

Rapid communication

## Seasonal development of nutrient composition, lipid oxidation and colour of fillets from Norwegian spring-spawning herring (*Clupea harengus* L.)

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### Abstract

The Norwegian spring-spawning herring (*Clupea harengus* L.) forms the basis of extensive fisheries off the Norwegian coast from September to January each year. Herring is filleted, frozen and exported, mainly to the East European markets. Fillets from herring caught late in the season tend to be discoloured and it was hypothesised that this was due to lipid oxidation. Herring fillets from fish caught throughout the season were sampled at a local filleting plant. The fillets had been frozen and stored at  $-30\text{ }^{\circ}\text{C}$  for varying periods. Dry matter and lipid content, fatty acid composition of lipids, vitamin C and E, lipid oxidation and the colour of the fillets were monitored. Lipid and dry matter content of the fillets decreased during the season from 175 to 75 g kg<sup>-1</sup> and 364 to 260 g kg<sup>-1</sup>, respectively. Vitamin C content decreased during the season and varied between 3.3 and 1.0 mg kg<sup>-1</sup>, while vitamin E varied between 21 and 7 mg kg<sup>-1</sup>. The change in fatty acid composition was characterised by a decrease in polyunsaturated and saturated fatty acids and an increase in monounsaturated fatty acids during the season. Thiobarbituric acid reactive substances (TBARS) varied between 43 and 122 nmol g<sup>-1</sup> and peroxide value (PV) between 2.6 and 6.8 μmol g<sup>-1</sup>. The fillets became darker, redder and yellower toward the end of the season, especially after spawning migration had commenced. There was a negative correlation between vitamin E content and both TBARS and PV, indicating that vitamin E protected against lipid oxidation or that vitamin E was consumed during lipid oxidation. Very little connection was found between lipid oxidation and colour development. It was concluded that the colour deterioration in the fillets was not due to lipid oxidation, but may have been caused by the change in proximate composition, less lipid leading to less white and more transparent fillets when the dark muscle and blood stains became more visible.

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### 1. Introduction

In 1997, the Norwegian spring-spawning herring (*Clupea harengus* L.) reached a spawning biomass of 9 million tonnes, after having recovered from a nearly extinct state in the late 1960s (Slotte, 1999). The stock spends the feeding period (April–August) scattered over most of the Norwegian Sea. During the wintering period (September–January), the herring congregate at large depths in Vestfjorden, Ofotfjorden and Tysfjorden in Northern Norway (Fig. 1). Spawning migration is

initiated in mid January to spawning grounds from Lofoten to Lista. During both the wintering and spawning migration periods, the herring do not feed. This leads to a cyclic change in fat and energy contents in the fish, lipid content being high in the summer and low in the winter, where lipid content is inversely correlated with water content (Slotte, 1999).

This stock is the basis for extensive fisheries off the Norwegian coast from September to March, and appreciable amounts of herring are filleted, frozen and exported, mainly to East European markets. Frozen herring fillets tend to become discoloured, especially when the fish are caught late in the season. We hypothesised that this may have been caused by rancidity, since lipid oxidation often causes yellow fluorescent

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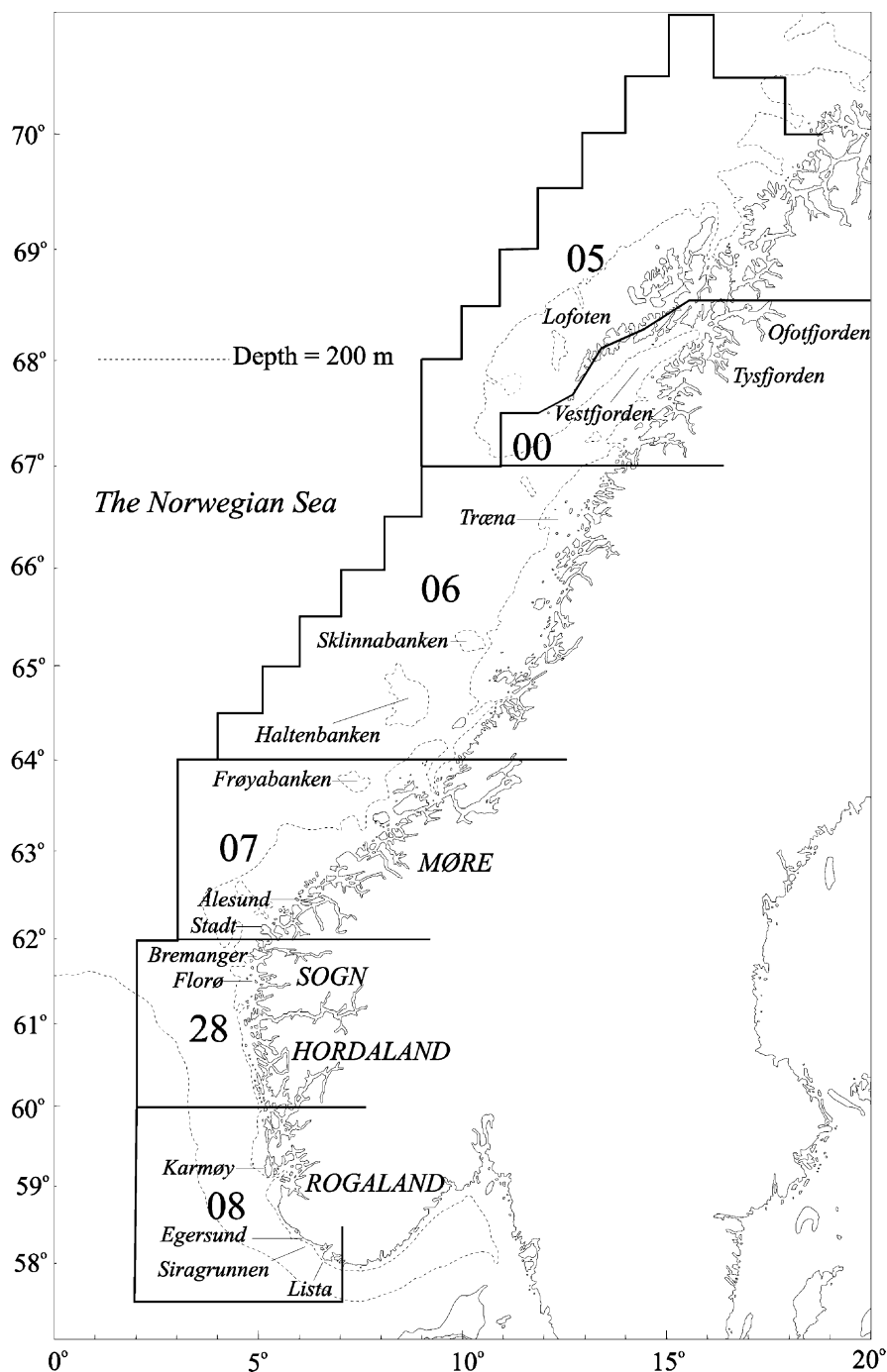


Fig. 1. Map of statistical fishing areas (two digits) off the Norwegian coast.

pigments to accumulate in the fillet. Further, herring are not bled and bloodstains appear on the fillets. By exposure to air, the blood darkens, and may be perceived as discoloration. The excess blood may also stimulate lipid oxidation. The present study maps the development of dry matter, lipid content, fatty acid composition, antioxidant levels, lipid oxidation and colour in fillets from herring caught during the season, with the purpose of identifying a possible connection between change in nutrient composition, lipid oxidation and discoloration of the fillets.

## 2. Materials and methods

### 2.1. Fish

Norwegian Spring spawning herring (*Clupea harengus* L.) caught in the regular fisheries off the Norwegian coast from September 1997 to March 1998 (fishing area given in Fig. 1) and filleted and frozen at Austevoll Fiskeindustri AS, Storebø, Norway, were sampled for analyses. The herring fillets were packed in 20-kg cardboard boxes

with plastic linings, frozen in a tunnel and stored at  $-30\text{ }^{\circ}\text{C}$ . Samples of three boxes per catch were taken on 3 November, 8 December and 5 March. The fillets were thawed overnight and 10 double fillets per box were sampled. One fillet from each fish was used for colour measurements, the other for chemical analyses, for which the 10 fillets per box were pooled.

## 2.2. Chemical analyses

Dry matter was determined gravimetrically after freeze-drying. Lipid content was determined gravimetrically after extraction with ethyl acetate and isopropanol (Lie, Waagbø, & Sandnes, 1988). Vitamin E was analysed, as described by Lie, Sandvin, and Waagbø (1994), vitamin C, according to Mæland, Rosenlund, Stoss, and Waagbø (1999), thiobarbituric acid reactive substances (TBARS), according to Hamre, Næss, Espe, Holm and Lie (2001) and peroxide value according to Undeland, Härröd, and Lingert (1998). Fatty acid composition was analysed by the method of Lie and Lambertsen (1991).

## 2.3. Colorimetric measurement

Flesh colour was measured at three points in the fillet (anterior, middle, posterior) using a portable Hunterlab Miniscan/EX instrument (10\* standard observer, illuminant D65, Hunter Associates Laboratory Inc, 11491 Sunset Hills Road, Reston, Virginia, USA) calibrated to a white and a black standard. The tristimulus  $L^*a^*b^*$  measurement mode was used as it relates the human eye response to colour. The  $L^*$  variable represents lightness ( $L^*=0$  for black,  $L^*=100$  for white),  $a^*$  scale represents the red/green,  $+a^*$  intensity in red and  $-a^*$  intensity in green.  $b^*$  scale represents the yellow/blue,  $+b^*$  intensity in yellow and  $-b^*$  intensity in blue.

## 2.4. Statistical analyses

The software Statistica (Statsoft Inc., Tulsa, USA) was used for the correlation analyses. Correlation coefficients were considered significant at  $P < 0.05$ .

## 3. Results

Dry matter content (Table 1) in the fillets varied from 260 to  $364\text{ g kg}^{-1}$  and showed a strong correlation (Table 4) with the variation in lipid content ( $r^2=0.97$ ), which varied from 72 to  $175\text{ g kg}^{-1}$  wet weight (Table 1). There was also a strong correlation between time from start of the season and both dry matter and lipid contents (Table 4,  $r^2=-0.85$  and  $-0.75$ , respectively), showing a decrease during the autumn and winter. Vitamin C content (Table 1) was also negatively correlated with time from start of the season (Table 4,  $r^2=-0.74$ ) and varied between 1.0 and  $3.3\text{ mg kg}^{-1}$  wet weight. Vitamin E content (Table 1) varied between 7 and  $21\text{ mg kg}^{-1}$  wet weight and was not related to time from start of the season, but correlated weakly and negatively with storage time (Table 4,  $r^2=-0.58$ ). The fatty acid composition (Table 2) was characterised by a decrease in n-3 polyunsaturated fatty acids (PUFA), n-6 PUFA and saturated fatty acids and an increase in monoenes through the season.

TBARS varied between 43 and  $125\text{ nmol g}^{-1}$  (Table 1) and showed a relatively strong negative correlation with vitamin E content (Table 4,  $r^2=-0.82$ ). Further, TBARS was weakly correlated with time from start of the season ( $r^2=0.47$ ), storage time ( $r^2=0.53$ ) and vitamin C content ( $r^2=-0.55$ ). Peroxide value varied between 2.6 and  $6.8\text{ }\mu\text{mol g}^{-1}$  lipid (Table 1), and was weakly and negatively correlated with vitamin C and E contents (Table 4,  $r^2=-0.55$  and  $-0.61$ , respectively).

Table 1

Contents of dry matter, lipid, vitamin C, vitamin E, thiobarbituric acid reactive substances (TBARS) and peroxide value (PV) in frozen stored fillets of Norwegian spring spawning herring caught between 2 September 1997 and 5 March 1998 (Fishing area is given in Fig. 1)

Date of catch	Fishing area	Catch to freeze (days)	Frozen stored (days)	Dry matter ( $\text{g kg}^{-1}$ )	Lipid ( $\text{g kg}^{-1}$ wet wt.)	Vitamin E ( $\text{mg kg}^{-1}$ wet wt.)	Vitamin C ( $\text{mg kg}^{-1}$ wet wt.)	TBARS ( $\text{nmol g}^{-1}$ wet wt.)	PV ( $\mu\text{mol g}^{-1}$ lipid)
2/9/1997	05	4	57	$364\pm 5$	$175\pm 4$	$20\pm 1$	$1.9\pm 0.1$	$62\pm 6$	$4.1\pm 0.2$
5/9/1997	05	4	54	$347\pm 6$	$161\pm 3$	$18\pm 2$	$2.2\pm 0.2$	$59\pm 12$	
12/10/1997	00	3	19	$350\pm 3$	$157\pm 2$	$21\pm 2$	$3.3\pm 0.4$	$44\pm 11$	$2.6\pm 0.2$
23/10/1997	00	3	8	$342\pm 12$	$154\pm 18$	$21\pm 2$	$2.9\pm 0.5$	$44\pm 4$	
27/10/1997	00	4	3	$334\pm 18$	$144\pm 23$	$18\pm 2$	$2.0\pm 0.1$	$43\pm 11$	
4/11/1997	00	4	67	$337\pm 18$	$158\pm 28$	$11\pm 4$	$2.0\pm 0.3$	$94\pm 5$	$6.1\pm 0.2$
5/11/1997	00	6	64	$338\pm 9$	$159\pm 8$	$10\pm 3$	$1.8\pm 0.2$	$116\pm 26$	
10/11/1997	00	5	60	$333\pm 5$	$154\pm 6$	$8\pm 1$	$1.8\pm 0.1$	$125\pm 0$	
26/11/1997	00	4	45	$337\pm 5$	$160\pm 8$	$13\pm 2$	$1.6\pm 0.1$	$95\pm 17$	$4.7\pm 0.8$
18/1/1998	00	4	51	$313\pm 9$	$130\pm 6$	$14\pm 4$	$1.1\pm 0.4$	$99\pm 22$	
19/1/1998	00	4	50	$300\pm 2$	$110\pm 4$	$7\pm 1$	$1.2\pm 0.1$	$122\pm 7$	$6.8\pm 1.9$
24/2/1998	28	2	17	$273\pm 2$	$89\pm 3$	$13\pm 3$	$1.2\pm 0.1$	$97\pm 17$	$5.5\pm 0.2$
2/3/1998	07	2	10	$302\pm 8$	$128\pm 10$	$28\pm 7$	$1.4\pm 0.4$	$62\pm 33$	
5/3/1998	28	4	5	$260\pm 7$	$72\pm 24$	$19\pm 4$	$1.0\pm 0.2$	$81\pm 16$	$4.3\pm 0.6$

Table 2  
Summary of fatty acid composition (% of total fatty acids) in frozen stored fillets of Norwegian spring spawning herring caught between 2 September 1997 and 5 March 1998

	Date of catch													
	2/9/1997	5/9/1997	12/10/1997	23/10/1997	27/10/1997	4/11/1997	5/11/1997	10/11/1997	26/11/1997	18/1/1998	19/1/1998	24/2/1998	2/3/1998	5/3/1998
Saturated	24.2±0.4	24.6±0.1	21.8±0.8	21.7±0.5	22.9±0.6	22.7±0.5	22.5±0.9	22.7±0.4	22.5±0.2	22.5±0.2	21.7±0.6	20.9±0.5	18.6±0.4	20.8±0.2
Sum 16:1	5.0±0.3	4.8±0.2	4.3±0.2	4.6±0.1	4.7±0.2	4.9±0.2	4.6±0.3	4.7±0.2	4.7±0.1	4.8±0.0	4.6±0.3	3.9±0.1	4.3±0.3	3.9±0.2
Sum 18:1	14.3±0.4	15.0±0.2	12.2±0.4	12.8±0.9	13.7±1.7	13.6±0.5	12.7±1.1	13.9±0.1	13.8±0.6	16.0±0.3	15.5±1.3	13.9±0.5	12.2±0.7	14.8±0.3
Sum 20:1	10.3±0.4	10.2±0.5	12.9±0.7	13.0±0.4	11.6±0.9	12.0±0.3	12.5±1.1	11.5±0.6	12.1±0.2	11.1±0.1	11.7±1.4	14.7±0.1	17.0±0.9	14.6±0.2
Sum 22:1	15.9±0.4	15.4±0.7	19.4±1.0	19.3±0.3	17.7±1.7	18.1±0.7	19.0±1.4	17.4±0.5	18.4±0.2	17.8±0.2	18.2±1.6	22.6±0.4	25.7±1.8	22.1±0.7
24:1n-9	0.7±0.0	0.7±0.0	0.8±0.0	0.8±0.1	0.8±0.1	0.8±0.0	0.8±0.1	0.8±0.0	0.8±0.0	1.1±0.1	1.2±0.1	1.2±0.0	1.1±0.1	1.2±0.0
Monoenes	46.2±0.4	46.1±0.4	49.6±1.8	50.5±1.4	48.5±0.7	49.4±0.7	49.6±1.1	48.2±0.7	49.8±0.6	50.8±0.2	51.2±1.8	56.4±0.4	60.3±1.8	56.6±1.4
Sum n-3	24.5±0.4	24.4±0.5	23.7±1.3	23.0±0.9	23.6±0.4	23.1±0.2	23.3±0.1	24.6±0.2	23.0±0.6	21.0±0.3	21.4±0.9	16.7±0.3	15.9±1.1	17.0±0.8
Sum n-6	1.9±0.0	2.0±0.1	1.8±0.1	1.9±0.0	1.9±0.1	1.9±0.1	1.9±0.0	1.9±0.1	1.9±0.1	1.8±0.1	1.8±0.1	1.6±0.1	1.5±0.2	1.6±0.1
Polyenes	26.7±0.4	26.6±0.7	25.7±1.3	25.0±0.9	25.8±0.5	25.2±0.2	25.4±0.1	26.6±0.3	25.1±0.6	23.0±0.3	23.4±1.0	18.6±0.4	17.6±1.3	18.8±0.9
n-3/n-6	12.6±0.4	12.3±0.4	12.8±0.7	12.4±0.2	12.5±0.5	12.4±0.5	12.1±0.1	13.2±0.5	12.3±0.3	11.9±0.9	11.9±0.2	10.6±0.9	11.0±1.0	10.6±0.5

The fillets were lighter, less red and more yellow at the anterior compared to the posterior end (Table 3). Further, they tended to become darker and redder with progress of the season (Tables 3 and 4). At the anterior end, the fillets also became more yellow with time. The development towards darker and redder fillets was correlated with the decreases in lipid and dry matter contents, whereas the development of yellowness was coupled to dry matter content, only. There were weak positive correlations between both redness and yellowness and vitamin E and between vitamin C and lightness. Except for a very weak negative correlation between TBARS and yellowness at the anterior end of the fillet, there seemed to be no connection between contents of lipid oxidation products and colour (Table 4).

#### 4. Discussion

The decrease in dry matter and lipid content from September to March is in accordance with previous studies (Slotte, 1999, and references therein) and is a result of the wintering and spawning migration periods, where the herring do not feed but depend on their body energy stores accumulated during spring and summer. The three last catches were taken in migrating fish in the spawning areas off the coast of Møre and Hordaland, where the herring caught in Hordaland had the lowest fat and dry matter contents. This is in agreement with Slotte (1999), who found that energy losses were several times higher in migrating, compared to wintering herring. The changes in fatty acid composition indicate that n-3 PUFA and saturated fatty acids are preferred for catabolism while the monoenes are spared. This is surprising, since the n-3 PUFA are essential fatty acids, while monoenes are probably dispensable. The decrease in lipid content and % n-3 PUFA cause the herring fillets caught late in the season to be less nutritious in terms of being a source of marine lipid.

The levels of TBARS showed large variation and were very high compared to the 25 nmol g<sup>-1</sup> found in Atlantic salmon after 8 months of frozen storage (Hamre, Berge, & Lie, 1998). Unpublished results indicate that herring is more susceptible to oxidation than most other fish species. This is in agreement with Hultin (1988) who found that enzymatic lipid oxidation of microsomes from light muscle of herring was 4–5 times higher than that from light muscle of winter flounder and red hake. The enzymatic lipid oxidation in dark muscle of herring was 3–4 times that in light muscle (Hultin, 1988). In spite of the high TBARS in the herring fillets, the levels of peroxides were acceptable at about half of the recommended maximum level for consumption. Lipid oxidation seemed dependent on antioxidant vitamins, primarily vitamin E. The results show either that vitamin

Table 3

Colour measurements on frozen stored fillets of Norwegian spring spawning herring caught in the period 2 September 1997 to 5 March 1998

Date of catch	Frozen stored (days)	L1	L2	L3	a1	a2	a3	b1	b2	b3
4/11/1997	67	64.0±1.1	60.1±1.3	53.6±2.4	2.5±0.3	3.1±0.7	3.5±0.7	19.5±0.7	19.0±1.4	15.8±0.9
5/11/1997	64	64.2±0.5	62.0±0.2	53.8±0.8	2.4±0.5	2.9±0.3	4.4±0.6	19.7±1.1	19.3±0.3	16.6±0.0
10/11/1997	60	64.8±0.2	60.7±1.9	52.4±1.1	2.4±0.1	3.7±0.5	4.9±0.1	18.7±0.3	19.9±0.0	16.3±0.4
26/11/1997	45	61.5±2.5	59.0±2.5	51.5±3.0	2.8±1.0	3.2±1.0	4.7±0.7	19.9±0.6	19.5±0.6	16.2±0.3
18/1/1998	51	64.3±1.4	60.4±2.0	52.4±1.9	2.4±0.4	2.8±0.5	3.6±1.0	19.8±0.6	18.3±0.6	15.4±0.9
19/1/1998	50	62.0±0.7	58.4±2.2	50.9±2.4	2.7±0.1	3.1±0.5	4.3±0.4	19.8±0.4	18.8±0.7	16.5±0.8
24/2/1998	17	59.1±1.5	56.0±1.8	49.7±1.6	3.8±0.3	4.0±0.5	4.2±0.6	20.1±0.5	18.8±0.1	16.1±0.2
2/2/1998	10	61.8±1.7	57.5±0.4	51.2±1.2	4.3±0.5	4.9±0.8	4.6±1.2	21.5±0.8	20.6±0.9	16.7±1.2
5/3/1998	5	59.1±0.6	55.2±0.6	48.1±1.2	4.5±0.8	4.5±1.1	4.5±0.9	20.1±0.4	19.7±0.2	15.8±0.1

L: Lightness, black=0, white=100. a: Redness;  $x < 0$  green,  $x > 0$  red. b: Yellowness;  $x < 0$  blue  $x > 0$  yellow.  $N=30$ . Figure codes indicate measurement points on the fillet; 1, anterior, 2, middle 3, posterior.

Table 4

Correlation between quality parameters, time from start of the season and storage time in frozen stored fillets of Norwegian spring spawning herring caught between 2 September 1997 and 5 March 1998

	TBARS	PV	Vitamin E	Vitamin C	Dry matter	Lipid									
<b>Lipid oxidation products and nutrient composition</b>															
Time from start of the season	0.47*	0.37	-0.15	-0.74*	-0.86*	-0.75*									
Storage time	0.53*	0.38	-0.58*	-0.11	0.45*	0.48*									
TBARS	1.00														
PV	0.64*	1.00													
Vitamin E	-0.82*	-0.61*	1.00												
Vitamin C	-0.55*	-0.53*	0.33*	1.00											
Dry matter	-0.27	-0.31	0.08	0.67*	1.00										
Lipid	-0.15	-0.28	0.05	0.58*	0.97*	1.00									
<b>Colour</b>							L1	a1	b1	L2	a2	b2	L3	a3	b3
Time from start of the season	-0.63*	0.72*	0.52*	-0.69*	0.55*	0.19	-0.64*	0.14	0.03						
TBARS	0.14	-0.38	-0.39*	0.17	-0.32	-0.27	0.06	-0.08	-0.07						
PV	0.07	-0.32	-0.04	0.16	-0.46	-0.43	0.23	-0.53	0.09						
Vitamin E	-0.24	0.59*	0.59*	-0.32	0.55*	0.42*	-0.17	0.13	0.05						
Vitamin C	0.48*	-0.36	-0.25	0.44*	-0.16	0.12	0.59*	0.03	0.18						
Dry matter	0.80*	-0.68*	-0.41*	0.80*	-0.46*	-0.12	0.73*	0.02	0.11						
Lipid	0.74*	-0.56*	-0.34	0.73*	-0.35	-0.08	0.68*	0.07	0.08						

TBARS, thiobarbituric acid reactive substances; PV, peroxide value. Significant correlations are marked with \* ( $P < 0.05$ ). Colour measurements were taken at three points on the fillet; 1, anterior; 2, middle; 3, posterior.

E was consumed during oxidation or that oxidation was slower at high levels of vitamin E. In the latter case, the large variation in TBARS could be explained by variation of vitamin E in the fillets. The vitamin E levels were similar to those found in fillet of Atlantic salmon fed between 100 and 250 mg kg<sup>-1</sup> dry weight vitamin E (Hamre & Lie, 1995), which is similar to levels found in copepods (unpublished data).

Lipid oxidation increased slightly with time from the start of the season and decreased slightly with increasing levels of vitamin C. Since vitamin C and time were strongly inversely correlated, their effects cannot easily be separated. Increased oxidation could, in addition to the decreasing vitamin C content, be caused by other compositional changes, such as change in lipid content and fatty acid composition. Ruff, FitzGerald, Gross, Hamre, and Kerry (2003) found that vitamin C up to 45

mg kg<sup>-1</sup> had very little effect on lipid oxidation in turbot fillet, and the vitamin C levels found here are so low that it can be questioned whether they can have a significant antioxidant effect. On the other hand, the compositional changes towards lowered lipid and polyunsaturated fatty acids levels would theoretically be expected to give lowered lipid oxidation.

Other parameters that might affect lipid oxidation are handling and storage time. Storage time was only weakly correlated with TBARS and not with PV. Handling during filleting and freezing of the fish was not monitored in the present study, although these factors may have a profound effect on lipid oxidation. It is possible that variation in handling conditions caused variation in lipid oxidation and, thereby variations in the production of TBARS and consumption of vitamin E.

The herring fillets were lighter, less red and less yellow at the anterior compared to the posterior end. The reason for this is probably that the dark muscle constitutes a larger part of the fillet at the posterior end, and is visible under the surface of white muscle. There was a very clear change in colour of the fillets during the season, the fillets becoming darker, redder and more yellow with time. This especially concerns the catches taken after the start of the spawning migration, where dry matter and lipid were also the lowest. The colour development was strongly correlated with dry matter and lipid contents and with time from the start of the season, but very little connection was found between lipid oxidation and colour. The slight positive correlation between redness and yellowness and vitamin E shows that vitamin E did not protect against colour deterioration, while the slight positive correlation between vitamin C and lightness is not easily separated from the other changes occurring with time. Of the lipid oxidation indicators, only TBARS showed a very weak negative correlation with yellowness in the posterior end of the fillet, indicating that increasing lipid oxidation caused less and not more yellow colour, as would have been expected. Thus, there is little reason to believe that lipid oxidation is the cause of colour deterioration in fillets from herring caught late in the season. The change in dry matter and lipid could be the actual reason for this quality change, where less lipid makes the fillet less white and more transparent so that the red muscle and blood patches become more visible. Our data indicate that quality related to colour is conserved during the wintering period to a large extent, but that it deteriorates quickly during the spawning migration.

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