Preparation of Omega-3 PUFA Concentrates from Fish Oils via Urea Complexation

By W. M. N. Ratnayake, B. Olsson, D. Matthews and R. G. Ackman *

The effect of weight ratio of urea to fatty acids and the urea-fatty acid adduct crystallization temperature on the enrichment of eicosapentaenoic acid from marine oil fatty acids was studied. The optimum ratio of urea to fatty acids was found to be 3 : 1 for laboratory scale preparations and the optimum temperature for the formation of urea-fatty acid adduct was 1° C. At very low temperatures (-12, -18, -35° C) the recovery efficiency for EPA was reduced. Using these optimum values, enrichment of EPA and other n-3 polyunsaturated fatty acids via urea complexation was carried out on a pilot plant scale on a variety of North Atlantic and North Pacific fish oils and a seal oil. Irrespective of the type of starting oil, all the oils gave a concentrate with 69–85 % total n-3 PUFA with an overall yield of 17–20 %. Menhaden is clearly an ideal oil for preparation of EPA concentrate, as the starting oil usually has a higher proportion of EPA to DHA than most of the other commercial fish oils.

Herstellung von Omega-3-PUFA-Konzentraten aus Fischölen durch Harnstoffkomplexbildung

Es wurde der Einfluß der Gewichtsverhältnisse von Harnstoff zu Fettsäuren und der Kristallisationstemperatur des Harnstoff-Fettsäureaddukts auf die Anreicherung von Eicosapentaensäure aus Fettsäuren von Fischöl untersucht. Es zeigte sich, daß das optimale Gewichtsverhältnis von Harnstoff zu Fettsäuren 3 : 1 bei der Herstellung im Labormaßstab und die optimale Temperatur zur Bildung des Harnstoff-Fettsäureaddukts 1° C betrugen. Bei sehr geringen Temperaturen (-12, -18, -35° C) wurde die Wiedergewinnungsrate für EPA verringert. Bei Nutzung dieser optimalen Werte wurde die Anreicherung von EPA und anderen n-3 mehrfach-ungesättigten Fettsäuren mittels Harnstoffkomplexbildung im Pilotanlagemaßstab an einer Vielzahl von Fischölen aus dem Nordatlantik und dem Nordpazifik und an einem Seehundsöl durchgeführt. Unabhängig von dem Ausgangsöl lieferten alle Öle ein Konzentrat von 69–85 % Gesamt n-3 PUFA bei einer Gesamtausbeute von 17–20 %. Das Menhadenöl ist ein ideales Öl für die Herstellung von EPA-Konzentrat, weil das Ausgangsöl normalerweise ein höheres Verhältnis von EPA zu DHA als die meisten anderen im Handel befindlichen Fischöle hat.

Introduction

It has been suggested that the effectiveness of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of fish oils in preventing atherosclerotic cardiovascular diseases lies both in their ability to interfere with the production of the 2-series of prostaglandins, especially thromboxane A_2 (TXA_2), and to promote the conversion of EPA to antiaggregatory prostaglandins of the 3-series, especially PGI_3[1-4]. For further clinical studies, and perhaps as a nutritional health supplement, a product enriched in EPA and DHA may be preferred to ordinary fish oil[5]. Concentrates keep the intake of calories and of other possibly physiologically active fatty acids as low as possible.

Fish oils could be concentrated in the form of the natural triglycerides or as modified triglycerides, as free fatty acids, or as the simple alkyl esters of these acids. Most of the fish oil products sold over the counter today are in the natural triglyceride form, but more recently are appearing in the free fatty acid or ethyl ester form. The amount of EPA and DHA in the simplest fish oil concentrates is usually claimed to total about 30 % (Table 1). Levels in the natural triglyceride form higher than this are difficult to achieve, and may also be economically less attractive. However, it has long been known[6,7] that much higher levels of EPA and DHA could be reached by first converting the triglycerides to the free acids or to alkyl esters. Of the several methods considered for concentration of such materials, urea complexation is one of the most promising as it allows handling of large quantities of material in simple equipment, and requires no organic solvents except ethanol. The milder conditions used in the urea complexation should not affect the molecular structures of the highly unsaturated fatty acids. The urea fractionation of fatty acids is based mainly on the degree of unsaturation, and straight chain and monounsaturated acids form urea inclusion compounds much more readily than do the polyunsaturated fatty acids (PUFA)[6-13].

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The present study initially investigated on a laboratory scale the influence of weight ratio of urea to fatty acids, and the urea-fatty acid adduct crystallization temperature, on the content of EPA in the fatty acid concentrates. This part of the study was carried out with menhaden *(Brevoortia tyrannus)* oil. Finally, knowing the optimum conditions for production of n-3 PUFA concentrate, a large-scale urea complexation procedure was developed and tested with oils from menhaden, Atlantic redfish *(Sebastes sp.),* Atlantic herring *(Clupea harengus),* Pacific dogfish *(Squalus acanthias),* Pacific salmon (mixed, but probably mostly from sockeye, *Oncorhynchus nerka*) and Atlantic harp seal *(Pagophilus groenlandica).* A preliminary report on the second part of this study was published elsewhere[11].

Experimental

Laboratory-Scale Urea Complexation

Several laboratory-scale urea complexations were conducted in order to determine the optimum weight ratio of urea to fatty acids and the optimum complexation temperature for the enrichment of EPA. Normally the laboratory experiments were carried out with 20 g of fish or other oil. Typically, fish oil (20 g) was saponified by refluxing for 1 hr, in an atmosphere of N_2, with a mixture of KOH (4.6 g), H_2O (8.8 ml) and 95 % ethanol (26.4 ml). The saponified mixture was diluted with water (50 ml), and the non-saponifiable matter was extracted with hexane (3 x 50 ml) and discarded. The aqueous layer was neutralized with 3N HCl with subsequent extraction of the free fatty acids with hexane (3 x 50 ml). The hexane extract was dried over sodium sulphate, evaporated to dryness and the free fatty acids were recovered.

Urea and fatty acids (18 g) were mixed with 95 % ethanol (90 ml) and the mixture was heated, with magnetic stirring, until the whole mixture turned into a clear, homogenous solution. Initially the excess urea and the urea-fatty acid adduct were allowed to crystallize overnight at room temperature and subsequently in a cold room, where the temperature was maintained at the desired levels (-35, -18, -12, 1 and 6° C), for 24 hours for further crystallization. The crys-

Table 1

*Claimed contents of EPA and DHA in some retail fish oil nutritional supplements sold in capsule form, on the basis of 1000 mg capsules, with product type**

Trade name, company, and label "features"	Fatty Acid EPA (mg/g)	Fatty Acid DHA (mg/g)	Product Type
Norwegian cod liver oil (Twin Lab)[a]	66–82	68–84	oil
Biosaumon (Medicorp [France])[b] – Natural and without danger – No physico-chemical processing	120	160	oil
MaxEPA 300 (Walgreen Labs Inc.) – Hi potency omega-3 fatty acids – Cold water natural fish oil	180	120	oil
MaxEPA (Solgar Co., Inc.) – Marine Lipid Concentrate	180	120	oil
Natural Omega-3 (Country Life) – Fish Body Oils[c]	180	120	oil
Cardi-Omega-3 (Solar Nutritionals Inc.) – All natural MaxEPA fish oil concentrate	180	120	oil
Your Life (P. Leiner Nutr. Prod. Inc.) – Natural fish oil concentrate	180	120	oil
proto-chol (E. R. Squibb & Sons Inc.) – Natural fish oils	180	120	oil
Natural brand OMEGA-3 (Sonergx Nutr. Prod.) – Fish oil concentrate	180	120	oil
Nature's Best (Nature's Best Food Suppl.) – Natural fish oil concentrate	180	120	oil
PROMEGA (Parke-Davis) – Natural fish oil concentrate	280	120	oil
Super EPA 500 (Walgreen Labs Inc.) – Hi potency omega-3 fatty acids – Cold water natural fish oil	300	200	ethyl ester
Cholesterex (Nutr. Prod. of Amer. Inc.) – Omega-3 fish oil concentrate – Purified natural fish oil concentrate	200	85	oil[d]
Omega-'3' EPA SUPER 500 (Schiff Bio-Food Proc.) – Unsaturated fish oils[e]	300	200	methyl ester
Health crafts EPA-Forte (Booker Health Products [U.K.]) – Selected marine lipid concentrate	310	210	ethyl ester
PROMEGA PEARLS (Parke-Davis) – Natural fish oil concentrate	300 "omega-3" fatty acids per pearl[f]		oil

* Published by *R. G. Ackman* in Chemistry and Industry, 7 March 1988, pp. 139–145. Copyright permission has been given by the Society of Chemical Industry, London.

[a] Vitamin A-1250 I.U., Vitamin D-130 I.U. per 500 mg capsule.

[b] Vitamin A-700 I.U., Vitamin D-70 I.U. per 1 g.

[c] Includes garlic 50 mg and ascorbyl palmitate 2 mg, "in a blend of glycerin, soya-bean oil, lecithin and lemon oil".

[d] Fat < 1 g per serving of two capsules, according to label.

[e] Willow bark (10 mg) included.

[f] PEARLS softgel size is 600 mg. Values are given per softgel.

tals formed (the urea-fatty acid adduct, also termed the urea complexing fraction, UCF) were separated from the liquid (non-urea complexing fraction, NUCF) by filtration on a *Buchner* funnel under suction. The NUCF was diluted with an equal volume of water, and acidified to pH 4–5 with 6 N HCl. The liberated free fatty acids were extracted with hexane. In a similar manner the fatty acids from the UCF were recovered after the addition of water and HCl.

Pilot Plant Scale Preparation of n-3 PUFA Concentrate

Pilot plant scale preparations were carried out with 40 kg of fish oil. A flow chart of the procedure is given in Fig. 1. The experimental details have been published previously[11].

The crude n-3 PUFA NUCF concentrate was converted to the ethyl esters using absolute ethyl alcohol/conc. H_2SO_4. The crude ethyl esters were purified by low-pressure distillation using a 6-inch (15.2 cm) diameter Pope wiped-film still (Pope Scientific Inc., P. O. Box 495, Menomonee Falls, Wisconsin), at a pressure of 0.2–0.5 Torr and with the still body temperature at 250° C. The throughput was 5 ml/min.

Analytical Techniques

Gas liquid chromatography of fatty acids

Samples of oil and fatty acids were converted to methyl esters by heating at 100° C in a nitrogen-flushed screw-cap centrifuge tube for 60 min (oils) or 30 min (fatty acids) with 10% BF_3-MeOH (1 ml) and benzene or hexane (1 ml). Gas

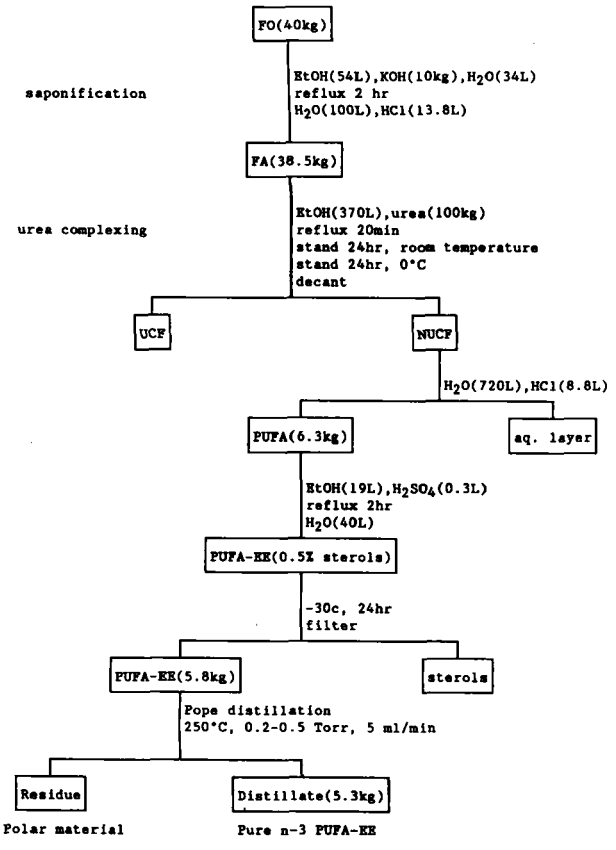

Fig. 1. Flow scheme for concentration of fatty acids in commercial oil by urea complexation, esterification and short-path wiped-wall distillation

liquid chromatography was executed with a Perkin-Elmer Sigma-3B GLC unit and a SUPELCOWAX-10 column, flexible fused silica, 30 m x 0.25 mm ID, operated either isothermally or with programmed temperatures [14,15]. The fatty acid ethyl esters were analysed directly using the same SUPELCOWAX-10 column.

Iatroscan TLC/FID

The purity or the composition of the starting marine oils and of the various fatty acid or ethyl ester fractions was examined by the Iatroscan thin-layer chromatography/flame ionization detector system [16]. Chromarods-SII were developed in hexane : diethyl ether : formic acid 97 : 3 : 1 (v/v/v).

Results and Discussion

PUFA Concentrating Methods

Very little has changed in the field of fish oil fatty acid concentration since *Gruger's* review of 1962 [7]. For human therapy there should be a major preoccupation with safety of PUFA [17], which rules out the iodination-chromatography-silver dehalogenation route [18], and other procedures of silver ion chromatography [19] would also have to be reviewed for potential toxicity. Supercritical fluid fractionation (SCF) with CO_2 is one more possibility [20-22], but even it was anticipated nearly four decades ago [23]. A recent paper by *Nilsson et al.* [22] indicates that the ethyl esters of EPA and DHA derived from menhaden oil can be obtained in high purity using SCF with CO_2. The SCF CO_2 fractionates fatty acid esters according to chain length, and therefore it is not possible to achieve a clean separation of fatty acid esters differing only in degree of unsaturation. According to *Nilsson et al.* [22] SCF works well with starting fatty acid mixtures having higher proportions of EPA and DHA, and lesser amounts of saturates, monounsaturates and dienes. Thus, prior to SCF, it is necessary to remove the saturated and less unsaturated fatty acids by urea fractionation. Removal of these makes it possible to obtain EPA and DHA in purities exceeding 90%. The SCF CO_2 of ethyl esters is still in the laboratory scale operation stage, but appears suitable for obtaining high purity EPA and DHA at g levels.

Laboratory Scale Urea Fractionation

The urea complexation could be performed either on the free fatty acids or on the methyl or ethyl esters. In this investigation we preferred the free fatty acids due to their solubility in the urea/ethanol solution being higher than those of the corresponding simple alkyl esters. This is a very important consideration in large scale preparations, as it minimizes the solvent (ethanol) used.

The influence of the urea/fatty acid ratio (w/w) on the percentage yield of n-3 PUFA in the concentrate was studied on a small scale using fatty acids derived from menhaden oil. In this particular set of experiments the weight of fatty acids was kept constant at 18 g, but the weight of urea was varied. The urea complex was allowed to crystallize at room temperature for 24 hours. The variation of EPA and DHA levels with the corresponding variation of the urea/fatty acid weight ratio is shown in Fig. 2. The concentration

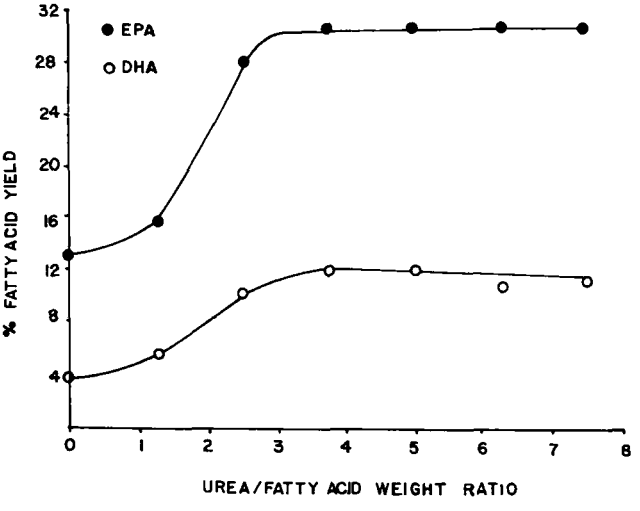

Fig. 2. Content of EPA and DHA in the non-urea crystalline fraction after urea crystallization at room temperature with various amounts of urea

of EPA and DHA reached a maximum at an approximate urea/fatty acid ratio of 3 : 1. Above this ratio the percentage of n-3 PUFA, especially of EPA, remained constant, but the overall yield of the NUCF decreased. When an excess of urea was used, more and more of the EPA in particular was complexed and found its way into the urea complexing fraction. These findings confirmed those of *Haagsma et al.* [9] who reported that with cod liver oil fatty acid methyl esters the efficiency of urea complexation reached the maximum when the urea/fatty acid ratio is about 3 : 1.

Table 2 shows the results of the study on the influence of the crystallization temperature on the yield and composi-

Table 2

The influence of the urea-fatty acid adduct crystallization temperature on the yield and fatty acid profile of the UCF and NUCF laboratory scale fractionation

Fatty Acid[1]	Starting Menhaden oil	Temperatures (° C) 6		1		−12		−18		−35	
		UCF	NUCF	UCF	NUCF	UCF	NUCF	UCF	NUCF	UCF	NUCF
14:0	14.0	15.5	2.0	15.8	1.8	18.7	1.4	19.3	1.6	14.5	0.7
16:0	20.1	33.0	1.8	17.9	0.8	27.9	0.6	37.4	1.4	31.7	0.3
16:1n-7	16.4	20.1	15.8	21.6	8.4	21.6	7.2	16.9	6.5	20.3	3.5
16:4n-1	2.0	–	4.7	–	5.5	–	7.3	–	6.8	–	7.4
18:0	2.9	4.6	–	4.1	–	4.3	–	4.8	–	4.6	–
18:1*	11.8	20.1	3.8	14.0	1.5	16.3	1.8	12.9	1.2	15.5	1.1
18:2n-6	1.6	1.1	1.6	1.3	–	1.1	1.6	0.8	1.2	1.1	0.8
18:4n-3	3.6	–	9.7	–	11.2	–	11.6	–	11.9	–	13.1
20:1*	1.6	2.5	–	1.5	–	1.8	0.1	0.7	0.1	1.4	–
20:5n-3	12.6	1.2	29.5	1.1	32.9	1.2	33.3	1.3	35.9	3.5	34.3
22:6n-3	4.4	0.4	10.9	–	11.1	0.3	11.3	TR	14.9	0.2	18.3
Yield (wt%)		52	38	51	35	52	32	45	28	61	23
% Recovery efficiency of EPA	100	4.9	89.0	4.5	91.4	4.9	84.6	4.6	79.8	16.9	62.6

* All isomers.

[1] Only major and important fatty acids have been listed.

tion of UCF and NUCF fractions. In this experiment the urea/fatty acid weight ratio was kept at the optimum level of 3 : 1. Among the saturates and monounsaturates, the longer chain length fatty acids formed urea adducts more readily than the shorter chain lengths. Even at very low temperatures (−18 or −35° C) it was difficult to completely remove the shorter chain lengths, especially the 14 : 0 and 16 : 1n-7 found in all fish oils. *Iverson* and *Wick*[10] observed a similar behaviour with methyl ester derivatives of shorter chain fatty acids.

Among the major n-3 PUFA, 18 : 4n-3 and DHA were found almost exclusively in the NUCF. Although the major proportion of EPA was always recovered in the NUCF, a slight proportion invariably complexed with urea and was detected in UCF. The amount of EPA in the UCF increased sharply below −18° C. These results showed that EPA has a slightly higher tendency to form urea adducts than the other two major n-3 PUFA, especially at very low temperatures. *Haagsma et al.*[9] have reported similar results for fatty acid methyl esters from cod liver oil. They reported 100% recovery of DHA and 18 : 4n-3 from the NUCF, but only 60% recovery of EPA.

At very low temperatures the overall yield of the NUCF is low (Table 2). The optimum temperature for maximum recovery of EPA is apparently about 1° C. At this temperature the recovery efficiency of EPA is very high and 91% of the total EPA present in the starting oil is found in the NUCF fraction.

Pilot Plant Scale Urea Fractionation

Using the results of the laboratory work as a guideline, we have developed an efficient multi-kg scale fractionation of fish oil as outlined in Figure 1. Hydrolysis of the oil is the first step. In scaling up the procedure from a g-scale to multi-kg-scale, an increase in the proportion of water was preferred for the saponification. This excess of water is required to minimize the formation of ethyl ester by-product; otherwise, transesterification becomes a significant reaction producing substantial quantities of fatty acid ethyl esters. High levels of ethyl esters in the fatty acid mixture tend to give low yields of NUCF during the subsequent urea complexation step. The saponification proportions outlined in Figure 1 usually produce less than 1% of fatty acid ethyl esters in the free acid product. In the pilot plant scale it is not necessary to have elaborate methods of fatty acid recovery using water-immiscible solvents or by other means. Thus a second solvent is essential only if one wants to remove non-saponifiable matter prior to urea fractionation. With most fish oils, composed of about 99% triglyceride (including free fatty acid, and mono- and diglycerides), this step is not required. Acidification, followed by a gentle water wash with a subsequent settling period of 2 hrs is adequate for recovery of 99% of the fatty acids in our procedure.

For the urea complexation step on this scale, a weight ratio of urea to fatty acids of 2.63 : 1 was used, which is slightly less than the optimum ratio of 3 : 1 found in the laboratory study. The deviation from the urea to fatty acid ratio of 3 : 1 has no large effect on the yield of NUCF or on EPA recovery. It is perhaps better to use the lowest possible ratio of urea to fatty acids as it economizes on the volume of ethanol required to dissolve the urea/fatty acid mixture into a homophase under reflux. The yields of the crude PUFA recovered from the NUCF of the oils tested ranged from 17 to 21%.

Refining of PUFA Concentrate through Low-Pressure Distillation

Usually the crude PUFA concentrate is dark in color and needs some refining. One of the convenient and efficient ways of cleaning up the crude PUFA concentrate is by low-pressure distillation of the ethyl esters. Initially distillation was attempted using a conventional *Stedman* distillation column, but this found to be unsuitable for the highly unsaturated PUFA concentrate. The conventional distillation produces a substantial proportion of artifact fatty acids, perhaps mostly with *trans* ethylenic unsaturation[24,25], and probably some polymeric materials. The geometric isomer artifacts were present in both the distillate and the residue[26]. The n-3 PUFA are thought to have a higher tend-

ency to produce artifacts than the other PUFA with more centrally located double bonds. There is, however, the possibility of both the bond closest to the carboxyl group and that nearest the methyl group being unusually labile in EPA and DHA, a subject currently under investigation in our laboratory[27].

The low-pressure distillation with a thin-film still of the Pope wiped-wall type was found to produce a product with no artifacts because of the short contact time, of at most a few minutes, dependent on the flow rate of the ethyl esters over the heated distilling surface. GLC and infrared analysis of the distilled ethyl esters indicated that there was no isomerization of bonds in the heat-labile constituents. Any residual ethanol and any other volatiles disappeared into the vacuum system, and the polymeric substances and the bulk of the pigments were retained in the non-volatile residue. The distillation is about 99% efficient with less than 1% retained in the residue. The main PUFA ethyl ester concentrate was a light straw color, almost free of odor as compared to most fish oils fatty acids or the undistilled PUFA ethyl esters.

Purity and the Fatty Acid Profile of the Distilled PUFA Ethyl Esters

Iatroscan TLC/FID analysis showed that the Pope-distilled PUFA ethyl ester concentrates, except that from dogfish liver oil, were quite pure, with more than 99% ethyl esters and only traces of cholesterol and free fatty acids. There was no polymeric or oxidized material at the Chromarod origin[28]. The starting dogfish liver oil was not a pure triglyceride oil and had only 49% triglyceride, the major component class being diacylglyceryl ethers (51%). Therefore the PUFA ethyl ester concentrate (distilled) prepared from this dogfish liver oil had only 83% ethyl esters, the balance being made up of monoalkylglyceryl ethers. These monoalkylglyceryl ethers did not interfere with the free acid-water phase separation or the urea complexing itself.

Urea fractionation removed most of the straight chain saturated fatty acids, but the branched chain fatty acids were enriched in the NUCF along with PUFA (Table 3). Among the branched chain fatty acids were the known iso, anteiso, centrally monomethyl branched, and the three isoprenoid structures[29,30]. Apart from these, redfish oil showed two novel fatty acids, namely, 2,2,6,10,14-pentamethylpentadecanoic acid and 2,3,7,11,15-pentamethylhexadecanoic acid, which have not been reported previously in any natural product (unpublished results of the authors).

Urea crystallization mainly fractionates by the degree of fatty acid unsaturation[13], but the chain lengths also influence the efficiency. The monounsaturated longer-chain fatty acids, especially of the C_{20} and C_{22} chain lengths, form urea adducts more readily than the shorter chain (C_{14} and C_{16}) acids[10]. In particular, the C_{20} and C_{22} monoethylenic fatty acids can be removed very easily as urea adducts, so only traces of these acids, sometimes of interest in animal physiology[31], are left in the PUFA concentrates (Table 3). Palmitoleic acid (16 : 1n7) is, as expected[10], hard to remove totally from the concentrate. It is known that the 20 : 1 and 22 : 1 fatty acids of fish oils from the northern latitudes are of exogenous origin[32]. The easy removal of these long chain monoethylenic fatty acids from herring, salmon, redfish and seal blubber oils by urea means that the concentrates made from these oils are similar to each other in their fatty acid compositions. Thus we have shown that the remaining fatty acids are a 'basic' composition for these oils, and that this basic fatty acid composition is represented by menhaden oil[11]. In effect any of the northern fish oils are equally as suitable as menhaden oil for urea complexing to give total n-3 PUFA concentrates. However, the proportion of EPA to DHA in the concentrate was higher when menhaden oil was used, due to the higher proportion of EPA in this type of oil[32].

Concluding Remarks

Most of the fish oils from temperate climates are suitable for producing an omega-3 PUFA concentrate through urea complexation. Most of the fish oils gave a concentration with 69–85% of total n-3 PUFA with an overall yield of 17–20%. The saturated and longer-chain monoethylenic fatty acids could easily be removed as urea complexes. Menhaden oil is the ideal oil for preparing the concentrates as the starting oil has higher levels of both EPA and of saturated fatty acids.

Table 3

Fatty Acid Composition of the Starting Marine Oils and the Concentrates Prepared via Urea Complexation on a Pilot-Plant Scale*

Fatty Acid	Atlantic Herring		Pacific Salmon A		Pacific Salmon B		Atlantic Menhaden		Atlantic Redfish		Harp Seal Blubber		Pacific Dogfish Liver	
	Starting	Concentrate	Starting	Concentrate	Starting	Concentrate	Starting	Concentrate	Starting	Concentrate	Starting	Concentrate	Starting	Concentrate
14:0[1]	10.9	1.7	3.6	0.3	4.3	0.6	8.8	2.7	3.1	0.9	5.2	1.1	1.7	0.4
16:0	13.5	-	11.7	0.2	14.1	0.3	17.8	0.6	6.8	TR	8.4	0.3	12.7	0.4
18:0	0.6	0.1	2.4	-	3.1	0.2	2.8	TR	1.5	-	1.3	-	4.6	0.4
Saturates[2]	28.7	5.4	19.8	2.7	23.5	2.5	30.8	5.3	12.8	3.3	16.3	4.0	28.6	9.8
16:1[3]	8.4	5.4	4.7	5.9	5.0	2.8	12.0	13.3	8.7	6.5	15.6	11.7	5.8	4.5
18:1[3]	6.4	0.2	18.0	2.9	16.8	3.5	11.4	0.1	11.0	0.4	19.2	1.6	24.1	2.3
20:1[3]	15.2	TR	13.9	TR	12.2	0.5	1.8	ND	23.1	0.1	17.2	0.6	5.3	0.1
22:1[3]	23.7	TR	8.7	TR	7.9	0.3	0.5	ND	27.5	-	9.9	0.9	2.2	0.1
Monoenes	52.9	5.6	49.7	8.8	42.0	7.5	25.7	13.4	70.3	6.9	62.2	14.9	38.3	7.1
16:2[3]	1.5	4.1	0.3	1.5	0.8	1.6	2.0	3.3	0.6	4.9	0.9	2.8	1.9	0.6
16:3[3]	1.4	2.8	0.7	1.1	0.8	0.6	2.8	4.4	0.5	2.3	0.2	1.2	2.3	0.8
16:4[3]	1.0	3.8	0.2	1.1	0.1	0.2	1.8	3.3	0.8	3.3	0.5	1.7	3.8	2.0
18:2n-6	0.7	1.6	1.4	3.2	1.2	1.5	1.3	0.7	0.5	1.2	0.9	2.0	2.7	2.0
18:3n-3	0.6	1.7	0.9	2.3	1.0	1.3	0.9	0.9	0.2	0.7	0.4	0.9	1.7	1.0
18:4n-3	2.0	13.1	1.6	5.2	1.4	3.8	3.3	7.0	1.0	5.1	1.0	4.1	0.4	2.1
20:4n-6	0.1	0.8	0.7	1.1	0.5	1.0	0.5	1.1	0.2	0.9	0.4	1.3	0.3	1.8
20:5n-3	5.5	30.0	8.2	25.3	6.8	18.4	14.8	34.9	6.0	32.5	6.6	25.5	3.6	18.6
21:5n-3	0.2	1.2	0.3	1.0	0.3	1.0	0.4	0.8	0.3	2.2	TR	1.5	0.3	1.7
22:5n-3	0.5	1.4	1.2	5.4	2.0	4.1	2.1	3.0	0.6	3.0	2.9	9.5	2.8	12.1
22:6n-3	4.3	25.7	11.6	35.0	16.8	50.7	9.4	26.7	4.4	29.2	6.1	25.3	6.6	33.0
n-3 PUFA	14.5	74.4	25.9	76.5	30.3	83.3	32.2	76.6	12.9	74.5	17.7	69.0	19.1	72.5

* WT.% (g fatty acid per 100 g GLC volatile fatty acids). ND = not detected; TR = trace

[1] 14:0 includes some 4,8,12-trimethyltetradecanoic acid (4,8,12-TMTD), which does not form urea complexes[29,30].

[2] The total saturates include a number of iso, anteiso and multibranched saturate acids. The proportions of these acids are important in dogfish liver oil and in all the concentrates.

[3] Includes all isomers.

Literature

[1] *J. Dyerberg* and *K. A. Jorgensen*, Prog. Lipid Res. **21**, 255 [1982].
[2] *H. C. Bronsgeest-Schoute, C. M. van Gent, J. B. Luten* and *A. Ruiter*, Amer. J. Clin. Nutr. **34**, 1752 [1981].
[3] *S. H. Goodnight, W. S. Harris, W. E. Connor* and *D. J. Illingworth*, Arteriosclerosis **2**, 87 [1982].
[4] *A. Leaf* and *P. C. Weber*, New Eng. J. Med. **318**, 549 [1988].
[5] *P. G. Norris, C. J. H. Jones* and *M. J. Weston*, Brit. Med. J. **293**, 104 [1986].
[6] *R. G. Ackman, R. D. Burgher* and *P. M. Jangaard*, Can. J. Biochem. Physiol. **41**, 1627 [1963].
[7] *E. H. Jr. Gruger*, United States Dept. Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries, Circular 296, Fishery Industrial Research **2**, 31 [1962].
[8] *O. S. Privett*, Prog. Chem. Fats Other Lipids **9**, 407 [1968].
[9] *N. Haagsma, C. M. van Gent, J. B. Luten, R. W. de Jong* and *E. van Doorn*, J. Amer. Oil Chemists' Soc. **59**, 118 [1982].
[10] *J. L. Iverson* and *R. W. Wick*, J. Assoc. Off. Anal. Chem. **50**, 111 [1967].
[11] *R. G. Ackman, W. M. N. Ratnayake* and *B. Olsson*, J. Amer. Oil Chemists' Soc. **65**, 136 [1988].
[12] *A. M. Abu-Nasr, W. M. Potts* and *R. T. Holman*, J. Amer. Oil Chemists' Soc. **31**, 16 [1954].
[13] *H. Schlenk*, Prog. Chem. Fats Other Lipids **II**, 243 [1954].
[14] *R. G. Ackman*, WCOT (Capillary) Gas-Liquid Chromatography, in: Analysis of Fats and Oils, edited by *R. J. Hamilton* and *B. A. Rossel*, Elsevier Applied Science Publishers, New York, pp. 137–206, 1986.
[15] *R. G. Ackman*, Acta Med. Scand. **222**, 99 [1987].
[16] *R. G. Ackman*, Methods in Enzymology **72D**, 205 [1981].
[17] *R. G. Ackman* and *W. M. N. Ratnayake*, Proc. M.I.T. Seagrant Conf. Oct. 8, 1987, in press.
[18] *D. Rubin*, United States Patent No. 4526902 (1985).
[19] *R. A. Adlof* and *E. A. Emken*, J. Amer. Oil Chemists' Soc. **63**, 1592 [1985].
[20] *R. J. Swientek*, Food Processing **32**, July 1987.
[21] *S. S. H. Rizvi, J. A. Daniels, A. L. Benado* and *J. A. Zollweg*, Food Technology **40**, 57, July 1986.
[22] *W. B. Nilsson, E. J. Gauglitz Jr., J. K. Hudson, V. F. Stout* and *J. Spinelli*, J. Amer. Oil Chemists' Soc. **65**, 109 [1988].
[23] *H. J. Passino*, Ind. Eng. Chem. **41**, 280 [1949].
[24] *R. G. Ackman, S. N. Hooper* and *D. L. Hooper*, J. Amer. Oil Chemists' Soc. **51**, 42 [1974].
[25] *A. Grandgirard* and *F. Julliard*, Rev. franç. Corp. Gras. **34**, 213 [1987].
[26] *R. G. Ackman*, Chem. and Ind. (London) (5) 139 [1988].
[27] *R. C. Wijesundera, W. M. N. Ratnayake* and *R. G. Ackman*, 79th Annual Meeting of the American Oil Chemists' Society, May 8–12, 1988, Phoenix, Arizona.
[28] *J. K. Kaitaranta* and *P. J. Ke*, J. Amer. Oil Chemists' Soc. **58**, 710 [1981].
[29] *R. G. Ackman* and *S. N. Hooper*, Comp. Biochem. Physiol. **32**, 117 [1970].
[30] *R. G. Ackman, J. C. Sipos* and *S. N. Hooper*, J. Amer. Oil Chemists' Soc. **54**, 199 [1977].
[31] *R. Ronneberg, G. Holmer* and *G. Lambertsen*, Ann. Nutr. Metab. **31**, 160 [1987].
[32] *R. G. Ackman, J.-L. Sebedio* and *M. I. P. Kovacs*, Mar. Chem **9**, 157 [1980].
[33] *R. G. Ackman*, Fatty Acid Composition of Fish Oils, in: Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil, edited by *S. M. Barlow* and *M. E. Stansby*, Academic Press, London, pp. 25–88, 1982.

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Composition and Mineral Distribution of Rapeseed Varieties Tested for Adaptation in Turkey*

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Rapeseed belongs to the world's most important oilseeds. The nutritive value of rapeseed meal has gradually improved to the point where it has become an acceptable alternative to soybean meal as a source of protein in rations. The objectives of the present work was to assess the composition and mineral distributions of low and high erucic rapeseed cultivars. Ten varieties including a local one were examined for their proximate composition. The fat content ranged between 40.5 and 45.6 %, while the crude protein content varied from 24.3 to 28.2 % on dry matter basis. Mineral analysis were conducted on seeds after wet ashing of samples. Total phosphorus content was found to be between 0.60 and 0.84 %, being nearly twice as compared to soybeans. Total sulphur, which predicts both the levels of S-bearing amino acids and glucosinolates varied greatly, from 0.40 to 1.11 %, in the examined rapeseeds. Important differences among the tested rapeseed cultivars were also valid for other minerals especially iron, manganese and calcium contents.

Zusammensetzung und Mineralstoffverteilung von Rapssaat-Sorten, die zum Anbau in der Türkei getestet wurden

Rapssaat gehört zu den weltweit bedeutendsten Ölsaaten. Der Nährwert von Rapsmehl ist ständig angestiegen, und Rapsmehl ist mittlerweile eine echte Alternative zum Sojamehl im Hinblick auf seine Eigenschaft als Eiweißquelle. In der vorliegenden Arbeit wurden die Zusammensetzung und die Verteilung der Mineralstoffe von Rapssaaten mit niedrigem und hohem Gehalt an Erucasäure untersucht. Es wurden 10 Sorten einschließlich einer einheimischen Sorte auf ihre ungefähre Zusammensetzung untersucht. Der Fettgehalt lag zwischen 40.5 und 45.6 %, der Gehalt an Roheiweiß zwischen 24.3 und 28.2 %, bezogen auf Trockenmasse. Nach Naßveraschung der Proben wurden Mineralstoffanalysen an den Saaten durchgeführt. Es zeigte sich, daß der Gesamtphosphorgehalt zwischen 0.60 und 0.84 % lag. Damit war er beinahe doppelt so hoch wie der von Sojabohnen. Der Gesamtgehalt an Schwefel, welcher sowohl die Schwefel enthaltenden Aminosäuren als auch die Glucosinolate umfaßt, schwankte bei den untersuchten Saaten erheblich, und zwar von 0.40 bis 1.11 %. Beachtliche Unterschiede zwischen den untersuchten Rapssaaten wurden auch bei anderen Mineralstoffen, besonders bei Eisen, Mangan und Calcium ermittelt.

Introduction

Rapeseed ranks fifth in total world production of edible, vegetable oils, but cultivation is limited to regions where soyabean and subtropical oilseed crops are poorly adapted. In Turkey between 1950 and 1960 rapeseed was harvested

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