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Seasonal Changes in Crude and Lipid Composition of Herring Fillets, Byproducts, and Respective Produced Oils

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Crude and fatty acid composition analyses were performed on fillets, byproducts, and oil originating from herring (*Clupea harengus*) caught off the North Sea from June 1999 to January 2001. Monthly statistical differences were found in the fat content, the range of variation being larger in fillets than in byproducts. The most consistent change observed in fillets was an increase of unsaturation from May to September reflected in a reduced percentage of monounsaturated fatty acids, whereas for byproducts and oil this trend was not so well defined. The results indicated that the lowest values of the total amount of polyunsaturated fatty acids (PUFAs) in the oil were found from January to March (∼14%), coinciding with the postspawning and starvation period. In contrast, the highest values were found from June to August (∼23%). Thus, the herring byproducts are all year an adequate raw material for fish oil production; however, during the summer they are richer in PUFAs.

KEYWORDS: Crude oil; crude composition; herring byproducts; PUFAs; seasonal changes; processing

INTRODUCTION

The composition of several fish species varies from season to season due to its natural cycle, maturity stage, geographic location, etc. (*1*−*8*). Herring, being a typical pelagic fatty fish, goes through a natural cycle showing considerable variations in lipid content and composition as well (*9*−*14*). The industry takes advantage of this fact by producing respective typical products such as maatjes, marinated, frozen, cured herring, and kippers, which are characteristic for different times of the year and encouraged to be consumed by health authorities as a source of the beneficial polyunsaturated fatty acids (PUFAs). During the processing of herring a considerable amount of byproducts is originated, which has to be processed further or disposed of. Following successful experiences upgrading herring byproducts into stable crude fish oil (*15*, *16*), showing that sorting is not required (*17*) and that good-quality fish oil could be produced from stored byproducts (*18*), knowledge is necessary to elucidate the oil composition changes over the year. An important question is whether it is possible to keep the composition of oils produced from herring byproducts constant when the lipid content and composition of herring fluctuates seasonally. Literature on the topic does not exist, and the availability of such data might bring practical implications to the fish-processing industry.

The present work was undertaken to examine possible seasonal changes over the year in crude composition and lipid content of herring with particular regard to the distribution and change of fatty acids composition among fillets, byproducts, and oil produced from byproducts.

MATERIALS AND METHODS

Sampling of herring (*Clupea harengus*) was carried out from June 1999 to January 2001 at the North Sea (**Figure 1**). Before the herring was processed in a fish-processing company, some specimens were collected, and total length, weight, and maturity stage were recorded. According to the collected data, and using the maturity scale recommended by the herring committee of International Council for the Exploration of the Sea (ICES) (*19*) and by others (*20*), the maturity categories IV−V describe herring approaching spawning condition and categories VI−VII are related to herring that are about to spawn or have recently spawned. Categories I−III and VIII concern fish unlikely to spawn for some time. In this study, fresh herring was used except for the months of June, July, and August; fish caught during these months have been deep-frozen before being processed. Processed fillets, deboned and skinned, were collected and characterized for crude and fatty acids composition. Obtained byproducts from the filleting operation were also characterized and used for fish oil production. In this way, data available on fillets, byproducts, and oil could be directly compared because they originated from the same batch. The same procedure was performed over the year. The study was carried out for all months except April.

Each fish oil production run required ∼1000 kg of mixed (heads, frames, skin, viscera, etc.) herring byproducts. Byproducts were minced and immediately pumped to an insulated scraped-surface heat exchanger indirectly heated by steam (95 °C). Finally, the mixture was separated

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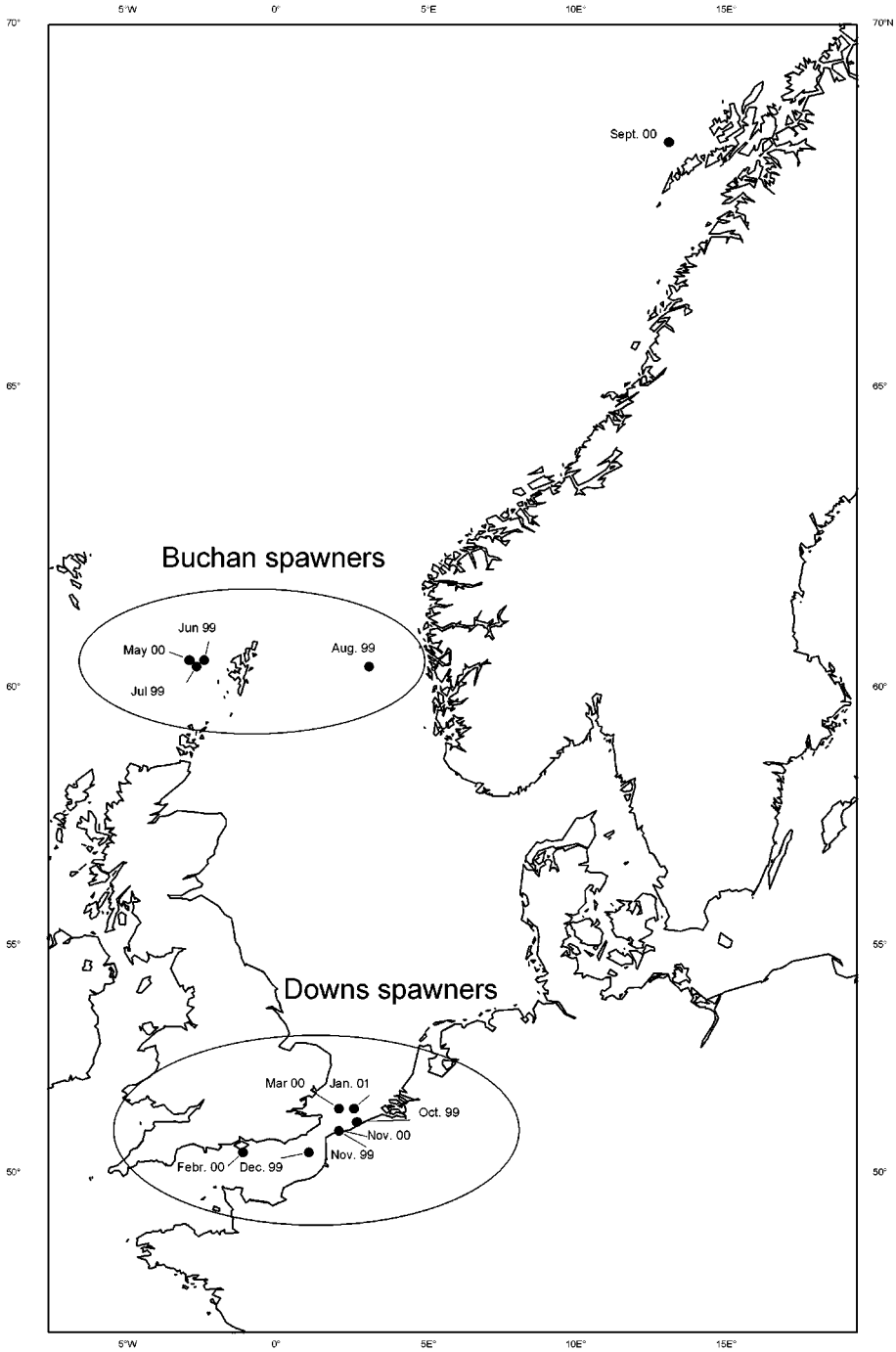

Figure 1. Periods and catching areas of the herring studied to evaluate seasonal changes over the year.

in a three-phase decanter into a phase containing solids (called the protein phase), a water phase (stickwater), and a lipid phase (oil). The same conditions and system as described earlier were used (*15*). On each occasion, samples of the intermediate mixture after the mincer and from the produced crude herring oil were collected. All samples were kept in the absence of light and oxygen and frozen at −80 °C until analyzed.

Fatty Acid Composition. Lipids from herring fillets and byproducts were extracted according to the method of Bligh and Dyer (*21*). Fatty acid methyl esters (FAMEs) of oil samples and of lipids extracts of fillets and byproducts were prepared according to AOCS (*22*) Official Method Ce 1b-89 and analyzed with regard to the content of individual fatty acids. On each occasion, three samples ($N = 3$) were analyzed once ($a = 1$). The different FAMEs were separated from each other by gas chromatography (GC) and identified using the conditions described previously (*15*). Results were expressed as a percent of the total fatty acids area.

Lipid Content. The total lipid content in the samples was determined gravimetrically after extraction according to the Bligh and Dyer (*21*) procedure ($N = 3$, $a = 1$). Results were expressed as grams of lipid per kilogram of samples.

Moisture. Moisture was determined as described earlier (*15*) ($N = 3$, $a = 1$). Results were expressed as percentage of wet weight.

Protein. Total nitrogen in the homogenized samples was determined using the Kjeldahl digestion method as described previously (*15*) ($N = 3$, $a = 1$). Results were expressed as percentage of wet weight.

Salt. Chloride content in all of the samples was titrated according to Volhard's method as described by Kolthoff and Sandell (*23*) ($N = 3$, $a = 1$). Results were expressed as percentage of wet weight.

In all cases, with the exception of the fatty acid composition analysis, internal reference materials were analyzed together with the samples.

Statistical Analysis. SPSS, version 10.0, software was used. The results were calculated by using General Linear Models (GLM), based on the least-squares method. Models of analysis of variance were used

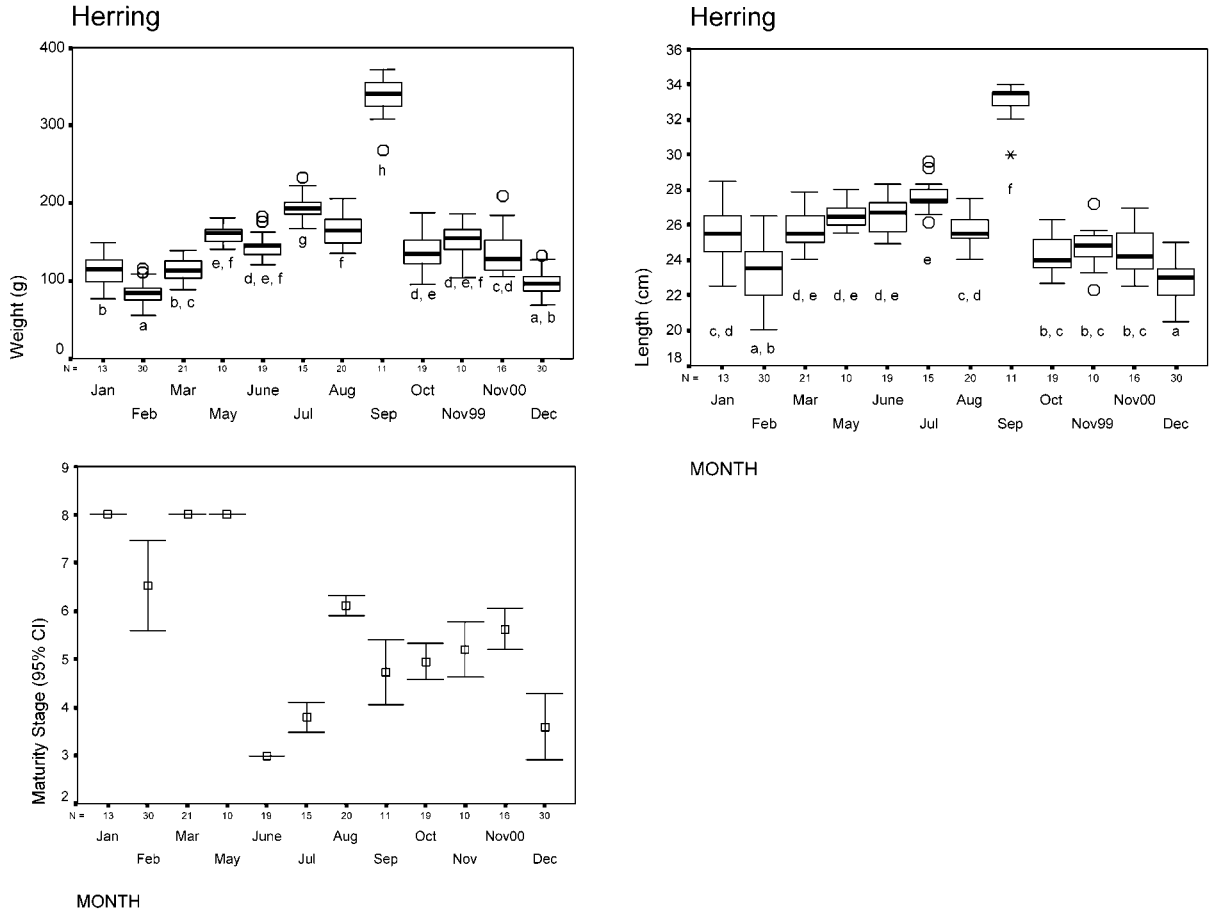

Figure 2. Box and whiskers plots of the weights and lengths of the caught herring: (□) interquartile range; (—) median; I, nonoutlier (maximum and minimum); (○) outlier; (∗) extreme; *N*, number of samples. Different letters under the box plots represent significant differences ($P < 0.05$). Error bars with 95% confidence interval represent the range of variation for the maturity stage.

to evaluate any effect of fatty acids and month of the year. When using one-way ANOVA, Pearson's bivariate two-tailed correlation was used for the total saturated, MUFAs, and PUFAs to check any existent relationship for the total groups of fatty acids of the fillets, byproducts, and oil. The relationship of fatty acids between fillets and byproducts and between byproducts and oil from the grouped data was also subjected to an ANOVA test. For comparisons of the fatty acids composition, the months were placed in three groups based on the maturity stage and PUFAs content of fillets over the year. The groups were designated stage I, which contains the months of December to March, stage II (May to August), and stage III (September to November). The residuals were tested for normality using normality probability plots. Observed power was used as a statistical tool to evaluate the strength of the drawn inferences related to the failure to reject the H_0 (*24*). In all cases *P* was set at 95%.

RESULTS AND DISCUSSION

In the first part of this section, the ecological (distribution/catching areas) and physiological (length, weight, and maturity stage) characteristics of herring used in this study are described. The crude composition from the processed fillets and the respective obtained byproducts after processing is also considered. In the second part, the changes of fatty acid composition of fillets, byproducts, and produced oils over the year is discussed. For this, first, the individual monthly composition is shown, followed by grouped studied periods for fillets versus byproducts and byproducts versus oil.

Ecological and Physiological Data. The total Northeast Atlantic herring stock is divided into two major groups: shelf and oceanic. This subdivision has been made from a series of observations on the morphological, physiological (spawning time, maturation cycle, pattern, and rate of growth, etc.), and ecological characteristics of herring spawning at different times and in different localities (*25*). In this study, the herring was caught at different locations throughout the year and their weight, length, and maturity stages were recorded. As can be observed in **Figure 1**, all of the herring belonged to the shelf group subdivision with the exception of the batch caught in September 2000, off the Norwegian Coast, which belonged to the oceanic group. These two groups present different biological characteristics concerning general distribution, growth, and spawning place and time. As noted in the literature (*25*, *26*), herring of the oceanic group are often larger and consequently heavier, as can be observed with our own collected data (**Figure 2**), where significantly higher values were found for the month of September. The shelf group herring can be classified as Buchan, Dogger, and Down spawners according to spawning time and location. At the bottom part of the map (**Figure 1**) the group belonging to the Downs spawning group is shown. In this group, spawning occurs late autumn−winter, mainly from the third week of November to early January (*27*). Therefore, spent herring were caught from January to March (**Figure 2**, maturity stage). Herring shoals of the Downs population move north anticlockwise and reach the northern and central North Sea during the summer feeding season (*28*). The herring caught from May to August belong to the Buchan group. This group is mainly composed of autumn-spawning herring, although spring spawners can also be found in high proportions on the Shetland Islands at various seasons (*28*).

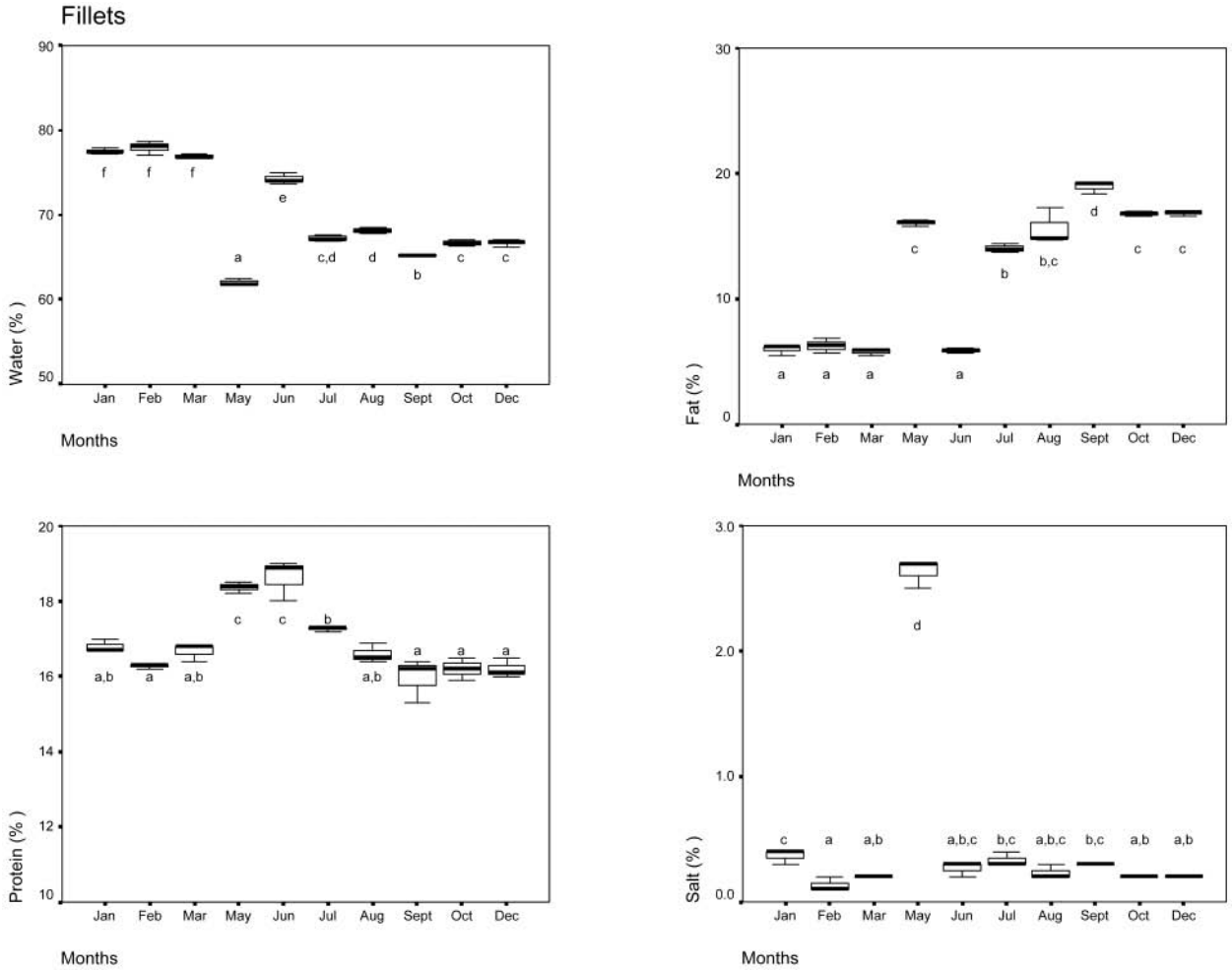

Figure 3. Box and whiskers plots of the crude composition over the year of the herring fillets originating the byproducts. Different letters under the box plots represent significant differences ($P < 0.05$), $N = 3$.

In general it can be seen that the weight increased from March until November and then decreased. The data also showed a quite good positive correlation between the length and the weight ($r = 0.911$). The month of November was sampled in the two consecutive years to see if yearly differences could be found. The results showed no significant difference between the samples. On the other hand, the month of April is missing in the study, because fish oil productions at the processing company never used fish from this month. With respect to the maturity stage it can be concluded that the investigated herring had a spawning period in autumn−winter with the exception of the fish caught in June, which were spring spawners. It is known that maturation is dependent on temperature and also on feeding conditions. Continuous recruitment of fish to the spawning population means that maturation within the stock can be asynchronous (*8*). In several sampling areas there was a mixture of fish in different gonad stages (**Figure 2**). Fish sampled in the months of January to March and June were unlikely to spawn for some considerable time.

Crude Composition. The crude composition of herring fillets and byproducts was determined over the year, and the results obtained are presented in **Figures 3** and **4**. The chemical composition of herring undergoes large fluctuations in response to a variety of factors. For example, the sexual maturity stage of the fish affects the lipid content due to increased consumption of fat reserves during the spawning period. Food availability and environmental water temperature are also important factors. Thus, fish will have various lipid contents, depending on the breeding cycle and time of year (*4*, *29*). Herring generally is known for its high fat content, although fat storage usually increases in summer when food is more available and may decrease to lower values in winter. Generally, fat and moisture contents are inversely proportional, whereas other body components (e.g., protein) remain fairly constant (*26*, *29*, *30*). Considering the fillets and byproducts, the water contents ranged from 62 to 77% and from 61 to 80%, respectively. As expected, the highest lipid content occurred in the month of lowest water content (May and September). Comparing fillets and byproducts for fat content, it is interesting to notice that the fat level in the byproducts is generally more constant than in the fillets. It is known that accumulation and storage of fat in herring prior the maturation take place in the muscle—both within the fibers themselves and between the fibers (*31*). Although the fillets collected in September were richest in lipid content (19%), it is in May that higher amounts of lipids were found in the byproducts (22%). Even though these results are reported for herring belonging to different groups with different morphological characteristics, a possible explanation for the differences found might be that herring recovers after a feeding period and has a tendency to accumulate fat in the dark muscle and skin rather than in the white muscle. Similarly, Ke and coauthors (*32*) found that white muscle of mackerel showed the largest seasonal variation in fat content. It is also known that dark muscle is the tissue used for continuous swimming and therefore is only used when lipids from the white muscle have been depleted. To support this theory, during the lean period the byproducts

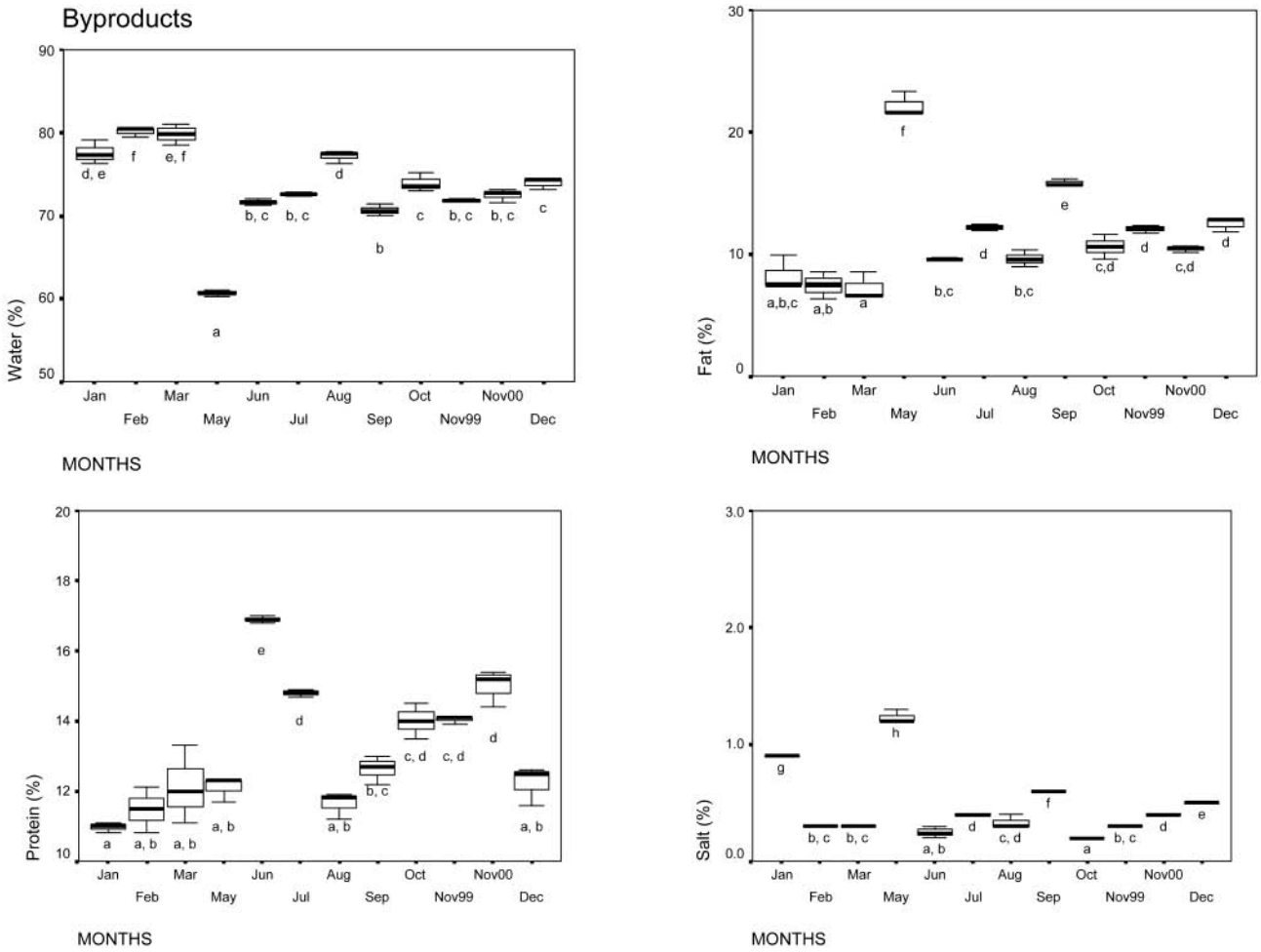

Figure 4. Box and whiskers plots of the crude composition over the year of the herring byproducts used for fish oil production. Different letters under the box plots represent significant differences ($P < 0.05$), $N = 3$.

(mainly composed of skin and dark muscle) presented also higher lipid values than the fillets (7.2 and 5.8%, respectively), suggesting that the reserves are used first from the white muscle that composes the fillets. During the winter period no food is available, and therefore the herring uses all of its fat resources, explaining the low values of fat content. The minimum value was reached in March, which coincides with the period of postspawning and starvation due to lack of food resources. A low fat value was obtained for the month of June. A very likely explanation is that the herring caught in that month belonged to a spring-spawning group (confirmed with the maturity stage III), being therefore recovering spent herring. There is a drop in fat content from January to March because of starvation and for the month of June after gonad maturation.

From a comparison of the two figures, it is not surprising that the fillets contained a higher amount of protein than the byproducts. In general, the protein values ranged between 16.0 and 18.9% in the fillets (September and June, respectively), whereas for the byproducts a variation between 11.0 and 16.9% was found (February and June, respectively). The salt content presented in May an extremely high value for the fillets and byproducts (2.6 and 1.2%, respectively). This can be easily explained due to the production of a popular and characteristic Dutch product, maatjes herring, where brine is added to the fillets (*15*), thereby increasing the salt content significantly. It can be stated that in general the byproducts contained a higher content of moisture than the fillets, which is expected because less fat and protein are present.

Fatty Acid Composition. Details of individual fatty acids composition of fillets, byproducts, and processed oil studied over the year are presented in **Table 1**, and the measured total saturated, monounsaturated fatty acids (MUFAs), and PUFAs are shown in **Figure 5**. As can be observed, fatty acids composition showed seasonal changes during the year. However, regardless of the season and maturity stage, MUFAs constitute the majority of fatty acids in the three products studied, followed by the saturated and PUFAs. The saturated fraction ranged from 22.5 to 35.8%. Within this group the major fatty acid was palmitic acid (16:0). The total monoenes content ranged from 34.9 to 58.8%, with 22:1 being the prominent monounsaturated fatty acid (comprising between 14 and 28% of the total). The high level of this fatty acid is characteristic for herring and in accordance with values reported in the literature (*15*, *33*−*35*), being originated in the fatty alcohols common in copepods (*10*, *33*, *34*). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were the major PUFAs in the fillets, byproducts, and herring oils, and changes in percentage of these fatty acids occurred over the year. Except in May, DHA was found in higher values than EPA. It has been reported (*5*) that in sardine, accentuated reduction in DHA content with a proportional increase for EPA occurred in the month of April, which was attributed to differences in diet. Because DHA is an important component of membrane structural lipids, its relative percentage can decrease during April, postspawning time, whereas EPA is a main fatty acid of plankton origin (*5*) and its level will therefore be more constant.

Table 1. Fatty Acid Profiles[a] of Fillets, Byproducts, and Respective Oils Produced from Herring (*Clupea harengus*) Byproducts over the Year

Fillets

fatty acid	Jan	Feb	March	May	June	July	Aug	Sept	Oct	Nov	Dec
14:0	10.4ab	11.4ab	9.6a	15.6c	9.2a	8.9a	10.6ab	11.1ab	9.3a	13.6bc	11.1ab
16:0	16.2ab	16.6ab	17.0b	15.8ab	20.3c	14.5ab	17.2b	16.5ab	13.7a	17.0b	16.2ab
18:0	2.0b	1.7ab	1.9b	2.3c	2.9d	2.0b	1.7ab	1.6a	1.5a	1.6a	1.6a
Σ[b]	28.6ab	29.8ab	28.5ab	33.7b	32.4b	25.4a	29.5ab	29.1ab	24.5a	32.2b	28.9ab
16:1	5.1abc	4.7a,b	4.8abc	5.6bc	6.7d	5.3abc	5.7c	5.1abc	4.6a	5.6c	5.2abc
18:1	11.8fg	11.6fg	12.8g	5.8a	7.9bc	6.6ab	8.5cd	13.0g	8.1bcd	9.7de	10.4ef
20:1	15.8b	10.9ab	14.9b	13.4ab	7.8a	9.9ab	11.4ab	12.4ab	12.0ab	13.3ab	13.6ab
22:1	22.8d	23.4d	20.1bcd	18.6abcd	13.8a	17.8abc	17.7abc	16.2ab	21.4cd	18.8bcd	22.5cd
Σ[b]	55.5e	50.7defg	52.5fg	43.3bc	36.1a	39.6ab	43.2bc	46.7cde	46.1cd	47.4cdef	51.7efg
18:2	1.7abc	2.1c	1.8bc	1.1a	1.7abc	1.5abc	1.6abc	1.4ab	1.9bc	2.0c	1.9bc
18:3	0.7a	0.9ab	0.7a	0.9ab	1.2bcd	1.3bcd	1.3bcd	1.2abcd	1.5d	1.5cd	1.2abcd
18:4	1.0a	1.5a	1.0a	4.4d	2.4b	2.8bc	3.9d	4.3d	2.8bc	3.1c	2.3b
20:5	3.7a	4.0a	4.3ab	6.4c	8.0d	8.0d	7.6d	6.4c	5.3b	4.5ab	4.6ab
22:6	6.1a	7.2ab	7.4ab	6.1a	9.7d	9.6cd	9.0bcd	8.5bcd	7.5abc	5.8a	6.0a
Σ[b]	13.2a	15.7abc	15.2ab	19.0de	23.0f	23.3f	23.4f	21.7ef	18.9cde	16.9bcd	16.1abcd

Byproducts

fatty acid	Jan	Feb	March	May	June	July	Aug	Sept	Oct	Nov	Nov 00	Dec
14:0	11.4abc	13.2c	11.0ab	17.1d	9.2ab	8.6a	11.2abc	11.3abc	8.4a	12.2bc	12.5bc	12.0bc
16:0	17.0ab	17.9ab	16.7ab	16.3ab	16.4ab	14.8a	19.4b	16.9ab	14.5a	19.2b	17.2ab	17.2ab
18:0	2.1cde	2.0bc	2.0bcd	2.4f	2.3ef	2.0bcd	2.2def	1.7a	1.8ab	2.0bc	2.2cde	1.8ab
Σ[b]	30.6abcd	33.1cd	29.7abcd	35.8d	27.9abc	25.4ab	32.8cd	29.8abcd	24.7a	33.3cd	31.8bcd	31.0abcd
16:1	5.1ab	6.0b	5.2ab	6.0ab	5.7ab	4.9ab	5.8ab	5.1ab	4.3a	5.9ab	5.3ab	5.6ab
18:1	12.0ef	11.2def	10.8cdef	6.0a	6.3ab	7.3ab	9.3abcde	13.8f	7.6abc	9.6bcde	8.1abcd	9.2abcde
20:1	13.3de	15.0fg	15.8g	13.6ef	8.3a	9.9ab	11.2bc	13.1de	11.7cd	12.9de	13.4ef	13.0de
22:1	19.4abc	19.9bc	23.5c	17.6ab	14.6a	17.5ab	16.4ab	17.4ab	21.0bc	18.7abc	19.1abc	19.7abc
Σ[b]	49.9de	52.2ef	55.4f	43.1bc	34.9a	39.6ab	42.7bc	49.4de	44.5c	47.2cd	45.9cd	47.5cde
18:2	1.6abcd	1.7abcd	1.4abcd	1.1a	1.5abcd	1.1ab	1.6abcd	1.3abc	1.8cd	1.7bcd	1.4abcd	2.0d
18:3	1.0	1.0	0.9	0.9	1.3	1.2	1.1	1.2	0.9	1.2	1.1	1.4
18:4	2.6b	1.6a	1.7a	3.8f	3.5ef	2.7bc	3.6ef	3.3de	2.7bc	2.4b	3.0cd	2.8bc
20:5	5.9de	3.3a	3.4a	6.0e	8.7f	8.2f	6.5e	5.1bcd	5.7cde	4.6b	6.1e	4.9bc
22:6	6.5bc	3.6a	4.0a	4.8ab	11.9f	9.3e	7.4cd	6.6cd	8.3de	6.0bc	7.2cd	6.6cd
Σ[b]	17.6bcd	11.1a	11.3a	16.7bc	26.8f	22.6e	20.2de	17.7bcd	19.4d	16.0b	18.8cd	17.7bcd

Oil

fatty acid	Jan	Feb	March	May	June	July	Aug	Sept	Oct	Nov	Nov 00	Dec
14:0	8.5a	8.6ab	9.3abcd	10.6bcd	9.2abcd	9.0abc	11.2d	10.8cd	9.1abc	8.9abc	14.7e	8.7ab
16:0	13.1abcd	12.2ab	12.6abc	11.5a	20.3f	14.2cd	16.8e	14.5d	13.3bcd	13.1abcd	17.2e	12.5abc
18:0	1.8abcd	1.7ab	1.6ab	2.1cde	2.9f	2.2e	1.9bcde	1.5a	1.7abc	1.6ab	2.1de	1.6ab
Σ[b]	23.4ab	22.5a	23.5ab	24.2ab	32.4de	25.4ab	29.9cd	26.8bc	24.1ab	23.7ab	34.0e	22.7a
16:1	5.2bcd	4.4ab	4.6abc	4.3ab	6.7d	5.5bcd	5.7bcd	4.9abcd	4.6abc	4.6abc	6.3cd	3.0a
18:1	10.0d	8.5c	8.6c	5.2a	7.9b	7.5b	8.7c	13.4e	7.4b	7.8b	8.5c	7.7b
20:1	15.7f	13.1de	13.3e	13.1de	7.8a	10.3b	11.7c	13.6e	12.0c	12.1c	12.4cd	11.9c
22:1	27.9g	25.0f	24.8f	20.9de	13.8a	17.9bc	17.8bc	19.0cd	21.5e	21.4de	16.5b	22.1e
Σ[b]	58.8e	51.0d	51.2d	43.5bc	36.1a	41.2b	43.9bc	50.8d	45.6c	45.9c	43.8bc	44.7bc
18:2	1.9	1.8	1.8	1.7	1.7	1.2	1.6	1.3	1.3	2.0	1.5	2.3
18:3	0.0a	1.1b	1.0b	1.1b	1.2b	1.2b	1.3b	1.2b	1.5b	1.5b	1.2b	1.4b
18:4	2.1ab	1.6a	2.1ab	4.1ef	2.4abc	3.2cde	4.5f	4.1ef	3.2cde	3.3cde	3.9def	2.9bcd
20:5	5.5bc	4.5a	4.3a	7.3e	8.0f	9.0g	7.7ef	5.9cd	5.8bc	5.9cd	6.4d	5.3b
22:6	6.1bc	4.9a	4.7a	6.3bc	10.7e	8.5d	7.7d	6.8c	6.5bc	6.6bc	5.8b	6.7c
Σ[b]	15.6a	13.8a	14.0a	20.5c	24.0d	23.1d	22.8d	19.2bc	18.3b	19.2bc	18.8bc	18.6bc

[a] Expressed as w/w % of total fatty acids. Mean values of three independent measurements are shown. Values in rows followed by different letters are significantly different ($P < 0.05$). [b] Values do not sum to 100% because minor fatty acids are not reported.

It has been suggested that the proportions of the different fatty acids varied with the lipid content (*4*); lean herring was reported to present significantly higher values of saturated fatty acids. This inverse relationship between lipid content and degree of unsaturation, as reported by other authors (*1*, *4*), was not observed. This implies, as previously suggested (*9*), the influence of food intake; that is, the composition of lipids in herring follows that in plankton.

The highest values for EPA, DHA, and total amount of PUFAs were found in general from May to August. The decrease in the percentage of PUFAs in herring fillets from September on may be an adaptation to spare these fatty acids for ovary construction, mainly using EPA and DHA (*3*). In gonadal herring lipids (*12*), it was observed that PUFAs were the major components, and it was assumed that a higher proportion of PUFAs than MUFAs was mobilized. In our case, increases of MUFAs, particularly 18:1 and 22:1, during maturation were observed.

To find whether any relationship existed between the total content of different unsaturation of fatty acids (**Figure 5**), Pearson's correlation was tested for the different individual fractions studied. Over the year, the amount of MUFAs in the fillets was consistently negatively correlated with PUFAs content ($r = -0.859$; $P < 0.01$; $N = 33$). In that respect the highest levels of PUFAs were found during the months of June, July, and August (~23%) and the lowest in the month of January

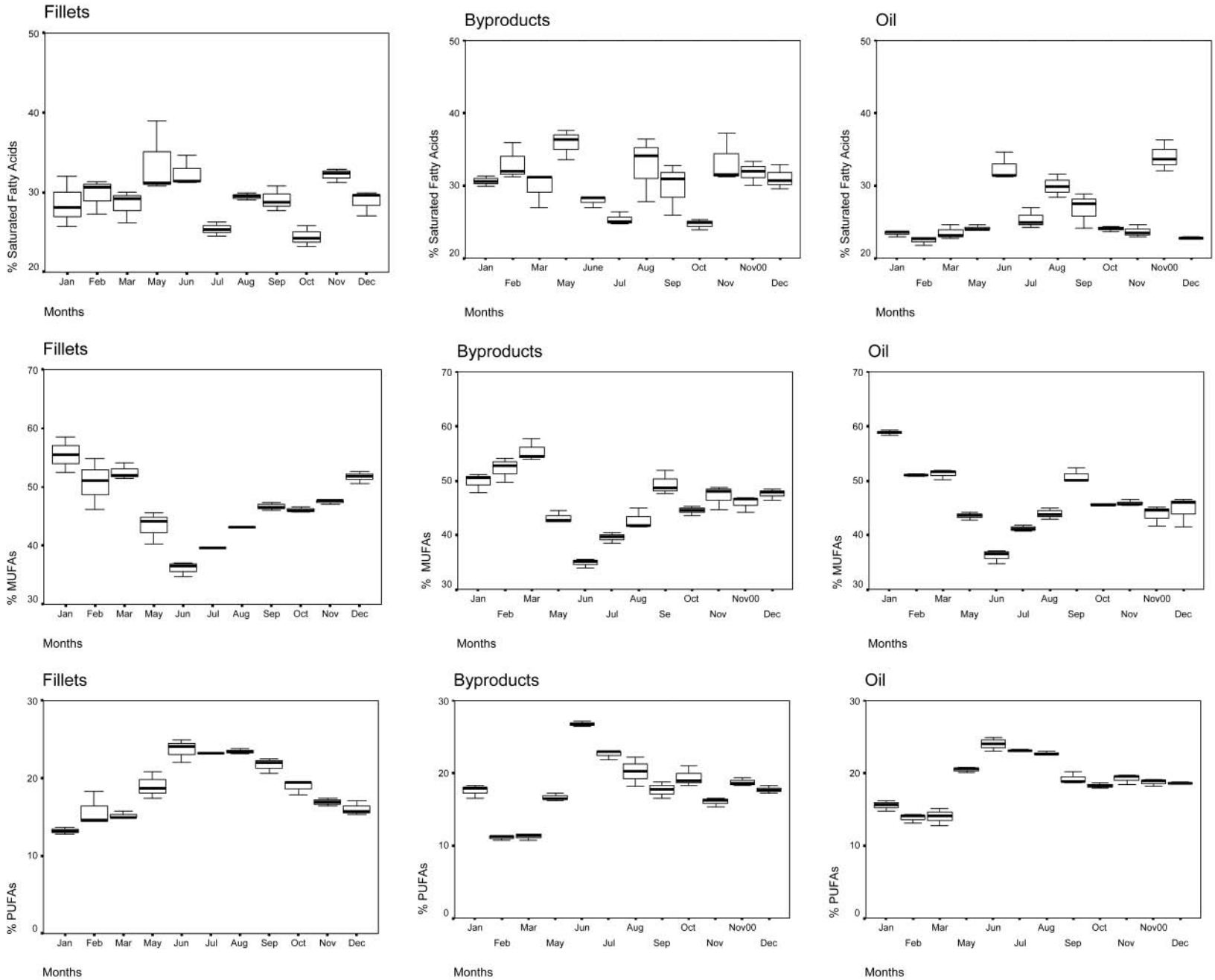

Figure 5. Box and whiskers plots representing the variation of the saturated, MUFAs, and PUFAs of the herring fillets, byproducts, and produced oil over the year ($N = 3$).

Table 2. ANOVA Table from the GLM of the Different Groups of Fatty Acids Measured on Fillet and Byproduct Data Comparing the Effect of Time of the Year (Stages), Type of Products (Fillets or Byproducts), and Interaction Effect[a]

measure	df	MS	*F*	*P*	measure	df	MS	*F*	*P*
saturated					**MUFAs**				
model	5	10.628	0.813	0.545	model	5	327.14	35.175	0.000
intercept	1	57433.2	4394	0.000	intercept	1	139334	14981	0.000
error	60	13.072			error	60	9.30		
effects					effects				
time	2	11.079	0.848	0.434	time	2	811.4	87.246	0.000
type	1	17.156	1.312	0.257	type	1	4.315	0.464	0.498
time × type	2	6.208	0.475	0.624	time × type	2	3.564	0.383	0.683
PUFAs					**EPA**				
model	5	128.011	17.197	0.000	model	5	25.324	32.8	0.000
intercept	1	21903	2942.4	0.000	intercept	1	2072.9	2684.6	0.000
error	60	7.444			error	60	0.772		
effects					effects				
time	2	312.10	41.927	0.000	time	2	62.998	81.58	0.000
type	1	15.030	2.019	0.161	type	1	0.052	0.067	0.797
time × type	2	0.988	0.133	0.876	time × type	2	0.298	0.387	0.681
DHA									
model	5	19.378	6.519	0.000					
intercept	1	3372.03	1134.4	0.000					
error	60	2.973							
effects									
time	2	40.951	13.78	0.000					
type	1	8.617	2.899	0.094					
time × type	12	2.602	0.875	0.105					

[a] MS, mean squares; df, degrees of freedom; *F*, *F* test; P, probability level. $Y_{measurement} = \beta_0 + \beta_{1,t} + \beta_{2,\tau} + \beta_{3,t\times\tau}$, where t (class variable) is the effect of the time of the year and τ (class variable) is the type of product studied. $t\times\tau$ represents the interaction term of the two class variables on the measurements studied. The estimation of the constant β_0 is given with the value of the intercept.

Table 3. ANOVA Table from the GLM of the Different Groups of Fatty Acids Measured on Byproducts and Oil Data Comparing the Effect of Time of the Year (Stages), Type of Products (Byproducts or Oil), and Interaction Effect[a]

measure	df	MS	*F*	*P*	measure	df	MS	*F*	*P*
saturated					**MUFAs**				
model	5	106.619	8.094	0.000	model	5	278.01	21.102	0.000
intercept	1	57563.2	4370.1	0.000	intercept	1	153692	11666	0.000
error	66	13.172			error	66	13.174		
effects					effects				
time	2	29.096	2.209	0.118	time	2	691.05	52.454	0.000
type	1	355.31	26.974	0.000	type	1	2.406	0.183	0.670
time × type	2	59.796	4.54	0.014	time × type	2	2.766	0.210	0.811
PUFAs					**EPA**				
model	5	125.115	20.395	0.000	model	5	24.209	36.20	0.000
intercept	1	24620.3	4013.3	0.000	intercept	1	2585.8	3866.6	0.000
error	66	6.135			error	66	0.669		
effects					effects				
time	2	303.701	49.505	0.000	time	2	57.482	85.953	0.000
type	1	18.078	2.947	0.091	type	1	6.052	9.050	0.004
time × type	2	0.04874	0.008	0.992	time × type	2	0.0150	0.022	0.978
DHA									
model	5	21.251	8.585	0.000					
intercept	1	3348.405	1352.8	0.000					
error	66	2.475							
effects									
time	2	51.380	20.758	0.000					
type	1	0.154	0.062	0.804					
time × type	12	1.671	0.675	0.513					

[a] MS, mean squares; df, degrees of freedom; *F*, *F* test; *P*, probability level. $Y_{measurement} = \beta_0 + \beta_{1,t} + \beta_{2,\tau} + \beta_{3,t\times\tau}$.

(~13%). It has been proposed (*3*) that the degree of unsaturation of pike neutral lipids reserves may influence the physical state (viscosity) of the same reserves and the speed with which they can be hydrolyzed to supply energy. This would explain the increase in the percentage of MUFAs during autumn and winter as an adaptation to compensate for reductions in PUFAs and maintain adequate viscosity at low water temperatures. Reciprocal changes in short- versus long-chain fatty acids also occurred. Declining food intake during winter may reduce the activity of elongases and desaturases enzymes (*36*, *37*) to such an extent that 18-carbon PUFAs are maintained while longer chain PUFAs decrease substantially. On the other hand, in the byproducts, the total amount of PUFAs was negatively correlated not only with the total MUFAs but also with the total saturated content ($r = -0.862$ and -0.465, respectively, with $P < 0.01$; $N = 36$, in both cases). For the produced oils, the PUFAs showed a significant positive correlation with the total saturated ($r = 0.518$; $P < 0.01$; $N = 36$) and a negative correlation to the content of MUFAs ($r = -0.785$; $P < 0.01$; $N = 36$). The total content of MUFAs, in the oil, correlated negatively over the

year with the amount of saturated and PUFAs content ($r = -0.534$ and -0.785, respectively, with $P < 0.01$and $N = 36$, in both cases). These results support previous statements (*38*) that fat is not metabolized continuously but in a stepwise manner; that is, fish build up energy reserves prior to spawning, during which the reserves are spent rapidly.

To organize the information and clearly perceive variations, the data were gathered into three stages. The stages were chosen on the basis of similarity of spawning period/maturity stage and total MUFAs and PUFAs content. Stage I corresponds to the beginning of the spawning and subsequent lean herring period, with a decrease and an increase of PUFAs and MUFAs, respectively, during the months from December to March; stage II from May to August is associated with a postfeeding period with an increase of fat and PUFAs content. Stage III, covering the remaining months, from September to November, is linked to the period that the herring is preparing to approach the spawning condition with the respective change in fatty acids content. To evaluate what influence of the time of year on the type of herring product (whether fillets or byproducts and byproducts or oil), concerning the fatty acids composition, all of the data were subjected to a least-squares estimates (LSE) test, which, for each fatty acids group, gave rise to the following parameters in the model:

$$Y_{\text{fatty acids}} = \beta_0 + \beta_{1,t} + \beta_{2,\tau} + \beta_{3,t\times\tau}$$

In this equation, t (class variable) is the effect of the period of time (stage) that the fish has been caught over the year, τ (class variable) is the type of product studied (fillets, byproducts, or oil), and $t\times\tau$ represents the interaction term of the two class variables on the fatty acids studied. The estimation of the constant β_0 is given with the value determined for the intercept.

Fillets versus Byproducts. Table 2 shows that, with the exception of the total saturated fatty acids measurements, all models were highly significant ($P \ll 0.05$). Within the significant models, in all cases only the influence of the period of time that the herring was caught was shown to have a significant effect ($P = 0.000$ and power $= 1.000$ except for DHA, for which a power $= 0.998$ was found). **Figure 6** represents schematically the statistical outcome for the variation in the total saturated, MUFAs, PUFA (PUFAs excluding EPA and DHA), EPA, and DHA fatty acids composition comparing herring fillets and byproducts over the year. The total amount of saturated fatty acids remained constant over the year (~31%), whereas the proportion of MUFAs and PUFAs changed seasonally. For the other groups of studied fatty acids, a consistently significant effect of the stages was observed. Herring caught in the first stage contained a significantly higher amount of MUFAs than the ones caught during the third stage, and the lowest significant level was found for the second stage. The total unsaturation level of the fillets and byproducts increased from its lowest level during stage I to the highest level in stage II. These changes are mainly due to the changes in EPA and DHA content. A similar tendency was observed in the EPA content, whereas the amount of DHA showed that the highest significant value was found in stage II, but no significant difference was detected between stages III and I.

Byproducts versus Oil. It was notable that in byproducts and oils during the months of February and March, the lowest values of EPA and DHA were measured (**Table 1**). Interestingly, those months corresponded to the postspawning period with spent or recovering spent herring. It is known that lipids are mobilized when fish starve or mature (*38*), because sexual maturation involves the mobilization of relatively large quantities

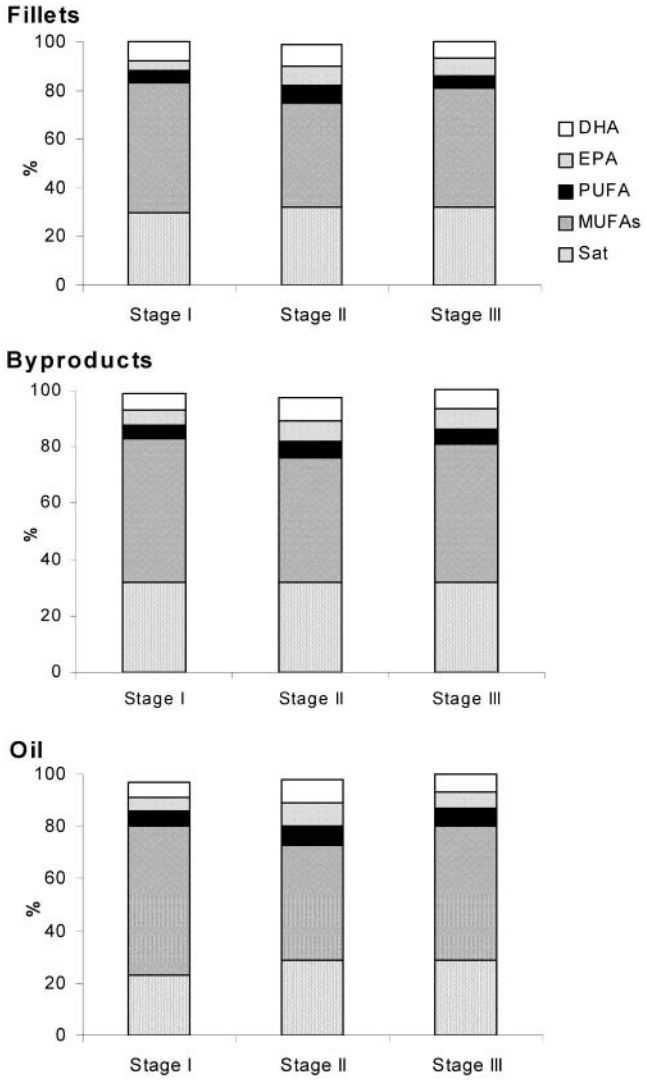

Figure 6. Seasonal variation on some groups of fatty acids influenced by the stages when comparing fillets versus byproducts and byproducts versus oil over the year.

of nutritive material for the developing offspring. A specific selection of fatty acids is mobilized into the developing roe (*12*). Love (*38*), summarizing the findings of several authors, suggests that DHA tends not to be mobilized from muscle, except when the lipid content of the muscle is very low, implying that PUFAs are broken down in the fish muscle only at a late stage of starvation. It is likely that it does not occur until the triglycerides have first been moved and the muscle cells have started to disintegrate (*38*).

A similar statistical procedure as described earlier was performed to study differences between byproducts and the respective produced oils. The fitted models (**Table 3**) were found to be all highly significant for the studied group of fatty acids. Concerning the group of MUFAs, PUFAs, and DHA, only the stage had a significant influence ($P \ll 0.05$, power $= 1.000$). Related to the total fraction of saturated fatty acids, the effect of the type of product (byproducts or oil) and the interaction effect between time and type were found to be significant ($P \ll 0.05$; power $= 0.999$ and 0.754, respectively). For the EPA content, not only time had a significant effect but also the type of product ($P \ll 0.05$, power $= 1.000$ and 0.842, respectively). **Figure 6** shows the different groups of fatty acids with the significant differences within the byproduct and oil. Significantly lower values of total saturated fatty acids were found for the

oil compared to the byproducts. To the other groups of fatty acids the influence of stages is important. As expected, the content of MUFAs reaches the maximum during stage I and the minimum during stage II. In contrast, the maximum contents of EPA, DHA, and total PUFAs are reached in stage II and the minimum levels during stage I. In all cases, significant differences among the three different stages of the year were found. Apparently, interconversion of those fatty acids occurred. In addition to the period of the year, for EPA also an interaction effect of time and type, either in byproducts or oil, was present.

In conclusion, it can be stated that the fillets contained a higher protein content than the byproducts, and a larger variation of the fat content over the year was observed. In the byproducts the fat content is less affected by seasonal variation, although certain variation occurred over the year, with a maximum reached in May and a minimum value obtained from January to March. This paper also confirms that fatty acid composition in the fillets fluctuates in an annual cycle depending on the maturity stage of the herring and food availability. However, the fatty acid composition in the byproducts is relatively stable. Nevertheless, the highest amount of total PUFAs in the oil was reached during the months of June−August in contrast to January−March, when the lowest levels were found, although never <14%. The upgrading process also offers a good opportunity to contribute to an environmental solution by reducing the amount of waste. Moreover, this type of valorization is highly interesting because it confers an added value to the herring byproducts originating from the fish industry during the entire year.

ABBREVIATIONS USED

EPA, 5,8,11,14,17-eicosapentaenoic acid; DHA, 4,7,10,13,-16,19-docosahexaenoic acid; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; *N*, number of samples analyzed; *a*, number of analyses per sample *N*; GLM, General Linear Models; MS, mean squares; df, degrees of freedom; *F*, *F* test; *P*, probability level; PUFA, PUFAs excluding EPA and DHA; Sat., saturated fatty acids.

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