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Composition and Stability of Herring Oil Recovered from Sorted Byproducts as Compared to Oil from Mixed Byproducts

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Herring oils produced from three different types of byproducts, only heads, mixed, and headless byproducts, were compared. Heads byproducts and its oil presented the highest oxidation levels and the lowest α-tocopherol content. Heads contained the lowest polyunsaturated fatty acids content and the highest amount of saturated fatty acids. No significant differences were found between the fatty acid composition of the mixed and the headless either in byproducts or in its oil. The oil was stored at two different temperatures (20 and 50 °C). Testing general linear models showed that oxidation was related to the peroxide value with a positive significant effect of the temperature, while the free fatty acids' model was more complex, with significant contribution of all of the effects studied. Fluorescence measurement was the one that correlated best with the oxidation progress.

KEYWORDS: Crude oil; PUFAs; heads; sorting; herring byproducts; lipid oxidation; storage; processing

INTRODUCTION

From several investigations, it has been suggested that the ω-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (C20:5, EPA) and docosahexaenoic acid (C22:6, DHA) are beneficial to the human body (*1*, *2*). It is known that fish, in particular fatty fish species, is one of the best sources of these types of fatty acids (*3*, *4*). The ω-3 PUFAs are highly susceptible to oxidation due to their high degree of unsaturation (*5*, *6*). This is one of the main difficulties during storage and shelf life of products containing considerable amounts of these fatty acids (*7*−*9*).

There are great differences in lipid storage between different fish species depending upon the organism constitution. Fat storage in fatty fish species, like herring, is dispersed, and the type of fat differs in various parts of the fish and organs (*10*, *11*). Lohne (*12*) found that the head and belly cavity of mackerel and capelin contain extensive lipid deposits. Tuna heads are known to be rich in DHA content (*13*). Nair and Gopakumar (*14*) reported that skin lipids from the oil sardine (*Sardinella longiceps*) had a higher proportion of monoenoic acids and lower proportions of PUFAs than muscle lipids. Similar results were found in the skin and muscle lipids of Atlantic mackerel (*Scomber scombrus*) by Ke et al. (*15*). German and Kinsella (*16*) reported that skin enzymes may constitute a significant source of initiating radicals leading to oxidation of fish lipids.

After successful and promising initial results in the valorization of herring byproducts reported earlier (*17*, *18*), the separation of the byproducts into its different constituents became of interest. In this way, it may be possible to influence the fatty acid composition and the lipid oxidation/stability of the resulting oil. The type of process used at the factory, after the filleting operation, gives the possibility of producing not only the normal mixed byproducts but also only the heads from the byproducts, and in this way, a headless byproducts variety is also created.

This paper compares the composition and storage stability from the oil obtained from different sorted byproducts (only heads and the headless byproducts) to the oil obtained using mixed byproducts: heads, skin, frames and fins, and visceras. In the first part of the study, a characterization of the different raw materials and the crude oils is made. In the second part, the stability of the oils was investigated. To follow the lipid oxidation process, several methods were used. Attention was given to the development of primary, secondary, and tertiary oxidation products. The peroxide value (PV), absorbance at 235 nm (measuring conjugated dienes, CD) and 270 nm (measuring conjugated trienes, CT), anisidine value (AV), and fluorescence compounds (FC) were measured. The loss of α-tocopherol and the change in the free fatty acids (FFA) content were also followed in time. General linear models (GLM) were used as a tool to evaluate whether any changes occurred on a statistically significant level during storage and to compare the different autoxidation process at the different temperatures and type of oil produced.

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Table 1. Composition of the Three Types of Herring Byproducts Used for Fish Oil Production[a]

	mixed byproducts	headless byproducts	head byproducts
crude composition			
protein (% w/w)	14.8 ± 0.1 a	14.3 ± 0.1 a	14.3 ± 0.6 a
moisture (% w/w)	72.6 ± 0.3 a	71.3 ± 0.4 b	66.9 ± 0.4 c
salt (% w/w)	0.4 ± 0.01 a	0.3 ± 0.03 a	0.7 ± 0.1 b
total lipids (g/kg wet sample)	122 ± 3 a	132 ± 6 a	129 ± 4 a
FFA (%)	0.7 ± 0.02 a	0.7 ± 0.01 a	0.8 ± 0.1 a
α-tocopherol (mg/100 g lipid)	0.5 ± 0.1 a	0.4 ± 0.1 a	0.0 ± 0.1 b
oxidation status			
PV (mequiv per/kg oil)	30.1 ± 2.8 a	47.8 ± 4.0 b	112.6 ± 6.3 c
AV	22.2 ± 0.2 a	33.9 ± 1.0 b	37.0 ± 3.7 b
FC (area units/pg oil)	198 ± 128 a	164 ± 32 a	230 ± 36 a
A235 (area units/μg oil)	51.7 ± 4.5 a	67.1 ± 13.3 ab	80.3 ± 10.7 b
A270 (area units/μg oil)	6.4 ± 1.7 a	7.6 ± 1.9 a	19.5 ± 1.7 b

[a] Mean values of three independent measurements and standard deviation are given. Values in the same row followed by different letters are significantly different ($P < 0.05$).

MATERIALS AND METHODS

The raw material used in this study was fresh byproducts obtained from frozen herring (*Clupea harengus)* caught in the North Sea in July 1999 off of 60.40 N 02.75 W. The fish were processed in October 1999 and had an average weight of 199 ± 15 g, length 28 ± 1 cm, and were found to be in maturity stage IV ($n = 15$).

Equipment. Each production run required ∼1000 kg of mixed (heads, frames, skin, viscera, etc.), heads, and headless byproducts. First, the byproducts were minced, and immediately after this stage, they were pumped to an insulated scraped-surface heat exchanger indirectly heated by steam (95 °C). Finally, it was separated in a three phase decanter into a solid phase (called protein phase), a water phase (stickwater), and a lipid phase (oil). The same conditions and system as described earlier were used (*17*).

Sampling Setup. A portion of the recovered herring oils was used for the storage stability experiment. In all cases, the oil was blanketed with an inert atmosphere (nitrogen), kept in closed, light impermeable containers at room temperature (≈20 °C) and in an oven (50 °C).

Two oil samples were taken, at regular intervals, from the different oils and analyzed for level of oxidation products, FFA formation, and α-tocopherol content. To avoid further oxidation, all of the samples were kept at −80 °C until analyzed. Prior to the analysis, the oil samples were thawed at room temperature for 30 min. The average of the two sample measurements was used to calculate the final results.

Fatty Acid Composition. Lipids from herring fillets and byproducts were extracted according to the method of Bligh and Dyer (*19*). Fatty acid methyl esters (FAMEs) of oil samples were prepared according to the AOCS (*20*) official method Ce 1b-89 and analyzed with regard to the amount of content of individual fatty acids. In each occasion, three oil samples ($n = 3$) were analyzed once ($a = 1$). The different FAMEs were separated from each other with gas chromatography (GC) and identified using the conditions described previously (*17*). Results are expressed as grams per kilogram of lipid.

α-Tocopherol. α-Tocopherol was analyzed according to the slightly modified method of Lie et al. (*21*) as described earlier (*17*) using reversed phase HPLC and fluorescence detection. On each occasion, $n = 2$, and $a = 1$. The repeatability of the method was 5.1% ($n = 1$, $a = 6$). Results are expressed as milligrams per 100 g of lipids.

Extraction of Lipids for Determination of PV, AV, FFA, and FC. Total lipids from the different types of herring byproducts were extracted according to the method of Burton et al. (*22*) as modified by Undeland et al. (*23*).

FFA. The amount of FFA of the oil samples was determined by titration according to AOCS (*20*) official method Ca 5a-40 ($n = 2$, $a = 1$). The percentage of FFA was calculated as oleic acid.

PV. The PV of the samples was determined according to the official AOCS (*20*) method Cd-8b-90 ($n = 2$, $a = 1$). The content is expressed in terms of mequivalents of peroxides per kilogram of lipid.

AV. The AV of the oil was carried out according to AOCS (*20*) official method Cd 18-90 ($n = 2$, $a = 1$).

Absorbance at 235 (A_{235}) and 270 nm (A_{270}). A_{235} and A_{270} nm of the oil samples were measured using flow injection analysis (FIA) as described by Undeland et al. (*24*) ($n = 2$, $a = 1$). Results were expressed as peak area units per nanogram of lipid. The relative standard devation percents were 2.0 and 4.3 ($n = 1$, $a = 6$) for A_{235} and A_{270}, respectively.

Fluorescence Compounds (FC). Total lipid soluble fluorescent lipid oxidation products with an excitation (ex) maximum at 367 nm and an emission (em) maximum at 420 nm were measured in the oil samples using FIA as described by Undeland et al. (*24*) ($n = 2$, $a = 1$). Results were expressed as peak area units per picogram of lipid. The repeatability was 7.0% ($n = 1$, $a = 6$).

Lipid Content. The total lipid content in the samples was determined gravimetrically after extraction according to the Bligh and Dyer (*19*) procedure ($n = 2$, $a = 2$). Results were expressed as grams of lipid per kilogram of samples.

Moisture. Moisture was determined as described earlier (*18*) ($n = 2$, $a = 2$). 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 (*18*). 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 (*25*) ($n = 2$, $a = 2$). Results were expressed as percentage of wet weight. In all cases, with the exception of fatty acid composition and lipid extractions, internal reference materials were analyzed together with the samples.

Statistical Analysis. The data were subjected to the analysis of variance (ANOVA) univariate method ($P < 0.05$) using SPSS software (version 10.0). Comparison of means after the ANOVA test was performed using the Tukey HSD test. The relation of fatty acid composition in raw material to that in the produced oils was also subjected to an ANOVA test. For comparisons, the type of byproducts was placed in three groups and the products were placed in two groups according to composition of the product: byproducts and oil. GLMs were tested to the stored oil data in order to describe whether there was a significant effect of the temperature, storage time, or oil type. The residuals were tested for normality using probability plots followed by one sample Kolmogorov−Smirnov test.

RESULTS AND DISCUSSION

In the first part of this section, the composition of the different raw materials and produced oils is presented. In the second part, the stability during storage of the two produced oils is discussed. Complications during processing enabled us to produce only a limited amount of heads oil, allowing for the characterization but not for the storage trial.

Compositional Data. *(i) Crude Composition.* The results of the crude composition analysis in the three types of byproducts are shown in **Table 1**. As can be seen, the composition is similar among the parts studied. In all cases, the highest value was found

Table 2. Fatty Acid Profile (g/kg of Lipid) in Total Lipids from Different Types of Byproducts and Respective Oils Produced from Herring (*Clupea harengus*) Byproducts[a]

	mixed		headless		heads	
fatty acids	byproducts	oil	byproducts	oil	byproducts	oil
saturated						
14:0	86 ± 4 a	90 ± 6 b	83 ± 1 a	88 ± 1 b	87 ± 8 a	94 ± 4 b
16:0	148 ± 5 a	142 ± 5 a	139 ± 1 ab	138 ± 0 ab	152 ± 8 b	145 ± 7 b
18:0	20 ± 0 a	22 ± 4 a	20 ± 0 a	20 ± 1 a	23 ± 1 a	23 ± 13 a
total	254 ± 9 ab	254 ± 15 ab	242 ± 2 a	246 ± 2 a	262 ± 17 b	262 ± 14 b
monounsaturated						
16:1	49 ± 1 a	55 ± 3 c	49 ± 1 a	54 ± 0 c	54 ± 1 b	56 ± 4 d
18:1	73 ± 3 a	75 ± 2 a	68 ± 2 a	72 ± 2 a	71 ± 3 a	71 ± 3 a
20:1	99 ± 3 a	103 ± 1 b	100 ± 2 a	103 ± 1 b	99 ± 2 a	99 ± 1 b
22:1*	175 ± 5 ab	179 ± 3 ab	175 ± 2 a	177 ± 2 a	189 ± 6 b	178 ± 3 b
total*	396 ± 12 a	412 ± 8 b	392 ± 7 a	406 ± 5 b	413 ± 12 a	404 ± 11 b
polyunsaturated						
18:2	11 ± 3 a	12 ± 4 a	13 ± 0 a	13 ± 3 a	12 ± 0 a	14 ± 1 a
18:3*	12 ± 2 a	12 ± 0 b	12 ± 1 a	14 ± 1 b	9 ± 1 a	14 ± 2 b
18:4*	27 ± 1 a	32 ± 0 d	30 ± 1 b	32 ± 0 e	23 ± 1 c	30 ± 1 f
20:5	82 ± 3 a	90 ± 3 c	85 ± 2 a	88 ± 1 c	79 ± 2 b	87 ± 2 d
22:6*	93 ± 1 a	85 ± 1 c	95 ± 2 a	81 ± 0 c	84 ± 1 b	80 ± 5 d
total*	225 ±10 a	231 ±8 c	235 ± 6 a	228 ± 6 c	207 ± 5 b	225 ± 11 d

[a] Mean values of three independent measurements and standard deviations are given. Values in the same row followed by different letters are significantly different ($P < 0.05$). The asterisk implies that an interaction effect was found between byproducts and produced oil. The values do not add up to 1000 because minor fatty acids are not reported.

Table 3. Oxidation Status of the Different Produced Herring Oils[a]

	PV (mequiv per/ kg fat)	AV	FC (area units/pg oil)	A235 (area units/μg oil)	A270 (area units/μg oil)	FFA (%)	α-tocopherol (mg/100 g lipid)
mixed oil	6.4 ± 0.1 a	9.1 ± 0.1 a	1.2 ± 0.1 a	193.5 ± 0.5 a	112.5 ± 0.5 a	2.5 ± 0.0 a	5.97 ± 0.68 a
headless oil	6.3 ± 0.1 a	6.8 ± 0.0 b	0.7 ± 0.0 b	194.5 ± 0.5 a	118.5 ± 0.5 b	2.3 ± 0.0 b	7.36 ± 0.02 b
heads oil	13.1 ± 0.1 b	21.2 ± 0.6 c				2.4 ± 0.0 a	2.16 ± 0.05 c

[a] Mean values of three independent measurements and standard deviations are given. Values in the same column followed by different letters are significantly different ($P < 0.05$). The blanks were not determined due to lack of sample.

for the moisture content followed by protein and fat content (≈70 and 14% and 125 g/kg wet sample, respectively). No significant changes among the byproducts composition were found for the protein and fat content. On the other hand, related to moisture content, it was found that the three fractions were significantly different among each other; the mixed byproducts were the ones that contained higher amounts of moisture in opposition to the heads that presented the lowest value (72.6 vs 66.9%, respectively). It is usual that the moisture content increases as the content of fat decreases (*26*). It is likely that the statistical difference found for moisture content in the fractions is related to the standard deviations of the measurements (larger for fat than for protein and moisture content determination). A significantly higher salt content was determined for the heads byproducts as compared to the other fractions (0.7%). The fact can be explained due to the heads, which are mainly composed of bones. No significant differences in FFA content were observed, implying that the hydrolysis occurred in a similar way to the different analyzed byproducts. No α-tocopherol was present in the heads byproducts contrary to the other two types of byproducts in which 0.4−0.5 mg/100 g lipids were found. The difference found on the α-tocopherol contents between heads vs mixed and headless byproducts was significant. The fact could be explained due to the herring heads, which are mainly composed of bones and skin and not so much of muscle tissue. Studies performed comparing α-tocopherol levels in lipids from skin and muscle of mackerel and herring showed that skin had the lowest α-tocopherol content (*10*, *27*) and also that skins oxidize much faster than the other parts of the fish (*10*, *28*).

Regarding the oxidation parameters, the amount of primary, secondary, and tertiary oxidation products in the heads byproducts was, clearly, the highest. This implies that the oxidation path progresses much faster in the heads than in the other byproducts processed. This agrees with the fact that the amount of α-tocopherol in this fraction was almost zero. It is known that this antioxidant can act as a protective tool against oxidation (*29*, *30*); hence, at lower α-tocopherol contents, higher values of oxidation products such as hydroperoxides, measured as PV, should arise, which was indeed observed. Consequently, those hydroperoxides compounds are gradually decomposed to the respective further oxidation products measured as CD, CT, and AV.

Endogenous enzymes liberated from the fish tissue itself can be a potential source of initiation of the peroxidation (*31*). In particular, peroxidases and lipoxygenases catalyze the formation of highly reactive hydroperoxides that can propagate the lipid oxidative chain reaction. Lipoxygenase activity has been reported in some fish species, and evidence supports the presence of different types of these enzymes in fish with varying distribution, activity, and stability (*16*, *32*) predominantly concentrated in the skin tissue of herring (*28*). Ke et al. (*15*) studied differential lipid oxidation in various parts of frozen mackerel. They reported an unusual difference in reaction rates between skin and muscle lipids, as observed by the difference in induction period and in the overall accumulation of oxidation products. Studies of oxidation in vitro show that the faster oxidation of skin lipids is probably related to some fat soluble enzyme in the mackerel skin lipids that catalyzes their oxidation (*15*). Apparently, heads herring byproducts liberate endogenous

Table 4. ANOVA Table from the GLM of the Different Measures on Fish Oil Data Comparing the Effect of Storage Days, Temperature, Oil Type, and Interaction Effects[a]

measure	df	MS	F	P
Log PV				
model	4	0.947	24.84	0.000
intercept	1	7.153	195.09	0.000
error	57	0.037		
effects				
days	1	0.0067	0.18	0.670
temp	1	2.97	81.15	0.000
type	1	0.000	0.001	0.979
temp*type	1	0.000	0.000	0.999
A 235 nm				
model	4	7.505	1.39	0.247
intercept	1	38729.05	7200.2	0.000
error	59	5.38		
effects				
days	1	6.07	1.129	0.292
temp	1	0.0126	0.002	0.962
type	1	18.547	3.448	0.068
temp*type	1	6.025	1.120	0.294
A 270 nm				
model	4	1.112	27.3	0.000
intercept	1	144.62	3544.2	0.000
error	59	0.0408		
effects				
days	1	0.922	22.6	0.000
temp	1	4.299	105.4	0.000
type	1	0.0704	1.726	0.194
temp*type	1	0.0826	2.025	0.160
AV				
model	4	26.141	31.0	0.000
intercept	1	1780.2	2111.2	0.000
error	59	0.843		
effects				
days	1	4.83	5.73	0.020
temp	1	62.23	73.79	0.000
type	1	37.45	44.42	0.000
temp*type	1	0.54	0.636	0.428
FC				
model	4	2706.26	25.98	0.000
intercept	1	17598.08	168.97	0.000
error	60	140.15		
effects				
days	1	1825.78	17.53	0.000
temp	1	10354.8	99.42	0.000
type	1	10.52	0.101	0.752
temp*type	1	282.92	2.716	0.105
FFA				
model	4	0.086	8.298	0.000
intercept	1	161.56	15581	0.000
error	60	0.010		
effects				
days	1	0.0569	5.492	0.022
temp	1	0.216	20.80	0.000
type	1	0.067	6.488	0.013
temp*type	1	0.048	4.618	0.036
α-tocopherol				
model	4	3.981	10.992	0.000
intercept	1	1225.67	3383.9	0.000
error	54	0.362		
effects				
days	1	2.386	6.586	0.013
temp	1	5.404	14.918	0.000
type	1	0.318	0.879	0.353
temp*type	1	0.105	0.289	0.593

[a] MS, mean squares; df, degrees of freedom; P, probability level. See text for further explanation. $Y_{measurement} = \beta_0 + \beta_1 t + \beta_{2,T} + \beta_{3,\tau} + \beta_{4,T^*\tau}$. Where t (covariate) is the storage time in days, T (class variable) is the effect of the storage temperature and τ (class variable) is the type of oil studied. $T^*\tau$ 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.

enzymes that associate to a greater surface/mass ratio of skin present in the heads promoted the lipid oxidation process.

Surprisingly, the lowest oxidation values were found for the mixed byproducts and not for headless byproducts as would be expected. The fact might be explained by the fractions collected of the headless byproducts that contain a large amount of viscera. These could be responsible for promoting the oxidative degradation. It is known that endogenous enzymes from internal organs of herring are of prime importance for the proteolysis, especially concerning the ripening process (*33*, *34*). Stoknes and coworkers (*35*) showed that the proteolytic activity of the intestinal and liver fractions dominated in herring. A strong and rapid development of rancid odor in very fresh unwashed mackerel fillets indicated that blood had a high prooxidative effect (*36*).

(ii) Fatty Acid Composition. The fatty acids pattern for each of the studied types of byproducts considered before and after respective fish oil production is shown in **Table 2**. In all of the cases, the fatty acids content showed the following ranking order: monounsaturated > saturated > polyunsaturated. The most abundant saturated and monounsaturated fatty acids in the different types of byproducts and oils were docosenoic acid (22:1) and palmitic acid (16:0). Among the PUFAs, EPA (20:5) and DHA (22:6) were the major components.

The heads byproducts have the highest level of total saturated fatty acids while the headless byproducts have the lowest (262 vs 242 g/kg of lipids). No significant difference was found between the byproducts and the respective oils produced.

The total amount of monounsaturated did not show any significant difference over the different types of byproducts, which was about 400 g/kg of lipids. However, with exception of the heads where an interaction effect for the model (tested as $Y_{\text{fatty acids}}$ = type + product + type*product) was found between the type of raw material used and the product (byproducts or oil), the oil fractions present a significant higher value of monounsaturated fatty acids than the respective byproducts, showing the dependency of the fat extraction process applied (solvent vs temperature), as reported earlier (*17*). The level of monounsaturated was highly dependent on the content of docosenoic acid, the major compound in the group. Its content was the highest in the heads and the lowest in the headless byproducts (189 vs 175 g/kg of lipids).

The lowest level of unsaturation was found in the heads; it differed significantly from the mixed and headless oil. Obviously, this difference is related to the fact that heads presented the higher content of saturated fatty acids. Significant differences were found between the total content of PUFAs of the byproducts and the respective oils. A similar pattern concerning type and composition was found for the EPA and DHA. In all cases, the heads byproducts presented a significantly lower value as compared to mixed and headless byproducts. After oil production, the level of DHA decreased significantly; the headless oil was the one with highest decrease and the heads had with the lowest (around 15 and 5% of the byproducts, respectively). On the other hand, the EPA content in the oil was significantly higher as compared to the byproducts. In this case, the heads and mixed oil have an excess of about 10% while approximately only 3.5% of that excess was found in the headless oil. The facts lead us to the conclusion that during oil production, the headless oil was the one that lost the most DHA, while on the other hand, EPA was mostly recovered from the heads. Although the total fat content did not differ significantly in the two fractions, there may be a difference on the content of neutral lipids and phospholipids present, although not studied further here, it represents an interesting topic for further research.

Initial Oxidation Status. The initial oxidation status of the three different produced herring oils is shown in **Table 3**. The

Table 5. Statistics Describing the Outcome of the Parameter Estimates[a]

	β_0	$\beta_1 t$	$\beta_{2,T}$		$\beta_{3,\tau}$		$\beta_{4,T*\tau}$
			20 °C	50 °C[b]	SM	SH[b]	20 °C*SM
Log PV	0.278 ± 0.07		0.495 ± 0.096	0			
A 270 nm	2.699 ± 0.084	0.003 ± 0.002	-0.607 ± 0.12	0			
AV	9.026 ± 0.50	0.00808 ± 0.06	-2.482 ± 0.704	0	1.355 ± 0.682	0	
FC	41.22 ± 4.14	0.148 ± 0.072	-29.63 ± 5.96	0			
FFA	2.588 ± 0.056	0.00083 ± 0.000	-0.189 ± 0.078	0	0.0101 ±0.076	0	0.109 ± 0.102
α-tocopherol	7.397 ± 0.246	-0.0054 ± 0.004	-0.710 ± 0.362	0			

[a] $Y_{\text{measurement}} = \beta_0 + \beta_1 t + \beta_{2,T} + \beta_{3,\tau} + \beta_{4,T*\tau}$ and the confidence intervals by least squares estimates for the fish oil data. Log PV is expressed as log mequiv perox/kg of lipid; A 270 nm as area units/ng of lipids; FC as area units/10 pg of lipids; FFA in percent as oleic acid; and α-tocopherol as mg/100 g lipids. Refer to text for more information. [b] This parameter is set to zero because it is redundant to the model. The blanks indicate that no significant effect was found.

PV of the heads oil was significantly higher as compared to the mixed and headless oil (13.1 vs 6.4 mequiv peroxides/kg lipids). The oil produced from heads presented also a significantly higher AV than those produced from mixed and headless byproducts. In fact, the headless oil shows the lowest found value for the AV (6.8). Obviously, it is related to the initial oxidation status of the raw material used; heads byproducts showed the higher level of oxidation products, and subsequently, the oil produced from it had also high oxidation values. It has been reported that as compared to muscle, skin is more sensitive to high temperatures, since this is found to be the only type of tissue that was almost completely inactivated by heat treatment (*10*, *37*, *38*). This suggests a purer enzymatic nature of the prooxidative activity in skin rather than in muscle, possibly because transition metal catalysis is more probable there. No differences could be found with respect to conjugated dienes, contrary to the conjugated trienes and FC where significant differences were found between the mixed and headless oil (112.5 vs 118.5 area units/μg oil and 1.2 vs 0.7 area units/pg oil, respectively). Because of a lack of sample, no data are available for these measurements concerning the heads oil. The FFA was significantly lower to the headless oil as compared to the mixed and heads oils (2.3 vs 2.5%). The α-tocopherol content of the oils varied from 2.16 to 7.36 mg/100 g of lipid. The headless oil had the significant highest content of α-tocopherol while the oil from the heads presented the lowest content, since most of it has been used while oxidation progressed. Note that although undetectable levels of α-tocopherol were found in the heads byproducts, since it is a liposoluble compound, small values could be measured in the respective oil. Apparently, the differences found in the α-tocopherol contents between the oils obtained from solvent method and by steam extraction from the same byproducts might be related to an extraction phenomena, originating a higher yield for the last procedure. Summarizing the results, it can be concluded that the heads oil was of a lower quality since it is the one with higher oxidation values and lowest α-tocopherol content. However, mixed and headless oils are of similar quality, probably because only a small part of the mixed oil consists of heads oil.

Oxidative Stability. Table 4 shows the outcome of the data analysis from the GLMs that were fitted to all of the data obtained from the chemical analyses. The data are related to the change in oxidation products and antioxidants during storage at two different temperatures.

Development of Oxidation Products. The first step in the statistical analysis is to check whether the data are normally distributed using probability plots followed by one sample Kolmogorov−Smirnov test. Only the PV data required a transformation in order to obtain normally distributed data.

To evaluate whether mixed or headless herring oil, stored at 20 and 50 °C, had the greatest effect on the oxidative changes, all of the data were subjected to a least squares estimates (LSE) test, which, for each measurement, gave rise to the following parameters in the model:

$$Y_{\text{measurement}} = \beta_0 + \beta_1 t + \beta_{2,T} + \beta_{3,\tau} + \beta_{4,T*\tau}$$

In this equation, t (covariate) is the storage time in days, T (class variable) is the effect of the storage temperature, and τ (class variable) is the type of oil studied. $T*\tau$ 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 determined for the intercept.

The fitted models (**Table 4**) showed that with exception of the A 235 nm measurement, all were highly significant ($P \ll 0.05$). Concerning the primary oxidation products (PV), a significant influence was detected only to the temperature. For CT (A 270 nm), FC, and α-tocopherol, the effect of storage time and temperature showed to be highly significant. Related to AV, not only were time and temperature significant but also the type of oil was significant. For the FFA, all four effects (time, temperature, type, and interaction effect between temperature and time) were significant. No studied parameter was found to be significant ($P > 0.05$) in to the measurements of CD (A 235 nm); consequently, the described model was also not significant ($P = 0.247$).

In the next step of the statistical analysis, nonsignificant effects were omitted stepwise; with this, new models were derived (**Table 5**). This technique provided the possibility to compare the overall rates at which the different oxidation products are produced. As can be seen in **Table 5**, in all cases, an initial value was estimated indicating that the initial intercept values differed from zero and were always positive. Because different units are used for the different measurements, no comparison of the values can be made. Concerning the storage time in days, it was shown that this did not have a significant effect to the log PV and had a negative influence on the α-tocopherol content ($\beta_1 = -0.0054$), which was expected. Because of the reduction in protection against oxidation that takes place with the decrease of α-tocopherol content, oxidation was formed. This is shown by an increase of secondary and tertiary oxidation products measured as conjugated trienes, AV, and FC. The largest influence of time was found for the tertiary oxidation products, measured as FC ($\beta_1 = 0.148$) and the smallest for the formation of conjugated trienes ($\beta_1 = 0.003$). Furthermore, FFA was somewhat dependent on the storage time ($\beta_1 = 0.000\,83$). This implies that during storage, some hydrolysis of the oil occurred. The time courses of the different signals during autoxidation for the mixed herring oil stored at 20 and 50 °C and their models are shown in **Figures 1** and **2**, respectively. The log PV values are not shown since they were found to be independent of the storage time.

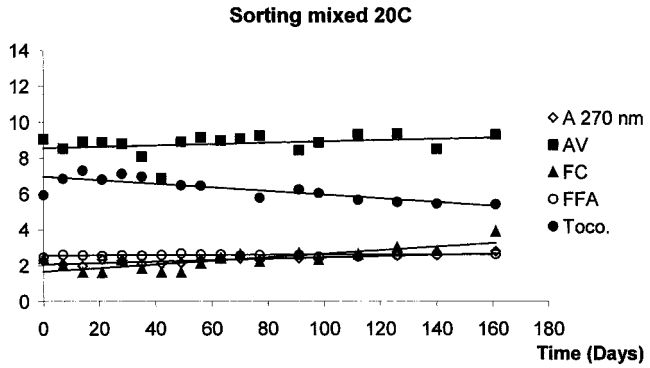

Figure 1. Trend lines and values determined for crude oil produced from mixed herring byproducts, stored under nitrogen and dark conditions (20 °C). α-Tocopherol is expressed as milligrams per 100 g of lipids; A 270 nm is expressed as area units per nanogram of lipids; FC is expressed as area units per 10 pg of lipids; and FFA is expressed in percent as oleic acid.

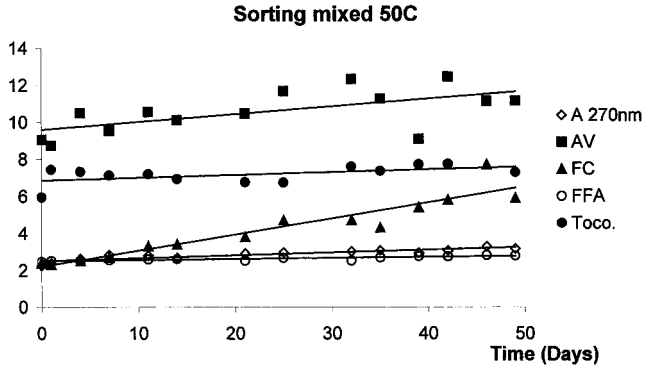

Figure 2. Trend lines and values determined for crude oil produced from mixed herring byproducts, stored under nitrogen and dark conditions (50 °C). Same units as described in the legend of **Figure 1**.

The storage temperature (**Table 5**) was found to be significant to all of the measurements. It can be observed that when the oil was kept at 20 °C, the log PV signal showed positive estimates in time while for the other signals negative estimates were found. The positive value of the log PV ($\beta_2 = 0.495$) means that higher values of hydroperoxides were determined at 20 rather than at 50 °C. The results illustrate the instability of the hydroperoxides concerning the effect of temperature, showing that these compounds are rapidly decomposed to further oxidation products at higher temperatures. The results are in good agreement with reported literature (*39*) and our previous findings (*18*). As the lipid oxidation process progresses, the development of CT, AV, and FC was in all cases significant and occurred in a lower rate at 20 rather than at 50 °C, since negative values were found. Once again, the FC has revealed to be the measurement that progresses more rapidly at 20 °C ($\beta_2 = -29.63$), as reported earlier (*17*) supported also by the findings of Aubourg (*40*). The increase in FFA level is significant, and it is more pronounced at a higher temperature of storage. The α-tocopherol content was found to be lower at 20 °C ($\beta_2 = -0.710$). The fact means, that a larger loss of this antioxidant seems to have occurred at 20 rather than at 50 °C. The reason of such phenomena might be related to a mechanistic reaction as reported previously (*18*) for oil produced from fresh herring byproducts.

The effect of origin of the oil from mixed or only headless byproducts showed to have a significant difference for the AV and FFA ($\beta_3 = 1.355$ and 0.0101, respectively). In both cases, the oil produced from mixed byproducts presented a higher value than the headless oil. A positive interaction effect between temperature (20 °C) and type of oil (SM) was only found for the development of FFA ($\beta_4 = 0.109$). For the other measurements, no significant difference was found.

CONCLUSIONS

It can be stated that heads byproducts oxidized much faster than the mixed and headless byproducts. With the exception of the CT, the headless oil has significantly lower initial values for the oxidation parameters and the highest content of α-tocopherol. In contrast, the oil produced from the heads had the highest oxidation values and presented the lowest α-tocopherol content. Heads contained the lowest PUFAs content and the highest amount of saturated fatty acids. No significant differences were found between the fatty acid composition of the mixed and the headless either in the byproducts or in the oil. Some differences were detected between the byproducts and the produced oil for the EPA, DHA, and total content of PUFAs. When stored over time, the headless oil only presented significantly lower values for the AV and FFA measurements. Considering the small differences found in the different types of produced oils, it seems that sorting of herring byproducts does not result in strongly different oil qualities. A relatively simple valorization procedure therefore seems to be suitable for the production of high quality and rather oxidation insensitive oil.

ABBREVIATIONS USED

EPA, 5,8,11,14,17-eicosapentaenoic acid; DHA, 4,7,10,13,16,19-docosahexaenoic acid; FFA, free fatty acids; PV, peroxide value; AV, anisidine value; FC, lipid soluble fluorescent oxidation products; A_{235nm}, absorbance measured at 235 nm = conjugated dienes (CD); A_{270nm}, absorbance measured at 270 nm = conjugated trienes (CT); PUFAs, polyunsaturated fatty acids; *a*, number of analyses of each sample preparation; SH, oil produced from headless herring byproducts; SM, oil produced from mixed herring byproducts; FIA, flow injection analysis; HPLC, high-performance liquid chromatography.

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LITERATURE CITED

(1) Schmidt, E. B.; Dyerberg, J. Omega-3 fatty Acids Current Status in Cardiovascular Medicine. *Drugs* **1994**, *47*, 405−424.
(2) Lanting, C. I.; Fidler, V.; Huisman, M.; Touwen, B. C. L.; Boersma, E. R. Neurological differences between 9-year-old children fed breast-milk or formula-milk as babies. *Lancet* **1994**, *344*, 1319−1322.
(3) Ackman, R. G. Fatty acids composition of fish oils. In *Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil*; Barlow, S. M., Stansby, M. E., Eds.; Academic Press: London, England, 1982; pp 25−88.
(4) Ackman, R. G. Seafood Lipids and Fatty Acids. *Food Rev. Int.* **1990**, *6*, 617−646.
(5) Hultin, H. O. Oxidation of lipids in seafoods. In *Seafoods: Chemistry, Processing Technology and Quality*; Shahidi, F., Botta, J. R., Eds.; Blackie, Academic & Professional: Glasgow, Scotland, 1994; pp 49−74.
(6) Eriksson, C. E. Oxidation of lipids in food systems. In *Autoxidation of Unsaturated Lipids*; Chan, H. W. S., Ed.; Academic Press: London, U.K., 1987; pp 207−232.

(7) Labuza, T. P. Kinetics of lipid oxidation in foods. *CRC Food Sci. Nutr.* **1971**, *2*, 355−405.
(8) Gray, J. I. Measurement of Lipid Oxidation: A Review. *J Am. Oil Chem. Soc.* **1978**, *55*, 539−546.
(9) Miyazawa, T.; Kikuchi, M.; Fujimoto, K.; Endo, Y.; Cho, S. Y.; Usuki, R.; Kaneda, T. Shelf life Dating of Fish Meats in Terms of Oxidative Rancidity as Measured by Chemiluminescence. *J. Am. Oil Chem. Soc.* **1991**, *68*, 39−43.
(10) Undeland, I.; Ekstrand, B.; Lingnert, H. Lipid Oxidation in Herring (*Clupea harengus*) Light Muscle, Dark Muscle, and Skin, Stored Separately or as Intact Fillets. *J. Am. Oil Chem. Soc.* **1998**, *75*, 581−590.
(11) Flick, G. J., Jr.; Hong, G.-P.; Knobl, G. M. Lipid Oxidation of Seafood During Storage. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; ACS Symposium Series; American Chemical Society: Washington, DC, 1992; pp 183−207.
(12) Lohne, P. Fat separation − new knowledge can open new processing possibilities. *Meld. S.S.F.* **1976**, *3*, 9−14.
(13) Chantachum, S.; Benjakul, S.; Sriwirat, N. Separation and quality of fish oil from precooked and nonprecooked tuna heads. *Food Chem.* **2000**, *69*, 289−294.
(14) Nair, M. R.; Gopakumar, K. *Fish & Marine Products, India*; Symposium on coastal aquaculture; p 170.
(15) Ke, P. J.; Ackman, R. G.; Linke, B. A.; Nash, D. M. Differential lipid oxidation in various parts of frozen mackerel. *Int. J. Food Sci. Technol.* **1977**, *12*, 37−47.
(16) German, J. B.; Kinsella, J. E. Lipid Oxidation in Fish Tissue. Enzymatic Initiation via Lipoxygenase. *J. Agric. Food Chem.* **1985**, *33*, 680−683.
(17) Aidos, I.; van der Padt, A.; Boom, R. M.; Luten, J. B. Upgrading of maatjes herring byproducts: production of crude fish oil. *J. Agric. Food Chem.* **2001**, *49*, 3697−3704.
(18) Aidos, I.; Lourenço, S.; van der Padt, A.; Luten, J. B.; Boom, R. M. Stability of Crude Herring Oil Produced from Fresh Byproducts: Influence of Temperature During Storage, submitted for publication.
(19) Bligh, E. G.; Dyer, W. J. A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911−917.
(20) *AOCS Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, IL, 1998.
(21) Lie, Ø.; Sandvin, A.; Waagbø, R. Transport of α-tocopherol in Atlantic salmon (*Salmo salar*) during vitellogenesis. *Fish Physiol. Biochem.* **1994**, *13*, 241−247.
(22) Burton, G. W.; Webb, A.; Ingold, K. U. A Mild, Rapid, and Efficient Method of Lipid Extraction for Use in Determining Vitamin E/Lipid Ratios. *Lipids* **1985**, *20*, 29−39.
(23) Undeland, I.; Härrod, M.; Lingnert, H. Comparison between methods using low-toxicity solvents for the extraction of lipids from herring (*Clupea harengus*). *Food Chem.* **1998**, *61*, 355−365.
(24) Undeland, I.; Stading, M.; Lingnert, H. Influence of Skinning on lipid Oxidation in Different Horizontal Layers of Herring (*Clupea harengus*) during Frozen Storage. *J. Sci. Food Agric.* **1998**, *78*, 441−450.
(25) Kolthoff, I. M.; Sandell, E. B. Argentimetric and mercurimetric determinations. In *Textbook of Quantitative Inorganic Analysis*; The Macmillan Company: New York, 1952; pp 545−546.
(26) Stroud, G. D. The herring. *Torry Advisory Note, n.57*; pp 1−16.
(27) Ke, P. J.; Ackman, R. G. Metal-catalyzed Oxidation in Mackerel Skin and Meat Lipids. *J. Am. Oil Chem. Soc.* **1976**, *53*, 636−640.
(28) Medina, I.; Saeed, S.; Howell, N. Enzymatic oxidative activity in sardine (*Sardina pilchardus*) and herring (*Clupea harengus*) during chilling and correlation with quality. *Eur. Food Res. Technol.* **1999**, 34−38.
(29) Giese, J. Antioxidants: Tools for Preventing Lipid Oxidation. *Food Technol.* **1996**, 73−81.
(30) Undeland, I.; Hall, G.; Lingnert, H. Lipid oxidation in fillets of herring (*Clupea harengus*) during ice storage. *J. Agric. Food Chem.* **1999**, *47*, 524−532.
(31) Lubis, Z.; Buckle, K. A. Rancidity and lipid oxidation of dried-salted sardines. *Int. J. Food Sci. Technol.* **1990**, *25*, 295−303.
(32) Wang, Y. J.; Miller, L. A.; Addis, P. B. Effect of Heat Inactivation of Lipoxygenase on Lipid Oxidation in Lake Kerring (*Coregonus artedii*). *J. Am. Oil Chem. Soc.* **1991**, *68*, 752−757.
(33) Schubring, R.; Oehlenschlager, J. Comparison of the ripening process in salted Baltic and North Sea herring as measured by instrumental and sensory methods. *Z. Lebensm. Unters. Forsch. A* **1997**, 89−92.
(34) Stefansson, G.; Nielsen, H. H.; Skara, T.; Schubring, R.; Oehlenschlager, J.; Luten, J.; Derrick, S.; Gudmundsdottir, G. Frozen herring as raw material for spice-salting. *J. Sci. Food Agric.* **2000**, *80*, 1319−1324.
(35) Stoknes, I.; Rustad, T.; Mohr, V. Comparative Studies of the Proteolytic Activity of Tissue Extracts from Cod (*Gadus Morhua*) and Herring (*Clupea Harengus*). *Comp. Biochem. Physiol.* **1993**, *106B*, 613−619.
(36) Richards, M. P.; Kelleher, S. D.; Hultin, H. O. Effect of washing with or without antioxidants on quality retention of mackerel fillets during refrigerated and frozen storage. *J. Agric. Food Chem.* **1998**, *46*, 4363−4371.
(37) Fujimoto, K.; Mohri, S.; Hasegawa, K.; Endo, Y. Oxidative Deterioration of Fish Meat. *Food Rev. Int.* **1990**, *6*, 603−616.
(38) Mohri, S.; Cho, S. Y.; Endo, Y.; Fujimoto, K. Lipoxygenase Activity in Sardine Skin. *Agric. Biol. Chem.* **1990**, *54*, 1889−1891.
(39) Davis, L.; Goodwin, L.; Smith, G.; Hole, M. Lipid oxidation in salted-dried fish: the effect of temperature and light on the rate of oxidation of fish oil. *J. Sci. Food Agric.* **1993**, *62*, 355−359.
(40) Aubourg, S. P. Assessment of antioxidant effectiveness on thermally treated marine lipids by fluorescence detection. *Eur. Food Res. Technol.* **2000**, *211*, 310−315.

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