Concentrating PUFA from Mackerel Processing Waste

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ABSTRACT: Mackerel processing waste comprising skins, viscera, and muscle tissue was evaluated for concentrating PUFA by urea complexation. Fish oil was extracted using either chloroform/methanol (2:1, vol/vol) or hexane/isopropanol (3:2, vol/vol). The yield of oil, as well as iodine, peroxide, and acid values, was determined for fresh fish oil extracts, and oil samples were stored at –70°C in the presence of 100 ppm α-tocopherol. PUFA concentrates were prepared from saponified fish oil. The mean oil yields were 9.18 ± 2.3, 9.2% ± 2.4, and 38.1 ± 3% for viscera, muscle, and skin, respectively. The mean baseline iodine value was 134 ± 5, which increased to 296 ± 7 after urea complexation. It was possible to concentrate PUFA from mackerel processing waste. The type of tissue used did not affect the amount of PUFA concentrated. Mackerel skin was most desirable because of its high oil content.

Paper no. J10427 in *JAOCS 80,* 933–936 (September 2003).

KEY WORDS: Docosahexaenoic acid, eicosapentaenoic acid, fish oil, fish skin, fish waste, mackerel oil, urea complexation.

Fish oils are a readily available source of long-chain PUFA, especially those of the n-3 series, mainly *cis*-5,8,11,14,17- eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA). Recognition of the roles played by these FA in human health and nutrition (1–3) and the resulting growth in new markets have stimulated much interest in methods of extracting and concentrating them from natural sources. These PUFA occur as TG in fish oils at levels between 10 and 25% (4).

Several methods have been reported for concentrating PUFA in marine oils, with varied yields. Among methods that concentrate PUFA as TG without prior hydrolysis are solvent fractionation, winterization, and molecular distillation (4). Concentrations of up to 30% EPA and DHA are feasible by using these methods. However, higher levels (65–80%) are attainable by processes combining either hydrolysis or esterification with methods such as supercritical fluid extraction, urea complexation, and molecular distillation. Concentrations beyond 90% are possible with HPLC (4).

Urea complexation has been applied extensively to concentrate PUFA from various sources including marine and vegetable oils (5–8). Ratnayake *et al.* (9) demonstrated pilot-scale (20 kg) urea complexation for concentrating n-3 PUFA. Compared with the other methods for producing PUFA concentrates, urea fractionation allows handling of large quantities of materials in simple equipment. Since the process requires only limited use of less toxic organic solvents such as ethanol, it is environmentally friendly. It is also cost-effective because urea is relatively inexpensive.

Urea forms complexes with molecules containing linear alkyl chains, which act as a template with which urea molecules complex in spiral-shaped structures as a result of several hours of cooling (8). Separation of urea complexes from the nonurea complexing fraction effectively removes saturated and long-chain monounsaturated FA and enriches the liquid extract in unsaturated FA.

Fish processing could generate wastes of up to about 50% of the body weight of the processed fish based on the body components of interest to the processor (10). Wastes from fish processing are used to produce fishmeal, with oil as a by-product, or to remediate soil. The oil content of fish waste ranges between 1.4 and 40.1% (10) depending on the species and tissue. Fish processing waste is therefore an important source of fish oil that could serve as a good source of PUFA while adding value to the waste. This study was undertaken to extract and characterize the oil from mackerel processing waste comprised mainly of skin, viscera, and muscle tissues and to assess the possibility of concentrating PUFA from oil extracted from these tissues using urea complexation.

MATERIALS AND METHODS

Materials. Mackerel samples were obtained from the Gaspé region of Québec, Canada. Samples were frozen and stored at –40°C as *ca.* 1.50-kg packages in sealed plastic bags until used. Organic solvents used were ACS grade or better. FA standards were purchased from Nu-Chek-Prep (Elysian, MN) and consisted of methyl esters of 32 FA ranging between $C_{8:0}$ and $C_{24:1}$. Urea was obtained from Fisher Scientific (Nepean, Ontario, Canada). BF_3/methanol (14%) and α-tocopherol were purchased from Sigma-Aldrich Company (Oakville, Ontario, Canada).

Extraction of fish oil. Two extraction methods were investigated, modifications of the chloroform/methanol (2:1, vol/vol) (11,12) and the hexane/isopropanol (3:2, vol/vol) methods (13). Duplicate samples of five batches of skin, muscle, and viscera were analyzed using each of the above methods.

Chloroform/methanol. Mackerel tissue (30 g) was homogenized without thawing for 2 min with a mixture of methanol (60 mL) and chloroform (30 mL) in a food blender. One volume of chloroform (30 mL) was added to the mixture and blended for an additional 30 s. About 30 mL 0.88% of KCl was then added.

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The homogenate was stirred and filtered through Whatman No. 1 paper. The organic phase was separated from the aqueous phase by using a separatory funnel. The organic solvent was then removed by using a rotary evaporator at 45°C. The oil extract was flushed with nitrogen and stored at –70°C until analyzed.

Hexane/isopropanol. About 20 g of mackerel tissue was homogenized as above with a 360-mL mixture of hexane/isopropanol (3:2, vol/vol) for 30 s. The homogenate was filtered through Whatman No. 1 filter paper. The homogenizer, funnel, and residue were washed twice with 40-mL portions of hexane/isopropanol followed by resuspending the residue each time and letting the solvent soak for 2 min before filtering. To the pooled filtrates was added 150-mL aqueous sodium sulfate made up of 1 g of anhydrous sodium sulfate salt in 15-mL distilled water. After 10 min, the homogenate was filtered to remove the sodium sulfate salt. The organic layer was separated in a separatory funnel and removed with a rotary evaporator.

In both cases, the final weights of extract, after evaporation, were determined. Three 2-mL aliquots of the final sample were evaporated to dryness under nitrogen to a constant weight and were used to estimate the weight of oil present in the sample.

Chemical properties of oil. Iodine, peroxide, and acid values (AOCS Methods Cd 1b-87; Cd 8-53; Cd 3d-63) of triplicate oil samples from the extracts were determined for five batches of each tissue type used (14).

Urea complexation. The method of Ratnayake *et al.* (9) with modifications was used to concentrate PUFA from fish oil. Fish oil (50 g) was saponified with 11.3 g of KOH, 40 mL 95% ethanol, and 12.5 mL of distilled water for 1 h under a stream of nitrogen. FA were extracted with hexane after partial cooling, followed by addition of 60 mL of distilled water and acidification to pH 1 using 6 N HCl. The hexane layer was separated and removed using a rotary evaporator. Urea complexation involved adding 10 g of FA to urea at a ratio of 1:3.5 (w/w); 95% ethanol was added at a ratio of 1:3.7 (wt/vol) based on weight of urea. The mixture was heated at 70°C with stirring to dissolve all the urea, producing a clear homogeneous solution. The sample was held at 0°C for 24 h after 6 h storage at room temperature to imitate crystallization. Urea crystals were separated by filtration, and the nonurea-complexing fraction was diluted with 100 mL of distilled water, acidified to pH 4.5 with 6 N HCl, and extracted twice with 50-mL vol of hexane. Hexane extracts were combined and dried on a rotary evaporator. The PUFA concentrates were flushed with nitrogen and stored at –70°C with 100 ppm of α-tocopherol.

FA analysis. FAME were produced by methylation with 14% boron-trifluoride. FAME analysis was performed on a gas chromatograph equipped with an FID and FID fitted with a 30-m SUPELCOWAX-10 fused capillary column (0.32 i.d., 0.25 mm film thickness; Bellefonte, PA). Separation was achieved by using the following temperature program: initial column temperature, 80°C; initial hold time, 1 min; final temperature, 220°C at a rate of 15°C/min; and final hold time, 25 min. Helium was used as carrier gas at a flow rate of 2 mL/min. The detector temperature was 260°C, and the injector temperature was 240°C. The 17:0 methyl ester was used as internal standard.

RESULTS AND DISCUSSION

Oil characteristics. Mackerel was chosen for this study because it is an underutilized fish with a limited range of value-added products developed from it. This is probably because of the highly perishable nature of the species. Being so oily makes the fish very prone to postmortem spoilage, such as rancidity, and presents processing challenges (15). However, in light of the nutritional value of marine oils, the oily nature of mackerel tissues presents opportunities. It is important to increase the information base available on mackerel to enhance exploitation. The baseline characteristics of mackerel oil in Table 1 indicate that the distribution of oil in the tissues is not proportional and the skin yields are the highest at *ca.* 38% compared with 9% for viscera and muscle. This disparity was evident irrespective of the solvent extraction system used. Consequently, the use of chloroform/methanol (2:1, vol/vol) for oil extraction was discontinued after the preliminary analysis, since we observed no inherent advantages over hexane/isopropanol (3:2, vol/vol) that would justify the risk of toxicity associated with exposure to both chloroform and methanol. Since mackerel skins are usually discarded during processing and they have a high oil content, skins are a most suitable material for producing PUFA because they will generate comparatively high volumes (*ca.* 38% w/w) of oil for small inputs, thus reducing bulk handling and transportation.

The PV were indicative of onset of autooxidation. The iodine values were lower than those reported for commercial mackerel oil; the iodine value of mackerel oil of 150 has been reported (16). The PV and acid value were typical of crude oil extracts (17). There was marked variation in these parameters between different batches of fish primarily as a result of the nonhomogeneous nature of fish-processing waste. The nonhomogeneous nature of fish-processing waste arises from the fact that the tissues are generated from different batches of fish and catches from different geographic locations and physiological states. Consequently, oil content in tissues from processing waste would not reflect that of a homogeneous population of fish and would vary between batches. It is a factor that needs to be considered in choosing materials for producing PUFA and emphasizes the need for preliminary screening to assess the batches with suitable baseline characteristics. However, for crude fish oil extracts, the acid value and PV were low (<6 mg/g and <4 meq/kg). They do, however, indicate that oxidation is a major problem in handling mackerel oil generated from processing waste and that frozen storage could retard but not eliminate oxidation.

TABLE 1
Baseline Characteristics of Mackerel Oil

	Tissue		
Parameters	Viscera	Skin	Muscle
Iodine value	134 ± 6	133 ± 5	134 ± 5
PV (meq/kg)	3.6 ± 0.8	3.3 ± 1.5	3.6 ± 1
Acid value (mg/g)	5 ± 1.3	4 ± 1	5 ± 1
Yield of oil (% w/w)	9.18 ± 2.3	38.1 ± 3.1	9.2 ± 2.4

TABLE 2
FA Content (wt%) of Saponified Mackerel Oil Before and After Urea Complexation[a]

	Mackerel oil			Urea concentrate		
FA	Muscle	Viscera	Skin	Muscle	Viscera	Skin
14:0	5.1 ± 0.9	1.7 ± 1.5	4.9 ± 0.2	0.5 ± 0.2	1.2 ± 1.06	0.5 ± 0.2
14:1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
16:0	19.9 ± 2.1	17.5 ± 0.5	15.3 ± 0.4	<0.5	0.6 ± 0.1	1.28 ± 0.2
16:1	5.8 ± 0.8	3.8 ± 0.2	6.9 ± 0.1	1.9 ± .3	2.0 ± 0.1	2.1 ± 0.4
18:0	3.6 ± 0.9	5.9 ± 0.2	2.9 ± 0.1	0.9 ± 0.6	<0.5	<0.5
18:1	9.1 ± 1.1	29.8 ± 2.2	10.9 ± 1	0.8 ± 0.6	1.8 ± 0.3	<0.5
18:2	1.3 ± 0.9	0.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	2.1 ± 0.2	1.35 ± 0.2
18:3	2.2 ± 0.8	<0.5	2.6 ± 0.8	8.7 ± 0.3	9.4 ± 0.2	9.2 ± 0.7
20:0	0.7 ± 0.2	<0.5	<0.5		<0.5	—
20:1	9.4 ± 1.7	5.1 ± 0.5	9.3 ± 0.7	—	1.7 ± 1.0	—
20:2	<0.5	<0.5	<0.5	—	—	—
20:4	<0.5	0.5 ± 0.2	<0.5	—	—	1.2 ± 0.1
20:5	8.0 ± 0.1	6.3 ± 0.7	8.5 ± 0.2	29.2 ± 2	28.7 ± 1.2	29.6 ± 1.8
22:1	13.6 ± 1.3	5.1 ± 0.7	12.1 ± 1.1	1.8 ± 0.3	—	—
22:6	9.7 ± 0.2	7.9 ± 0.5	10.2 ± 0.7	40.9 ± 3.1	33.0 ± 0.5	42.1 ± 4.4
24:0	1.1 ± 0.8	1.82 ± 0.1	1.3 ± 0.1	—	—	—
Others	10.5	14.1	13.8	14.0	19.5	12.7
Total PUFA	21.2	15.2	22.6	80.1	73.2	83.5
Total mono.	37.9	43.8	39.2	4.5	5.5	3.8
Total n-3	19.9	14.2	21.3	78.8	71.1	80.9

[a]Results are expressed as means ± SD (*n*-3). Total mono. = total monounsaturated.

Urea complexation. The FA composition of mackerel oil TG is given in Table 2. The major saturated and monounsaturated FA include 14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 20:0, 20:1, and 22:1. Of these, 22:1, 20:1, 16:0, and 18:1 concentrations were high. The basic FA that define most fish oils according to Ackman *et al.* (5), *viz.* 14:0, 16:0, 18:1, 20:1, 22:1, 22:5, and 22:6, were present although in disproportionate amounts in the three tissue types. The amounts of these FA are representative of values recorded for commercial mackerel oils (18,19). Mackerel oil has a uniquely high level of 22:1 compared with menhaden and sardine, the other fatty fish species (18), where the concentration of 22:1 ranges from trace to low. The amounts of these FA were present in all the tissues studied; however, there was a very high level of 18:1 in the viscera compared with the muscle and skin. The EPA and DHA levels were not markedly different between the three tissues types. This indicates that most tissues in mackerel could be viable sources.

The results of urea complexation are also shown in Table 2. Urea complexation resulted in 91.6–97% reduction in 16:0, and 47–69% and 90–98% reduction in 16:1 and 18:1, respectively. There were undetectable levels of 20:1 and 22:1. On the other hand, EPA and DHA increased between three- and fourfold based on the baseline values and those of the urea concentrates. EPA increased from 6.3–8.5% to between 28.7–29.6% and DHA from 7.9–13.6% to between 33–42.1%. Up to sixfold increments of DHA after urea complexation have been reported (5).

The total n-3 FA content of the oil based on the amount of EPA, DHA, and the major isomer of linolenic acid ranged between 71.1 and 80.9% The iodine value increased from *ca.* 134 in the oil to 296 ± 7 in the PUFA concentrates with the resulting increase in degree of unsaturation.

Tissue type did not affect the results of urea complexation. This is because the minor differences existing between the constituent FA of skin, viscera, and muscle occurred predominantly within the composition of the major mono- and diunsaturated FA, and these were preferentially removed following the urea complexation process. There were similar degrees of enrichment of PUFA in oils from the different tissue types. There was no inherent advantage in any tissue type. Therefore, the most suitable tissue would be one that provides more oil with limited bulk. The skin appears to be highly suited for producing PUFA from mackerel processing waste.

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[Received August 19, 2002; accepted March 12, 2003]