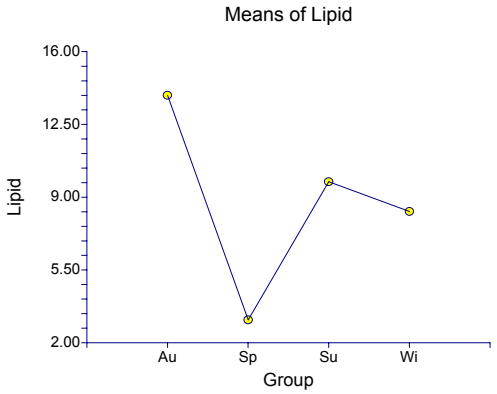
Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Group	3	178.9753	59.65844	2383.39	0.000000*	1.000000
S	8	0.2002473	2.503092E-02			
Total (Adjusted)		11	179.1756			
Total	12					

* Term significant at alpha = 0.05

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	12	8.762		8.762
A: Group				
Au	3	13.9	9.134352E-02	5.138
Sp	3	3.096667	9.134352E-02	-5.665333
Su	3	9.741333	9.134352E-02	0.9793333
Wi		3	8.31	9.134352E-02	-0.452

Plots Section



Page/Date/Time 2 8.2.2001 13:30:10
Database M:\MARGRET\1290\NYTT\SKRIF\LOKASKYRSLA\NCSS\CAPELIN.S0
Response Lipid

Tukey-Kramer Multiple-Comparison Test

Response: Lipid
Term A: Group

Alpha=0.050 Error Term=S DF=8 MSE=2.503092E-02 Critical Value=4.528845

Group	Count	Mean	Different From Groups
Sp	3	3.096667	Wi, Su, Au
Wi	3	8.31	Sp, Su, Au
Su	3	9.741333	Sp, Wi, Au
Au	3	13.9	Sp, Wi, Su