Multivariate Evaluation of Lipid Hydrolysis and Oxidation Data from Light and Dark Muscle of Frozen Stored Rainbow Trout (*Oncorhynchus mykiss*)

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Lipid hydrolysis and oxidation were evaluated by multivariate statistical methods in farmed rainbow trout (*Oncorhynchus mykiss*). The trout were acclimatized at 5 or 12 °C and then frozen as fillets and stored at −15 °C for up to 34 weeks. Results from analyses of lipid hydrolysis and lipid oxidation during frozen storage of light and dark muscle revealed that the major cause of lipid deterioration is hydrolysis. Hydrolysis resulted in an increase in free fatty acids by between 1 and 10%, particularly in the nutritionally important polyunsaturated $n-3$ fatty acids, 20:5 and 22:6. The phosphatidylcholine content of the muscle decreased by 25–50% during frozen storage. Phosphatidylethanolamine was stable in dark muscle but decreased in light muscle. By multivariate statistical evaluation it was possible to group the samples according to storage time or acclimatization temperature. A lower water temperature (5 °C) resulted in reduced lipid stability during frozen storage.

Keywords: *Rainbow trout; lipid; frozen storage; multivariate evaluation; oxidation; hydrolysis*

INTRODUCTION

One of the main limiting factors of frozen storage of fatty fish is hydrolytic and oxidative deterioration of lipids (Han and Liston, 1987; Shewfelt, 1981) resulting in rancidity and off-flavor as well as impaired nutritional quality.

There are also changes in texture during frozen storage due to interactions between proteins and lipid oxidation products [reviewed by Sikorski et al. (1976), Bremner and Hallett (1989), and Haard (1992)]. For instance, lipid oxidation products such as malondialdehyde (MDA) may cause cross-linking of proteins, which decreases their solubility (Kwon, 1965).

Neutral and polar lipids are subjected to hydrolysis during frozen storage, at different rates depending on lipid composition and storage temperature (Bosund and Ganrot, 1969; de Koning and Mol, 1990; Ingemansson et al., 1992). Polar lipids are hydrolyzed by the action of phospholipases; phosphatidylcholine (PC) is hydrolyzed more rapidly than phosphatidylethanolamine (PE) in fish muscle (Bosund and Ganrot, 1969). Hydrolysis itself activates the enzyme phospholipase A_2 and enhances phospholipid hydrolysis in frozen fish muscle (Han and Liston, 1987). However, there is no clear relationship between the overall activity of phospholipase A_2 and the amount of released free fatty acids during frozen storage (Aaen, 1993). Deterioration of phospholipids in frozen fish muscle may result in increased fragility of the sarcoplasmic reticulum membrane (Hanaoka and Toyomizu, 1981).

The relationship between free fatty acids (FFA) and lipid oxidation is a matter of debate in the literature. The fact that FFAs are oxidized more readily than esterified fatty acids is generally accepted (Labuza, 1971) but, in tissue with remaining intact lipid deposits, the FFAs are reported both to enhance lipid oxidation (Shono and Toyomizu, 1973; Dyer and Morton, 1956; Awad et al., 1969) and to inhibit it (Castell et al., 1966; Mazeud and Bilinski, 1976). Shewfelt (1981) found that hydrolysis of triacylglycerols led to increased oxidation, while hydrolysis of polar lipids had the opposite effect. Supplementation with natural antioxidants, e.g. α-tocopherol and carotenoids, via the fish feed is another important factor for post-mortem protection (Ingemansson et al., 1993b).

Several investigators have studied biochemical deterioration in fish muscle during frozen storage [for reviews, see Lovern (1962), Bilinski (1969), Shewfelt (1981), and Haard (1990, 1992)]. Many factors influence the composition of the lipid phase in fish muscle and its deterioration: species of fish, composition of diet, nutritional status, water temperature, salinity, migrations, genetic factors, and season of catch (fat content, development of gonads); and in post-mortem handling, temperature and time of frozen storage and the presence of oxygen and light. In fish, as in many other organisms, there is also a so-called homeoviscous adaptation; *i.e.*, the pattern of structural fatty acids is affected by the water temperature (Hazel, 1984). Acclimatization of rainbow trout to a low environmental temperature resulted in a general increase in the content of polyunsaturated fatty acids (PUFA), especially 20:5$n-3$ (eicosapentaenoic acid, EPA) and 22:6$n-3$ (docosahexaenoic acid, DHA) (Ingemansson et al., 1993a). Decreasing the acclimatization temperature induces an increase in capacity for oxidative metabolism, accompanied by a marked rise in the number of mitochondria in fish muscle (Lin et al., 1974; Smit et al., 1974; Johnston and Maitland, 1980; Egginton and Sidell, 1989). There is also an increase in the activity of tricarboxylic acid cycle enzymes in fish muscle acclimatized to low temperatures (Lehman, 1970; Hazel, 1972). Accordingly, the capacity of the muscle for aerobic metabolism increases with cold acclimatization (Smit et al., 1974).

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The purpose of this work was to study the extent of lipid hydrolysis and oxidation in the muscle tissue of frozen rainbow trout and to elucidate the effects of acclimatization temperature and storage time on these mechanisms using multivariate statistical methods.

MATERIALS AND METHODS

Fish. Farmed rainbow trout (*Oncorhynchus mykiss*), 1-year old, ranging in body weight from 500 to 700 g, all female, were obtained from Antens Trout Hatchery near Gothenburg, Sweden. The fish were kept in the laboratory in 1000-L tanks at 5 and 12 °C water temperatures on a 12-h light/12-h-dark regime for 20 weeks prior to use. Air was passed into the tanks to supply as much oxygen as possible at these temperatures. The fish were fed a 2% body weight per day ration of commercial trout pellets (ET 92) from Ewos AB (Södertälje, Sweden) via an automatic feeding box. The fish were killed by a blow to the head. The wet weight of viscera, liver, and gonads was determined, giving the viscerasomatic (VSI), hepatosomatic (HSI), and gonadosomatic indices (GSI) according to the relationship

$$\text{index} = (\text{wet wt of organ/wet wt of whole fish}) \times 100$$

Storage Conditions. We used a paired fillet design to compensate for individual variability. The fillets with skin were glazed and tunnel-frozen. One fillet of a pair was subjected to frozen storage at −15 °C in sealed aluminum bags while the other was immediately thawed, analyzed, and used as a control. The fillet samples were analyzed after 0, 2, 4, 8, 16, and 34 weeks of frozen storage.

Lipid Analyses. The frozen fish was thawed, the skin was removed, and light and dark muscles were separated by manual dissection. The muscle was homogenized with an Ultra-Turrax (Janke & Kunkel, IKA-Werk, Germany). Lipids were extracted with chloroform/methanol (2:1 v/v) according to the method of Bligh and Dyer (1959), as modified for muscle homogenates by Hanson and Olley (1963). The lipid residues were stored in chloroform at −40 °C, in an argon atmosphere. Lipid classes were separated and quantified by means of normal-phase HPLC using an ACS 750/14 light scattering detector according to the procedure of Ingemansson et al. (1991). A Lichrospher Diol 100 column (Merck, Germany) and a linear gradient of hexane/2-propanol/acetic acid (49:49:1 v/v) to hexane/2-propanol/water/acetic acid (10:65:25:1 v/v) were used.

The FFAs were converted to ammonium salts and separated on a Sep-Pak silica cartridge according to the method of Ansari and Shoeman (1988). Methyl ester derivatives of FFAs were prepared using acidic methanolysis according to the procedure of Kates (1972). Methyl ester derivatives of lipid fractions were prepared using an alkali methanolysis procedure according to that of Olsson et al. (1990). The methyl esters were analyzed using a capillary GC equipped with an on-column injector, a fused silica column (DB-Wax), and a flame ionization detector, according to the method of Ingemansson et al. (1991, 1993b).

Determination of Conjugated Dienes and Trienes. Lipids were extracted, solvents were removed with oxygen-free nitrogen, and the remaining lipids were weighed and dissolved in spectrograde cyclohexane to a concentration of 0.1 mg/mL. Spectra of the lipids were taken against cyclohexane from 190 to 400 nm (Janero and Burghardt, 1988) in a DU-8 UV−vis spectrophotometer (Beckman Instruments GmBH, München, Germany).

Determination of TBA-Reactive Substances. The measurements of TBA-reactive substances (TBA-RS) were performed by modification of the methods of Uchiyama and Mihara (1978) and Lee and Csallany (1987): 0.5 mL of 10% (w/v) homogenate in cold 0.020 M potassium phosphate buffer, pH 7.1, was placed in a 14-mL screw-capped test tube. Three milliliters of 1% phosphoric acid and 1 mL of 0.6% TBA solution were added and mixed for 1 min. The samples were incubated for 45 min in a boiling water bath. The reaction was immediately stopped by placing the test tubes in an ice−water bath and adding 4 mL of 1-butanol while mixing vigorously. The butanol phase was separated by centrifugation at 2000*g* for 30 min. The absorbance of the butanol phase was measured at 532 nm using a Perkin-Elmer Hitachi 200 spectrophotometer.

Analysis of Malondialdehyde. Free MDA was extracted according to the method of Lee and Csallany (1987) with the following modifications: The muscle was homogenized with an Ultra-Turrax (Janke & Kunkel, IKA-Werk) in cold 0.020 M potassium phosphate buffer, pH 7.1, 10% (w/v). The homogenate was immediately filtered through a 10-mL Amicon cell equipped with a YM2 membrane (Amicon, Danvers, MA) (cutoff 1000 MW) at low temperature (4 °C) under 55 psi (3.7 atm) of pressure of nitrogen gas. The filtrate was immediately analyzed by means of HPLC. Bound MDA was extracted by alkaline hydrolysis according to the procedure of Lee and Csallany (1987).

A stock solution of MDA was prepared from 1,1,3,3-tetraethoxypropane (TEP) purchased from Merck-Schuchardt, München, Germany by hydrolysis with 1% sulfonic acid at room temperature according to the method of Esterbauer et al. (1984). The TEP solution was stored in a refrigerator to avoid polymerization (Halliwell and Gutteridge, 1989). After 2 h, the hydrolyzed TEP solution was adjusted to pH 7.3 with a strong base, diluted, and checked for absolute MDA concentration by measurement of the UV absorption at 267 nm:

$$(E = 34\ 000\ \text{M}^{-1}\ \text{cm}^{-1})\ \text{(Bull and Marnett, 1985)}$$

An isocratic ion-pair HPLC (Bull and Marnett, 1985; Janero and Burghardt, 1988) was used for separation and quantification of MDA, with a solvent delivery system, Shimadzu LC-6A (Shimadzu Ltd., Kyoto, Japan), together with a UV detector, Shimadzu UV-6. The analytical column (250 mm × 4.6 mm) was packed with Kromasil C8, 5 μm (Eka Nobel, Surte, Sweden). The mobile phase was made up of acetonitrile/50 mM myristyltrimethylammonium bromide (MTEAB, Janssen Chimica, Geel, Belgium) in 20 mM potassium phosphate buffer, pH 7.1/1-propanol (12/85/3), at 1 mL/min flow rate at 25 °C. Detection was based on UV absorbance at 267 nm and quantification by peak area integration, with reference to MDA standard, on a Shimadzu C-R3A integrator.

Experimental Design. The experimental design used in this study was a modified two-factor, two-level factorial design. One of the basic requirements of a statistical experimental design is that it must be possible to change the experimental factors independently of each other and in a random manner. Time has direction and cannot be changed in the same manner as temperature and other experimental factors. This can be solved by centering the factorial design at 16 weeks. The factors studied were two different environmental temperatures during acclimatization for the live fish (5 and 12 °C) and different frozen storage times (0, 2, 4, 8, 16, and 34 weeks). Number of samples was $n = 4$ for all sets of parameters except for storage time of 0 weeks, where $n = 6$.

Multivariate Evaluation. The data were evaluated using the following multivariate statistical methods: principal component analysis (PCA) and partial least-squares correlation (PLS) (Wold, 1976, 1982; Wold et al., 1983). The strategy of these methods is to consider each experimental variable as a coordinate axis in a k-dimensional space (k = number of variables), the measurement space (M-space). Each object is described using a vector containing the values of the experimental variables and represented as a point in the M-space. Using the same measured variables, a set of objects forms a swarm of points in M-space and objects similar to each other would be near each other, so that distance would constitute a measure of similarity/dissimilarity. According to the methods used, new variables that are uncorrelated and represent a maximum amount of the systematic variance in the data were calculated as linear combinations of the original experimental variables. The new variables are called the latent variables, and they describe the underlying variance structure of the system and can often be fewer in number than the experi-

Table 1. Lipid Content and Lipid Class Composition in Light and Dark Muscle—Initial Values before Frozen Storage[a]

	5 °C		12 °C	
	light	dark	light	dark
total lipids[b] (g kg^{-1})	23 ± 2	76 ± 6	25 ± 2	103 ± 9
total FFA[c] (wt %)	0.12 ± 0.03	0.12 ± 0.02	0.13 ± 0.01	0.20 ± 0.04
lipid class[c] (wt %)				
neutral lipids	41.7 ± 1.3	57.0 ± 2.3	43.8 ± 0.8	61.4 ± 1.1
PC	41.8 ± 0.8	29.0 ± 3.2	44.9 ± 0.7	27.0 ± 1.6
PE	13.9 ± 1.1	8.3 ± 0.4	9.3 ± 0.2	7.1 ± 0.2
SPH	2.4 ± 0.3	3.3 ± 0.5	2.1 ± 0.3	3.0 ± 0.3

[a] Values are mean ± SEM. [b] $n = 6$. [c] Weight percentage (wt %) of total lipids; $n = 4$.

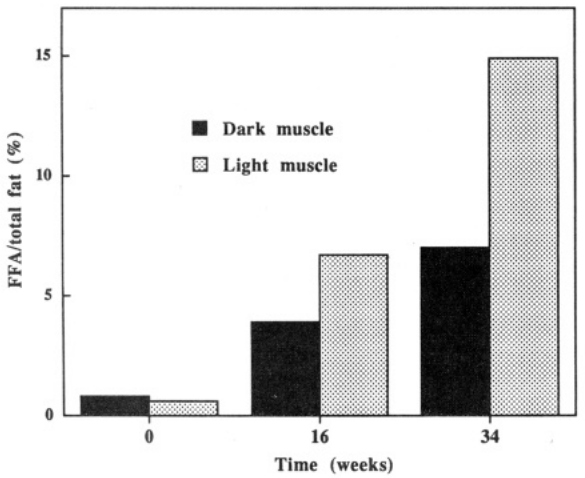

Figure 1. Lipid hydrolysis during frozen storage. (The amount of free fatty acids is given as a percentage of the total fat content.)

mental variables. The latent variables (principal components and PLS components) can be plotted, thus revealing natural groupings that can be utilized in classification.

RESULTS AND DISCUSSION

Fish Data. No diseases were observed, and no abnormal stress behavior could be seen among the fish. The hepatosomatic (HSI) and viscerasomatic (VSI) indices were similar among the fish acclimatized to 5 and 12 °C (HSI 9.2 ± 0.9 and 9.5 ± 0.6, respectively, and VSI 1.3 ± 0.1 for both groups). The stage of gonadal development (GSI) was slightly higher in the fish acclimatized to 12 °C (0.15 ± 0.03 for 5 °C and 0.5 ± 0.1 for 12 °C). The total fat content in the muscle was 4–5 wt % in all samples.

Lipid Hydrolysis. The average lipid class composition in the samples used in this study is shown in Table 1. Generally the dark muscle cells have a metabolism that is more aerobic in character and more dependent on lipid oxidation for energy requirements. The light muscle cells are more anaerobic in the aspect of being able to use glycogen as energy source even under restricted oxygen supply, thereby producing lactate with a decreasing intracellular pH as effect. These differences in energy metabolism are reflected in the lipid composition of the different muscle tissues. The dark muscle tissue has more lipid deposits rich in neutral lipids (triglycerides), and the relative lack of such deposits in the light muscle tissue gives a higher proportion of polar membrane lipids (PC, PE, etc.). The fatty acid composition in rainbow trout muscle lipids has been presented earlier (Ingemansson et al., 1991).

As can be seen from Figure 1, the initial content of free fatty acids is low in fresh fish (0.1–0.2%) and increases almost linearly during frozen storage. The slow increase in FFAs during storage also supports the notion that the handling of fish prior to extraction does not in itself cause significant deterioration. There is release, in particular, of the nutritionally important polyunsaturated $n-3$ fatty acids, 20:5 and 22:6 [cf. Ingemansson et al. (1992, 1993a,b)]. The major phospholipids in rainbow trout muscle are PC and PE. The phospholipids form a substrate for the major part of the lipid hydrolysis during frozen storage, especially in the light muscle tissue. The PC content decreased by about 50% in dark muscle during frozen storage, from 27–29 to 13.4 ± 0.5 wt %, and decreased from 42–45 to 30.6 ± 1.7 wt % in light muscle. The PE was stable in dark muscle but decreased from 9–14 to 7.6 ± 0.2 wt % in light muscle. As a result, the lyso forms of PC and PE were detected during the study and showed a tendency to increase, especially lyso-PC. The content of SPH was unchanged. Hydrolysis of phospholipids has been suggested to be caused by the activity of the enzyme phospholipase A_2 (EC 3.1.1.4), which is associated with the microsomal fraction of the muscle (Bilinski and Jonas, 1966; Shewfelt, 1981). However, in cod, no simple correlation between this enzyme and lipid hydrolysis could be found (Aaen, 1993), and it is possible that other catalytic mechanisms are involved, such as phospholipase A_1, C, lyso-phospholipases, or other esterases (Bilinski and Jonas, 1966; Cohen et al., 1967).

Lipid Oxidation. The lipid oxidation was monitored using three different measurements: UV absorption of conjugated dienes and trienes, formation of MDA, and the content of TBA-RS. The content of conjugated dienes and trienes is an indicator of early stages of lipid oxidation (Halliwell and Gutteridge, 1989; Rossell, 1989). MDA and TBA-RS are indices of later stages of lipid oxidation (Draper and Hadley, 1990; Janero, 1990). For a discussion of the spectrum obtained by measurement of the UV absorbance, see Ingemansson et al. (1993b). In this study, the UV spectra displayed no differences in diene and triene conjugates in fillets stored frozen for 34 weeks, whether they were acclimatized to 5 or 12 °C. There were small but insignificant increases in conjugated dienes and trienes in both light and dark muscle during frozen storage. Measurement of conjugated dienes by UV absorption in the lipid phase is complicated by a high background absorbance and therefore found not to be a very sensitive method for monitoring lipid oxidation in fish (Erickson, 1993).

The content of TBA-RS was about 3 times higher for dark muscle than for light muscle (Table 2). There was a small increase in the absorbance of TBA-RS in light muscle between the initial controls and fish stored for 34 weeks, but for dark muscle there was no siginificant difference.

The total content of MDA in light and dark control fish muscle was low, in the range of 0.5–1 μg g^{-1} of wet

Table 2. TBA-RS and MDA Content in Light and Dark Muscle Tissue as Indicators of Lipid Oxidation[a]

	0 weeks at 15 °C		34 weeks at 15 °C	
	light	dark	light	dark
TBA-RS (A_{532})	0.18 ± 0.03	0.69 ± 0.05	0.24 ± 0.04	0.63 ± 0.05
MDA (mg g^{-1} of wet tissue)				
total	0.58 ± 0.14	1.02 ± 0.16	1.40 ± 0.16	5.0 ± 0.2
free	0.51 ± 0.16	0.30 ± 0.13	ND[b]	ND
bound	0.07 ± 0.01	0.72 ± 0.16	ND	ND

[a] Values are mean ± SEM (n = 8). [b] Not determined.

tissue (Table 2). After 34 weeks of frozen storage, the content of MDA in light muscle had increased by about 50% to a total value of 1.4 $\mu g\ g^{-1}$ of wet tissue and in dark muscle about 5-fold to a total value of 5.0 $\mu g\ g^{-1}$. No differences were seen between fishes acclimatized to 5 and 12 °C, respectively.

The relatively low contents in MDA can be seen as an indication that lipid oxidation was taking place only to a low extent. The cause of this is actually only that the samples were stored for too short a time for more marked lipid oxidation to occur. Another contributing factor was the glazing of the samples, which is known to be an effective protection against lipid oxidation.

Multivariate Evaluation. The partial least-squares method was used to display the time dependency of the data, using the compositional data as the independent block and time (0, 2, 4, 8, 16, and 34 weeks) as the dependent block. Separate models for light and dark muscle were developed, as previous experiences have shown that combining these in the model building process yields a model that only emphasizes the differences between dark and light muscle (Ingemansson et al., 1993a).

With the model developed for light muscle, three PLS components for light muscle can be calculated, with 41% (30 + 9 + 2) of the variance in the compositional data explaining 97% (63 + 28 + 6) of the time variance. For dark muscle two significant PLS components can be calculated, with 34% (25 + 9) of the variance in the compositional data explaining 90% (73 + 17) of the time variance. In Figure 2, the strong time dependency of the data can be seen as three time-separated groups of fish for both light and dark muscle. It looks like some early changes are taking place soon after the beginning of frozen storage and that the next step in deterioration requires a longer storage time. Therefore, there are three groups, so that the control fish (0 weeks), the group of fish stored for 2, 4, 8, and 16 weeks, and, finally, the group stored for 34 weeks represent three levels of lipid degradation (Figure 2).

The compositional variables measured in light and dark muscle are shown in Tables 3 and 4. The relationship of the original experimental variables to the model, for each PLS component, can be expressed as the modeling power of that experimental factor. This is a quantity that varies between 0 and 1 and is essentially the amount of variance of each of the experimental variables contained in the component (Sjöström et al., 1986). The PLS models for light and dark muscle are mainly influenced by the FFA content and the lipid classes (Table 3). The strongest influence from the lipid classes comes from the NL fraction, PC, PE, and lyso-PC in both light and dark muscle. The contribution among the fatty acids in the NL fraction was from 16:0, 16:1$n-7$, and 18:1$n-9$, in PC from 20:5$n-3$, and

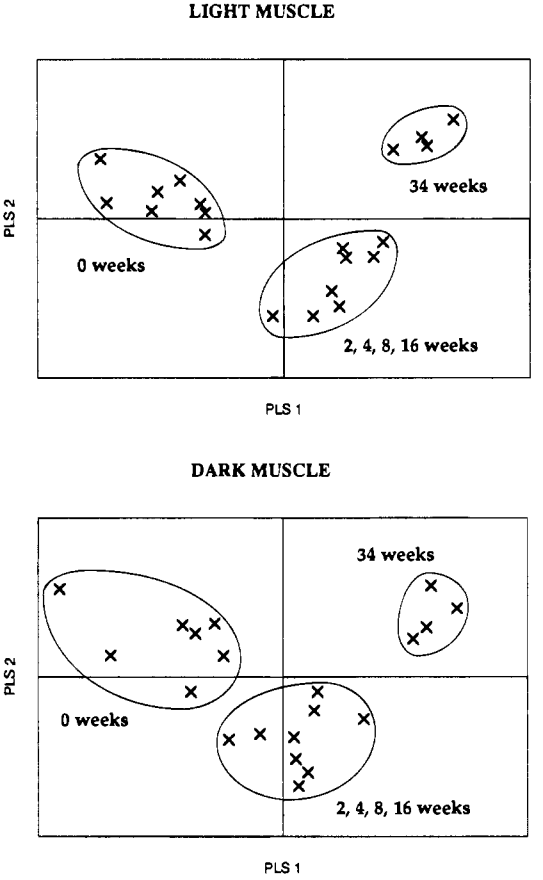

Figure 2. Score plot from a PLS correlation analysis, extracting the time effect in the data of frozen stored light and dark muscle of rainbow trout.

Table 3. Modeling Power in PLS Analysis with Respect to Time

variable	light muscle	dark muscle		light muscle	dark muscle
TBA-RS	0.15	0.16	NL-FA		
MDA total	0.10	0.06	16:0	0.11	0.17
MDA free	0.16	0.39	16:1$n-7$	0.16	0.24
conj trienes	0.05	0.01	18:1$n-9$	0.09	0.08
neutral lipids	0.38	0.45	18:2$n-6$	0.00	0.09
PC	0.40	0.46	20:5$n-3$	0.07	0.04
PE	0.39	0.07	22:1$n-11$	0.00	0.01
SPH	0.01	0.00	22:6$n-3$	0.02	0.16
lyso-PC	0.56	0.32	PC-FA		
lyso-PE	0.00	0.00	16:0	0.20	0.00
FFA			20:5$n-3$	0.23	0.36
14:0	0.43	0.14	22:6$n-3$	0.00	0.01
16:0	0.24	0.29	PE-FA		
16:1$n-7$	0.56	0.49	16:0	0.17	0.05
18:0	0.52	0.35	18:0	0.20	0.00
18:1$n-9$	0.61	0.19	18:2$n-6$	0.12	0.04
18:1$n-7$	0.44	0.32	20:5$n-3$	0.12	0.00
18:2$n-6$	0.40	0.41	22:6$n-3$	0.32	0.11
18:3$n-3$	0.47	0.25			
18:4$n-3$	0.53	0.59			
20:1$n-9$	0.02	0.02			
20:5$n-3$	0.66	0.35			
22:1$n-9$	0.03	0.02			
22:5$n-3$	0.47	0.47			
22:6$n-3$	0.47	0.41			

in PE from 22:6$n-3$, but they were all weak. The contributions of TBA-RS and total and free MDA to the models were weak, except for free MDA in the dark muscle (Table 3).

The next step was to analyze the effect of the acclimatization temperature (5 and 12 °C) on lipid stability. The results were not clear-cut, partly due to

Table 4. Modeling Power in PLS Analysis with Respect to Acclimatization Temperature

variable	light muscle	dark muscle
TBA-RS	0.25	0.43
MDA total	0.06	0.00
MDA free	0.05	0.21
conj trienes	0.14	0.00
neutral lipids	0.04	0.26
PC	0.04	0.10
PE	0.42	0.31
SPH	0.09	0.00
lyso-PC	0.08	0.09
lyso-PE	0.18	0.22
FFA		
14:0	0.15	0.14
16:0	0.09	0.22
16:1$n-7$	0.43	0.49
18:0	0.00	0.03
18:1$n-9$	0.52	0.11
18:1$n-7$	0.39	0.40
18:2$n-6$	0.57	0.66
18:3$n-3$	0.57	0.24
18:4$n-3$	0.33	0.36
20:1$n-9$	0.30	0.73
20:5$n-3$	0.20	0.00
22:1$n-9$	0.30	0.64
22:5$n-3$	0.23	0.00
22:6$n-3$	0.30	0.50

the high degree of natural variation between individuals. The data were normalized to minimize these variations by subtracting the initial value for each compositional variable, measured on the initial fillet, according to the formula

$$^{\text{fish,temp}}X_{i,\text{time}} - {}^{\text{fish,temp}}X_{i,\text{initial}}$$

Two significant PLS components can be calculated for each muscle type with 30% (25 + 5) of the variance in the compositional data explaining 98% (83 + 15) of the variance in temperature for dark muscle and with 33% (25 + 8) of the variance in the compositional data explaining 94% (74 + 20) of the variance in temperature in light muscle. Two groups are distinguished in both light and dark muscle, which clearly shows the temperature dependency in the data and the change in lipid stability due to different acclimatization temperatures (Figure 3).

The modeling power of the temperature dependency in the experimental variables is presented in Table 4. Among the lipid classes, PE contributed most, especially in light muscle. The strongest influence from the FFA came from 16:1$n-7$, 18:1$n-7$, and 18:2$n-6$ in both light and dark muscle, from 18:1$n-9$ and 18:3$n-3$ in light muscle, and from 20:1$n-9$, 22:1$n-9$, and 22:6$n-3$ in dark muscle. The strongest influence of the lipid oxidation variables on the model for temperature dependence could be attributed to TBA-RS in dark muscle.

Conclusions. The results from these analyses show a major increase in FFA due to hydrolysis of especially phospholipids, mostly PC, in frozen rainbow trout muscle stored at −15 °C up to 34 weeks and an increase in the formation of lipid oxidation products (most evident in the MDA content). It can be concluded that the major cause of deterioration of the lipids during frozen storage in our experiments was hydrolysis, not oxidation. This may have several causes, but the samples were glazed and stored in sealed aluminum bags, which provides relatively good protection against oxidation. Therefore, hydrolysis was strongly favored.

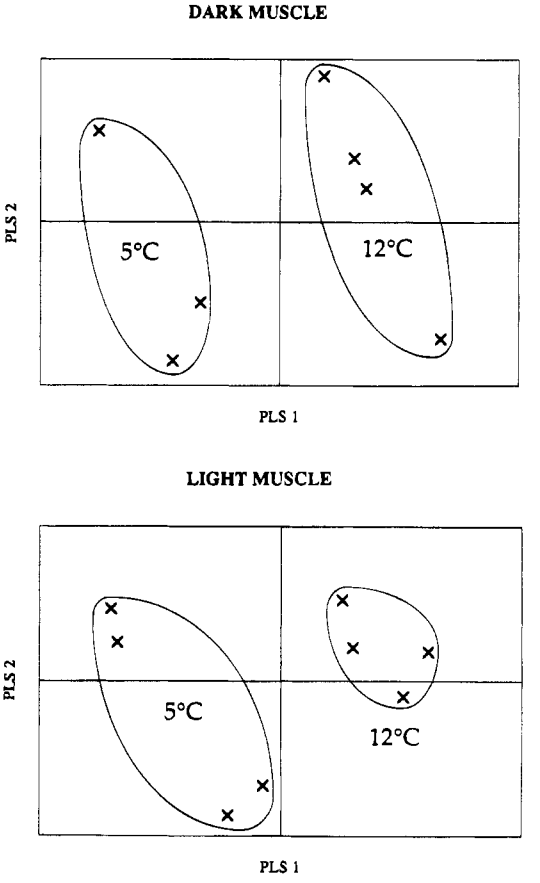

Figure 3. Score plot from a PLS correlation analysis, extracting the temperature effect in the data of frozen stored light and dark muscle of rainbow trout.

The storage time was also relatively short. Other experiments have shown that rainbow trout is fairly stable during frozen storage for up to 13 months at −20 °C under favorable conditions.

The strong influence in the multivariate modeling from TBA-RS and FFA shows that acclimatization to lower water temperature (5 °C) resulted in decreased lipid stability related to both oxidation and hydrolysis during frozen storage.

The antioxidant activity in fish muscle establishes a balance between deterioration and resynthesis. This balance is interrupted post-mortem. For instance, the carotenoids and α-tocopherol deteriorate during frozen storage of fish muscle (Ingemansson et al., 1993b). There is evidence that lipid oxidation does not accelerate until the amount of antioxidants has fallen to a certain level. Therefore, it is of great importance to ensure that sufficient amounts of antioxidants are present for the post-mortem protection of the lipid phase in the muscle.

In general, it is important to investigate the factors that affect lipid stability in frozen stored rainbow trout and to gain more knowledge about the degree to which changes take place in the lipid phase, through hydrolysis and oxidation, as these factors will not only determine the useability and potential of frozen storage of fish but also influence the possibility for further proccessing, such as curing and smoking. We believe that multivariate statistical evaluation of a large set of data, obtained by compositional and other chemical analyses, is a good and valuable complement to the traditional interpretation and can provide a better basis for optimization of processing and handling.

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