Upgrading of Maatjes Herring Byproducts: Production of Crude Fish Oil

Isabel Aidos,*[,†,‡] Albert van der Padt,[‡] Remko M. Boom,[‡] and Joop B. Luten[†]

Netherlands Institute for Fisheries Research (RIVO), P.O. Box 68, 1970 AB IJmuiden, and Food and Bioprocess Engineering Group, Biotechnion, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Fish oil has been extracted from byproducts of the maatjes (salted) herring production using a pilot plant consisting of a mincer, heat exchanger, and three-phase decanter. The crude herring oil obtained had an initial peroxide value (PV), anisidine value (AV) and free fatty acids (FFA) level of only 3 mequiv of peroxide/kg of lipid, 8.9, and 2.9%, respectively. 5,8,11,14,17-Eicosapentaenoic acid (EPA) and 4,7,10,13,16,19-docosahexaenoic acid (DHA) were present in considerable amounts (99 and 91 g/kg, respectively). During storage of the oil, no photooxidation could be detected. Storage at room temperature led to significant autoxidation over time, apparent from primary and tertiary oxidation products, measured by a decrease of hydroperoxides and an increase of fluorescent compounds (FC). Storage at 50 °C resulted in significant increases in secondary (AV) and tertiary oxidation (FC) products. At all storage conditions, the FFA contents remained low (<3%) and the α-tocopherol content remained constant. These results open the possibility for fish oil production of good quality using salted herring byproducts.

Keywords: *Maatjes herring; byproducts; crude oil; lipid oxidation*

INTRODUCTION

There is a sizable and growing world market demand for high-quality fish meals and oil, and production can be quite profitable if suitable raw material is available. Clupeids (e.g., herring and sprat) provide the largest single source of raw material for production of fish meal and oil. They may be classified as fatty, although the fat content may vary from 2 to 30% depending on species and seasons (*1*, *2*). The fish industry would be wise to handle the byproducts from gutting, filleting, and other fish-processing operations with care because these have proven to be a good raw material for fish meal and oil production. Skåra and Cripps (*3*) have found that byproducts from farmed salmon can be used to produce fish oil of a quality that is well suited for human consumption. Besides salmon, byproducts from other fatty fish species such as herring and mackerel might be used as a source of raw material.

In The Netherlands the main fatty fish species that is processed for human consumption is herring. At present, the Dutch fish industry sells their byproducts to fish meal plants in Germany and Denmark. The byproducts are transported to these countries and then converted into fish meal, fish oil, and pet food of rather poor quality due to long transport times. For this reason it would be better to process the herring waste near the source where it is produced. If possible, the fish byproducts should be held under chilled conditions to minimize the effect of microbial and enzymatic attack on the fish tissue. This spoilage is responsible for increased free fatty acid (FFA) contents and increased oxidative breakdown by, for example, peroxidases (*4*, *5*).

Maatjes herring, a very popular product in The Netherlands and surrounding countries, is produced mainly from fresh herring landed and gibbed in Denmark and subsequently lightly salted (*6*, *7*). Production is also possible from herring frozen in blocks. Gibbing is the removal of gills, liver, heart, intestines, and stomach but not the pyloric appendix. The gibbed, lightly salted herring is transported from Denmark to The Netherlands in barrels. After arrival, it is sorted and packed in smaller buckets. Brine is added, and the herring is frozen in order to kill nematodes and preserve the herring. Large processors produce maatjes herring fillets to be sold to retailers. In lightly salted herring the ripening effect by the enzymes from the pyloric appendix is mainly on the texture of maatjes herring and not on the taste or odor of the product (*6*−*8*).

The byproducts of maatjes herring processing consisting of heads, frames, skin, and fins could be used for the recovery of oil. The total amount of maatjes herring byproducts available from the major Dutch companies is estimated at ∼2100 tons/year. However, these byproducts have been treated with salt, which may affect the quality and stability of the oil (*9*, *10*). Oxidation may be initiated and promoted by several organic and inorganic substances such as copper and iron salts, often present in salt (*11*−*13*).

Fish oil, rich in polyunsaturated fatty acids (PUFAs), is very sensitive to oxidation. In general, three oxidation steps are recognized: initiation, propagation, and termination. The first stage of the oxidation process is characterized by the production of hydroperoxides, which is usually measured as the peroxide value (PV). The amount of hydroperoxides in oil usually increases with time to a certain maximum and then decomposes rapidly to the secondary oxidation products, leading to a subsequent decrease of the PV. The second stage is

* Author to whom correspondence should be addressed (e-mail isabel@rivo.wag-ur.nl; fax +31 255 564 644).
† Netherlands Institute for Fisheries Research (RIVO).
‡ Wageningen University.

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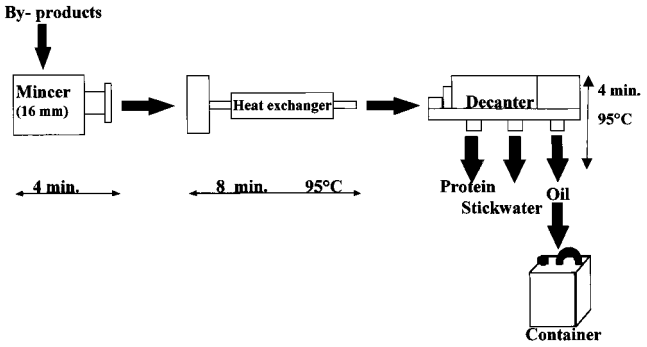

Figure 1. Scheme of the fish oil plant with processing time and temperature. Samples were taken after mincing and at the outlet of the decanter.

represented by the further degradation of lipids through a radical oxidation process initiated by the hydroperoxides. The resulting nonvolatile secondary end-products (high molecular weight saturated and unsaturated carbonyl compounds in triacylglycerols) can be measured by the anisidine value (AV). In many cases the AV is associated with the term "oxidative rancidity": an objectionable level of off-odors and off-flavors (*14*). This type of deterioration can be serious in poorly processed oils containing $n-3$ PUFAs (*15*).

Lipid deterioration compounds (peroxides and carbonyls) cause the formation of interaction compounds with fluorescent properties, the fluorescent lipid oxidation products (FC). The production of these so-called tertiary oxidation products is among others a function of temperature (*16*). The measurement of FC has been successfully applied to marine oils (*17*). The FFA content is usually measured because this is still one reliable parameter for oil quality (*1*). Furthermore, high levels of FFA can be a presage for lipid oxidation development (*18*).

Another approach to monitor the progress of oxidation is to study the decrease in antioxidant content. For that reason, for example, the α-tocopherol content in the oil, a major antioxidant in these oils, can be followed.

The aim of this paper is to investigate the possibility of using the salted byproducts of the maatjes herring industry as a raw material source for fish oil production. Therefore, a pilot extraction facility was developed and used to upgrade the herring byproducts by the production of high-quality herring oil. The paper focuses on the oil quality change during processing and storage of the oil from maatjes herring byproducts. First, a characterization of the raw material has been made, and mass balances of the process and oil yields achieved have been calculated. In the second part, a stability study was performed following the expected loss of α-tocopherol, development of FFA, and formation of oxidation products in the oil when stored at different conditions. Linear regression models were used as a tool to evaluate whether any changes were occurring at a statistically significant level during storage and to compare the different stability measurements.

MATERIALS AND METHODS

The raw material used in this study was herring byproducts from frozen herring (*Clupea harengus*) caught at the North Sea and used for maatjes herring production.

Equipment. The oil recovery process is schematically represented in Figure 1. A batch of ~1000 kg of maatjes herring byproducts (heads, frames, skin, viscera, etc.) was minced (mincer type, SAB, 49-033.2). Immediately after mincing, the minced byproducts were pumped (monopump, SW 032, speed drive 50 Hz, representing a typical flow of 250 kg/h) to an insulated scraped-surface heat exchanger indirectly heated by steam. The heated suspension was then separated in a three-phase decanter (Alfa Laval, Denmark, NX 409S-11G, set at 5600 rpm) into a semisolid phase (called protein phase), a water phase (stickwater), and lipid phase (oil). All equipment parts exposed to the products, with the exception of the mincer, were made of stainless steel.

Sampling. Samples were collected from four consecutive stages of the process, that is, the minced byproducts, the oil, the stickwater, and the protein phase, and these were analyzed.

Part of the recovered herring oil was divided into three containers and exposed to three different storage environments: at room temperature, in a closed dark container, flushed with nitrogen; at room temperature in closed transparent glass bottles placed in front of the window during the months of July and August 1999; in an oven at 50 °C in closed dark containers.

Two oil samples were taken, at regular intervals, from each storage condition. To avoid further oxidation, all of the samples were kept in an −80 °C freezer until being analyzed further. Prior to the analysis, the oil samples were thawed at room temperature for 30 min. The samples were then analyzed for the level of oxidation products, FFA formation, and α-tocopherol content.

Analytical Methods. *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) from the lipid extractions and the oil samples were prepared according to AOCS (*20*) Official Method Ce 1b-89 and analyzed with regard to the amount of content of individual fatty acids. In each occasion three samples of fillets, byproducts, and oils ($n = 3$) have been analyzed once ($a = 1$). The different FAMEs were separated from each other with gas chromatography (GC) using a Fisons 8130 instrument, equipped with an autosampler (Carlo Erba A200S) and detected with a flame ionization detector (FID). A fused silica capillary column (0.25 mm i.d. × 50m) coated with CP Sil-88 for FAMEs (film thickness = 0.20 μm), from Chrompack (Middelburg, The Netherlands) was used. The chromatographic conditions applied were as follows: column oven temperature, 190 °C; injection port and detector temperatures, 250 °C; sample size, 0.1 μL; split flow, 60 mL/min. Helium was used as carrier gas with an inlet pressure of 145 kPa. FAMEs were identified by comparison of their retention times with those of chromatographic standards (from Alltech and Sigma). Quantification was performed, using TurboChrom software (version 4.2, Perkin-Elmer), by integrating peaks on the chromatogram. Results are expressed as grams per kilogram of lipid.

Extraction of Lipids for Determination of Lipid Classes, Peroxide Value, and Fluorescent Oxidation Products. Total lipids from the herring fillets and byproducts were extracted according to the method of Burton et al. (*21*) as modified by Undeland et al. (*22*).

Determination of Lipid Class Distribution. The contents of neutral lipids (NL), phospholipids (PL), and FFA existent in the total lipids from herring fillets and byproducts and in the fish oil were determined gravimetrically by solid phase extraction (SPE) according to the method of Kaluzny et al. (*23*) ($n = 2$, $a = 2$). Disposable extraction columns Bakerbond SPE Amino (NH_2-500 mg) were used (J. T. Baker, Deventer, The Netherlands). Results are expressed as grams per kilogram of lipid.

α-*Tocopherol.* α-Tocopherol was analyzed by reversed phase HPLC (analytical column, 3 × 100 mm, inert Sil 3ODS3, Varian, The Netherlands) with fluorescence detection (excitation, 295 nm; emission, 330 nm), according to the slightly modified version of the method described by Lie et al. (*24*). Briefly, the homogenized samples were prepared for analyses by saponification in 5 mL of ethanol and 0.5 mL of 50% KOH and extraction of the unsaponifiable material in 2 × 2 mL hexane. Ascorbic acid was added before saponification, to

Table 1. Compositional Data of Frozen Raw Material Used for Production of Maatjes Herring (*C. harengus*): Fillets, Byproducts, Oil, Protein, and Stickwater Phase

	fillets	byproducts	herring oil	protein phase	stickwater
crude composition					
protein[a] (% w/w)	16.7 ± 0.6	11.7 ± 0.3	−[c]	24.9 ± 0.9	9.9 ± 1.0
moisture[a] (% w/w)	64.6 ± 0.6	68.6 ± 1.4	0.09 ± 0.01[b]	64.2 ± 0.8	77.8 ± 0.3
salt[a] (% w/w)	1.67 ± 0.05	2.94 ± 0.07	−	3.48 ± 0.05	4.07 ± 0.01
lipid components					
total lipids[a] (g/kg of wet sample)	164 ± 7	162 ± 0.5	−	56.2 ± 0.5	78.5 ± 12.5
neutral lipids[a] (g/kg of lipid)	937 ± 20	901 ± 1	971 ± 4	−	−
phospholipids[a] (g/kg of lipid)	13 ± 2	8 ± 4	7 ± 3	−	−
free fatty acids[a] (g/kg of lipid)	38 ± 11	71 ± 5	31 ± 4	−	−
α-tocopherol[b] (mg/100 g of lipid)	1.4 ± 0.4	4.2 ± 0.4	2.8 ± 0.1	−	−
trace elements					
Fe[a] (mg/kg of wet sample)	9.2 ± 0.3	26 ± 3	0.8 ± 0.1	51 ± 1	17.1 ± 0.2
Cu[a] (mg/kg of wet sample)	0.66 ± 0.08	0.45 ± 0.06	<0.1	1.06 ± 0.04	0.41 ± 0.02

[a] $n = 2$, $a = 2$, results are given as mean value ± (max−min)/2. For each of the two samples, $a = 2$. Mean values from these two analyses were used to establish the sample variation. [b] $n = 3$, $a = 1$, mean ± SD. [c] −, not measured.

prevent oxidation of the sample. Determination of the content was done by calibration with an external standard. On each occasion $n = 2$ and $a = 1$. The repeatability of the method for analyzing α-tocopherol was 5.1% ($n = 1$, $a = 6$). Results are expressed as milligrams per 100 g of lipids. Internal reference oil materials were analyzed together with the samples.

Free Fatty Acids. 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. Internal reference materials were analyzed together with the samples.

Peroxide Value. The peroxide value (PV) of the herring oil samples was determined according to AOCS (*20*) Official Method Cd-8b-90 ($n = 2$, $a = 1$). The content is expressed in terms of milliequivalents of peroxides per kilogram of lipid. Internal reference materials were analyzed together with the samples.

Anisidine Value. The anisidine value (AV) of the herring oil was carried out according to AOCS (*20*) Official Method Cd 18-90 ($n = 2$, $a = 1$). Internal reference materials were analyzed together with the samples.

Fluorescence Products (FP). 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. (*25*) ($n = 2$, $a = 1$). 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. Internal reference materials were analyzed together with the samples.

Moisture. Moisture content of the oil samples was determined according to the Karl−Fischer method ($n = 2$, $a = 2$). In the other samples moisture was determined by weighing the samples, until a constant weight was achieved, after drying in an oven at 105 °C ($n = 2$, $a = 2$). Results were expressed as percentage of wet weight. Internal reference materials were analyzed together with the samples.

Protein. Total nitrogen in the homogenized samples was determined using the Kjeldahl digestion method. Protein content was calculated as N × 6.25 ($n = 2$, $a = 2$). Results were expressed as percentage of wet weight. Internal reference materials were analyzed together with the samples.

Salt. Chlorine content in all of the samples was titrated according to Volhard's method as described (*26*) ($n = 2$, $a = 2$). Results were expressed as percentage of wet weight. Internal reference materials were analyzed together with the samples.

Analysis of Copper (Cu). To make copper available for analysis, wet sample digestion was used. The samples (about 0.5 g of oil and 1.0 g of the other samples) were destructed with concentrated nitric acid in a microwave oven for 45 min in Teflon destruction vessels. After destruction, the acid was partially evaporated and the samples were diluted to 50 mL with Milli-Q water. The copper content in each sample was determined with a Perkin-Elmer 5100 graphite furnace atomic absorption spectrometer (AAS) with Zeeman background conditions (*27*) ($n = 2$, $a = 2$). Results are expressed as milligrams per kilogram of wet sample. The limit of detection was 0.1 mg/kg. The repeatability of the method was 6.0%. Certified reference materials were analyzed together with the samples.

Analysis of Iron (Fe). About 1.0 g of each sample was ashed at 500 °C (*28*). The ash was dissolved in 5 mL of 6.0 M hydrochloric acid and diluted to 50 mL with double-deionized water. The concentration of iron was then measured with a flame atomic absorption spectrometer (Perkin-Elmer 5100, Norwalk, CT). In the case of the oil, samples were diluted with xylene and immediately measured with a graphite furnace atomic absorption spectrometer (Perkin-Elmer 5100). In both cases, a deuterium background correction (*29*) was used ($n = 2$, $a = 2$). Results are expressed as milligrams per kilogram of wet sample. The limits of detection were 0.01 and 0.1 mg/kg, and the repeatabilities of the methods were 10.0 and 5.0% for the oil and the other samples, respectively. Internal or certified reference materials were analyzed together with the samples.

Statistical Analysis. Data from the α-tocopherol and stability measurements were subjected to regression analyses to fit a model describing whether there was a significant increase or decrease over time. Lack of fit tests showed that linear models ($y = \beta t + \alpha$) could be used and therefore were tested. This technique also provided the possibility to compare the overall rates at which the different oxidation products are produced. Analysis of variance for testing the hypothesis H_0: $\beta = 0$ against H_A: $\beta \neq 0$ was used. Confidence intervals for the parameter β (regression coefficient) and α of different measurements were estimated. The p value was set at 95%.

RESULTS AND DISCUSSION

In the first part of this section the composition of the raw materials and produced oil is presented. In the second part, the stability of the oil during storage is discussed.

Compositional Data. *(i) Crude Composition.* The herring byproducts contain a high moisture content (68.6%), whereas the oil and protein contents were 16 and 11.7%, respectively (Table 1). The relatively high oil content can be explained by the fact that maatjes herring production uses catches only from the end of May/beginning of June, when it is known that the herring has a high fat content. Note that the total lipid contents in the herring fillets and the byproducts are very similar (16%). After processing, the protein and stickwater fraction contain 5.6 and 7.9% of oil, respectively. The relative distribution of NL, PL, and FFA

Table 2. Fatty Acid Profile (Grams per Kilogram of Lipid)[a] in Total Lipids from Fillets, Byproducts, and Oil Produced from Maatjes Herring (*C. harengus*) Byproducts

fatty acid	fillets	byproducts	oil
saturates			
C14:0	90 ± 4	89 ± 3	98 ± 6
C16:0	143 ± 7	138 ± 2	147 ± 8
C18:0	18 ± 1	18 ± 0	21 ± 2
total	252 ± 11	245 ± 5	266 ± 16
monoenes			
C16:1	50 ± 2	46 ± 1	52 ± 4
C18:1	60 ± 6	59 ± 1	72 ± 1
C20:1	139 ± 11	127 ± 3	130 ± 15
C22:1	202 ± 2	182 ± 3	166 ± 12
total	451 ± 20	414 ± 8	369 ± 29
polyunsaturates			
C18:2	13 ± 3	15 ± 1	13 ± 2
C18:3	15 ± 2	13 ± 0	13 ± 2
C18:4	46 ± 2	47 ± 0	61 ± 5
C20:5	68 ± 2	71 ± 0	99 ± 13
C22:6	70 ± 3	72 ± 1	91 ± 11
total	212 ± 12	217 ± 3	277 ± 33

[a] Mean value ± SD ($n = 3$, $a = 1$).

from fillets, byproducts, and oil shows that in all cases the NL are the major lipid constituents, followed by FFA and PL. The higher FFA levels indicated more hydrolysis on the herring byproducts than in the fillets (7.1 versus 3.8%). As expected, a higher protein content was determined in the fillets comparatively to the byproducts (16.7 versus 11.7%). The produced herring oil shows a moisture content of <0.1%. This value meets the quality assurance specification given by Young (*30*), who reported for a crude fish oil a maximum of 0.3%, whereas according to Bimbo (*12*) it should range on a usual basis from 0.5 to 1% maximum.

An overall mass balance was calculated on the basis of the crude composition of the raw material (100 kg of byproducts) and the end-products, giving 9.3, 17.6, and 73.1 kg of oil, protein, and water phase, respectively. The total yield of the oil was therefore 59%. This value can probably be further increased by optimizing the decanter. However, it should be emphasized that our aim was not to optimize high yields, and thereby stress the oil, but to isolate oil of high quality. The low yield of the protein phase indicates a good protein/water separation.

(ii) Fatty Acid Composition. It is known that within the same fish species the fatty acid composition can vary widely due to geographical location, fishing season, and parts of the body analyzed. Table 2 shows the fatty acid composition of the fat from the three different stages of the process: herring fillets, byproducts, and obtained oil. High concentrations of C20:1, C22:1, C20:5, and C22:6 fatty acids were measured. It is likely that these fatty acids originated from the feed of the herring, mainly copepods (natural marine zooplankton) from relatively high latitudes (*31*−*33*). The fillets and byproducts fractions showed the following ranking order for the amount of saturated, monounsaturated, and polyunsaturated fatty acids of their fat: monoenes > saturates > polyunsaturates; whereas for the oil fraction this order was monoenes > polyunsaturates > saturates. The absolute amounts of saturates are quite similar, showing that palmitic (C16:0) acid, as in most of animals (*34*), is the principal saturated fatty acid (∼14% of the total fatty acids). The byproducts and the oil had nearly the same concentration of monoenes, whereas oil from fillets had a somewhat higher concentration. Most of the difference can be explained by the content of C22:1, with the fillets having up to 18% higher levels. The total content of polyunsaturated fatty acids of the fat from fillets and byproducts was very similar but lower than in the fish oil fractions. The large differences regarding concentrations of individual polyunsaturates, especially C20:5 and C22:6, may possibly come from some oxidation during lipid isolation from the fillets and byproducts. This reveals that the fish oil extraction method used for a large-scale process seems to be more suitable for recovery of PUFAs. Higher values were found, implying that there were fewer losses.

(iii) Trace Elements (Fe and Cu). As shown in Table 1, the iron and copper contents varied substantially among the different fractions analyzed. Maatjes herring byproducts contained almost a 3 times higher iron content compared to the fillets, whereas concentrations of copper were higher (by ∼32%) in the fillets than the byproducts. These results reflect the high levels of various hemoproteins (*35*, *36*) and low molecular weight iron (*37*) in byproducts. Concentrations of copper were much lower than iron concentrations in all fractions. The protein fraction contained the higher metal content, whereas the oil had the lowest. The herring protein contained ∼64 times as much iron and at least 3 times as much copper as the corresponding oil. Values of 0.8 and <0.1 mg/kg of wet weight have been determined in the present crude herring oil for iron and copper, respectively. For herring oil, Notevarp and Chahine (*38*) reported values ranging between 10.2 and 0.5 mg/kg for iron and from 0.12 to 0.03 mg/kg for copper. Young (*13*) recommended as acceptable levels 1.5 and 0.2 mg/kg maximum for iron and copper contents, respectively. Bimbo (*12*) proposed values of 0.5−7.0 mg/kg for iron and <0.3 mg/kg for copper. This implies that the oil obtained is of acceptable quality. Oxidative stability of the capelin oil showed no correlation with the content of iron but some tendency to correlation with copper (*38*). In addition, Young (*13*) stated the importance of metals content, in particular, copper as a catalyst for the oxidation of oils and fats. Therefore, it can be concluded that our low oxidation levels (initial and developed over time) might be partly due to the low content of copper present in the crude herring oil.

(iv) α-*Tocopherol.* The α-tocopherol content showed large distribution variations. However, it should be pointed out that in some previous studies a large individual difference has been found (*39*). Lipids from herring byproducts contain 3 times as much α-tocopherol as the fillets. These results are in accordance with the results reported for mackerel dark muscle versus light muscle lipids (*35*, *36*, *40*). According to these authors, a possible explanation would be the abundance of mitochondria in dark muscle. The amount of α-tocopherol present in the oil, 2.8 ± 0.1 mg/100 g of lipids, is lower than the values reported in the literature for herring fish oil, for which values of 13, 21, and 14 mg/100 g of lipids have been described (*38*, *41*). Following the α-tocopherol content for the three different storage conditions has shown that there was hardly any loss of this antioxidant. This is confirmed by the fact that no significant losses or gains were found over time, as is shown in Table 3.

Table 3. Statistics Describing the Outcome of the Linear Regression Analysis of the Data Obtained at Three Different Storage Conditions[a]

measure	dark ($n = 20$)	light ($n = 13$)	50 °C ($n = 13$)
α-tocopherol			
α	3.62 ± 0.41	3.62 ± 0.56	3.65 ± 0.70
β	0.003 ± 0.009	0.013 ± 0.025	0.00207 ± 0.040
P	0.477	0.272	0.912
r^2	0.0285	0.108	0.0012
FFA			
α	2.73 ± 0.06	2.78 ± 0.09	2.62 ± 0.14
β	0.0013 ± 0.0014	−0.0003 ± 0.0040	0.0062 ± 0.0080
P	0.074	0.868	0.115
r^2	0.167	0.0026	0.2097
PV			
α	1.27 ± 0.407	2.50 ± 1.48	2.388 ± 1.41
β	−0.0106 ± 0.0088	0.038 ± 0.0666	0.023 ± 0.080
P	**0.021**	0.234	0.542
r^2	0.261	0.126	0.035
AV			
α	7.26 ± 0.35	7.59 ± 0.97	7.36 ± 1.87
β	−0.0073 ± 0.0077	−0.019 ± 0.044	0.17 ± 0.107
P	0.059	0.349	**0.004**
r^2	0.184	0.080	0.539
FC			
α	19.05 ± 1.55	13.27 ± 3.74	13.98 ± 4.94
β	0.165 ± 0.034	0.031 ± 0.168	1.678 ± 0.272
P	≪**0.05**	0.691	≪**0.05**
r^2	0.856	0.015	0.949

[a] For all the cases average values, obtained from duplicated measurements, were used. α and β represent the intercepts and slopes, respectively, with the corresponding confidence intervals; r^2 is the correlation and P the significance level found. Values given in bold represent significant differences.

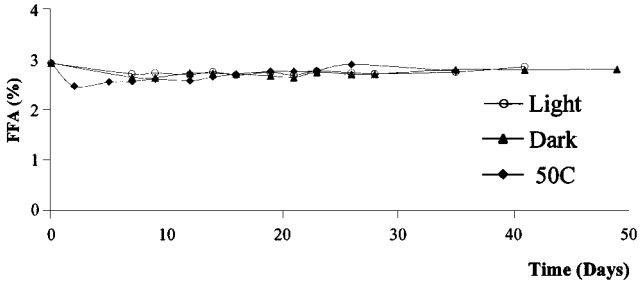

Figure 2. Free fatty acids of maatjes herring oil during storage (lines are only to guide the eye).

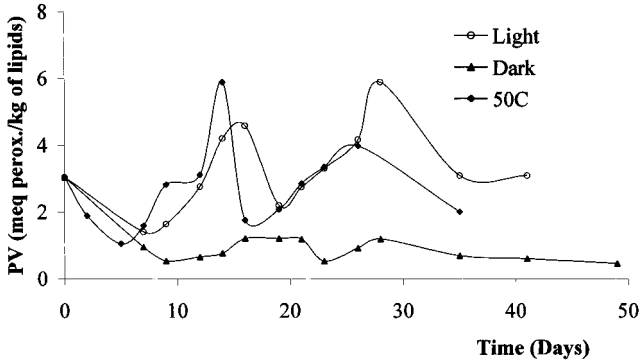

Figure 3. Peroxide value of maatjes herring oil during storage (lines are only to guide the eye).

Oxidative Stability. The value of FFA in oil is an important quality parameter, not only because usually the FFA value is checked due to contractual reasons (between the retailer and purchaser of the oil) (*1*) but also because the FFA are more susceptible to oxidation than esterified fatty acids (*18*, *42*). As quality specifications for crude fish oil, Bimbo (*12*) suggested that the FFA content should range between 1 and 7% but usually ranges between 2 and 5%, whereas Young (*30*) suggested maximum acceptable values of 4.0%. The results (Figure 2) show that the amount of FFA present in the maatjes herring oil was low (2.9%) and remained almost constant during storage time for all three different storage conditions. In fact, no significant change over time was indeed revealed for the referred studied storage conditions (Table 3). This suggests that significant hydrolysis of the oil did not occur during the storage period, possibly due to the low moisture content present in the oil (Table 1). Nambudiry (*9*) showed that with an increase in the salt content, the rate of FFA production in sardine muscle tissue decreases. In addition, in crude capelin oil (*38*) a positive correlation between iron content and FFA was found: oils with low iron contents tend to have low FFA values. Addison et al. (*43*) reported consistent results showing that the FFA of several herring oils had arisen primarily through hydrolysis of phospholipids, which occurs before or during oil production. In our case, low values of iron and phospholipids were determined, and it could be that this reduces FFA production.

The primary oxidation products were measured as the hydroperoxides, presented by the peroxide value (PV). The herring byproducts had a PV of 10.2 ± 2.1, whereas the oil presented a value of only 3.0 ± 0.3 mequiv of peroxide/kg of lipid. The decreased PV measured may be due to rapid hydroperoxide breakdown during the heating step of the process (*10*, *44*). During storage of the maatjes oil, two reproducible peaks of peroxide values are shown for the conditions at 50 °C and placed under light (Figure 3). This phenomenon can be caused by two different oxidation reactions with different speeds or initiation times (autoxidation and photooxidation, for the two studied conditions, respectively). It is known that the oxidation of unsaturated lipids is accelerated by exposure to light, there being two types of sensitizers recognized for photosensitized oxidation (*15*). Autoxidation and photooxidation produce hydroperoxides. However, no significant increase in time was found for PV at these storage conditions (Table 3). In

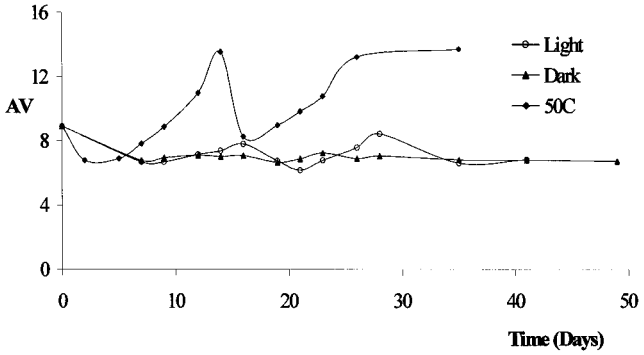

Figure 4. Anisidine value of maatjes herring oil during storage (lines are only to guide the eye).

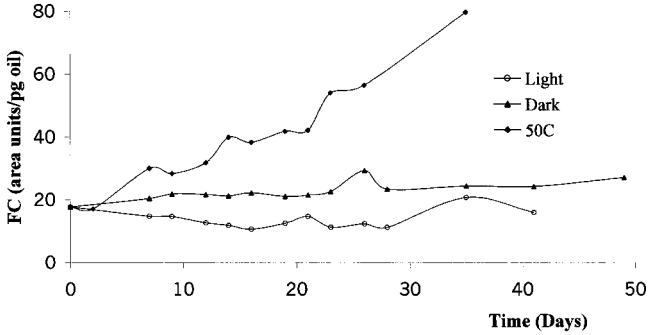

Figure 5. Fluorescent compounds of maatjes herring oil during storage (lines are only to guide the eye).

the oil stored under dark and nitrogen conditions, the peroxide value remains very low and decreases significantly over time ($\beta = -0.0106$; $P = 0.021$). This is unexpected, taking into account that fish oil is rich in polyunsaturated fatty acids and consequently susceptible to oxidation. A possible explanation for the low PV measured is the rapid degradation of hydroperoxides during the thermal treatment, as stated previously. The result is that the rate of degradation in the oil is much higher than the rate of formation, and low hydroperoxides result, which become even lower in time due to the propagation reactions.

The hydroperoxides are very unstable and decompose into secondary oxidation products (volatile and nonvolatile end-products). The nonvolatile secondary oxidation products can be followed with the anisidine value (AV). The byproducts presented an AV of 7.5 ± 0.4, whereas the oil had an AV of 8.9 ± 0.5. Figure 4 shows the AVs measured in the oil stored over time. In the oil kept at 50 °C, the development of secondary oxidation products initiates within a few days after storage. This implies that the primary oxidation products are rapidly decomposed to secondary oxidation products. In this case a significant, positive slope was found for the development of AV with storage time ($\beta = 0.17$). Although the correlation was not very high ($r^2 = 0.539$), the change was significant ($P = 0.004$). The two maxima present for peroxide value at 50 °C (Figure 3) are also observed in the anisidine value at 50 °C (Figure 4), which explains the low r^2. In the oil samples stored under light conditions, only two small peaks are present. Possibly, for the photooxidation process, a termination reaction is favored above a propagation reaction, under the conditions studied. There is hardly any change in the AV for the oil kept in dark conditions. The oil under this condition is stable. In both cases (light and dark, room temperature), slightly negative slopes were obtained with poor correlation values. The slopes were not significantly different from zero (Table 3). Therefore, it can be concluded that in the presence of light and at room temperature the low amounts of secondary oxidation products are caused by a lack of oxygen.

The tertiary oxidation products are shown in Figure 5 as fluorescent compounds (FC). An initial value of 17.7 area units/pg for the oil was determined. The maatjes herring oil stored at 50 °C shows an increase of FC values over time. This fact is confirmed with a positive slope, high correlation value, and significant increase over time ($\beta = 1.678$, $r^2 = 0.949$, and $P \ll 0.05$, respectively). The reaction from secondary to tertiary oxidation products clearly takes place. The oil stored under the light condition show a very low FC value, with a nonsignificant change over time. It is obvious that the low FC is due to low AV formed. Apparently, the primary oxidation products, formed in this case via photooxidation reaction, are only partly transformed into secondary oxidation products. A negative, although nonsignificant, slope found for the AV over time for this condition corroborates this hypothesis. The oil stored under dark conditions show a stable value for the FC, which persuades us that the high amounts of tertiary oxidation products are inhibited by a lack of oxygen and light when the oil is stored at room temperature. During storage of the oil under dark conditions a positive slope and a good correlation were found for the development of fluorescent lipid oxidation compounds with storage time ($\beta = 0.165$, $r^2 = 0.856$), and the change was significant ($P \ll 0.05$). For 50 °C the formation of fluorescent compounds progresses 10 times more quickly than at room temperature ($\beta = 1.678$ versus $\beta = 0.165$). Consequently, it can be concluded that there is a significant effect of temperature. Assuming Arrhenius kinetics, the activation energy has been estimated as ~74 kJ/mol. Such values are expected for chemical reactions.

Concluding Remarks. The results show that it is possible to produce good-quality fish oil from salted (maatjes) herring byproducts. This oil, like the oils from fatty fish species, contains a high percentage of the valuable polyunsaturated $n-3$ fatty acids, mainly EPA and DHA. The oxidation values found reveal that the crude herring oil obtained has low initial values of FFA, PV, AV, and FC. Low values of copper and iron were determined in the herring oil, explaining partly the low oxidation values.

From the herring oil stability study, it can be emphasized that linear models were not good to describe the effect of light, because for this condition all r^2 values were below 0.15.

For the studied oil, α-tocopherol and FFA contents did not change over time for the applied storage conditions. The oil kept under dark conditions at room temperature shows hardly any oxidation with a very slow decrease of PV and a slight increase of FC. For the dark conditions at 50 °C the AV and FC increase rapidly over time.

Only a significant increase over time of fluorescent lipid oxidation products was found both for the dark conditions at room temperature and at 50 °C. The formation of FC was under the last condition 10 times faster than under the former condition. Therefore, it can be concluded that temperature has a strong effect on preservation of the studied oil. Further FC can be used as a valuable rapid method to evaluate the progress of

lipid oxidation in oil. The oil presents low oxidation products over time when it kept under dark conditions, in the absence of oxygen, and at ambient temperatures. This shows that usage of maatjes herring byproducts is very promising for fish oil production of good quality.

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; AAS, atomic absorption spectrophotometry; PUFAs, polyunsaturated fatty acids; PL, phospholipids; NL, neutral lipids; SD, standard deviation; *a*, number of analyses of each sample preparation; FIA, flow injection analysis; HPLC, high-performance liquid chromatography.

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