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Characterization of Fish Oil Extracted from Fish Processing By-products

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Abstract

To improve the utilization of fish processing by-products, fish oils were extracted from hoki, yellowfin sole, mackerel, and horse mackerel, and their compositions were examined. The proximate compositions obtained for these 4 species of by-product revealed they were composed of 68.1～78.1% moisture, 1.2～1.6% ash, and 13.8～18.8% protein. Fish oils extracted from the hoki, yellowfin sole, mackerel, and horse mackerel were 5.5, 9.4, 13.4, and 10.3%, respectively. The total lipids extracted from the by-products of the 4 species were 6.21, 10.43, 12.81 and 10.06%, of which neutral lipids accounted for 77.38, 77.46, 87.21 and 86.79%, respectively. Neutral lipid analysis by TLC showed that triacylglycerol was the major component, while 1,3- and 1,2-diacylglycerols, free fatty acids, free sterols, and sterol esters were present as minor components. The major fatty acids were palmitic acid, stearic acid, and oleic acid. DHA and EPA were contained at levels of 0.2～4.7% and 3.7～9.5%, respectively, in the 4 types of fish oil. The fish oils extracted from the dark muscle fish, mackerel and horse mackerel, had greater polyunsaturated fatty acid (PUFA) contents than those of the white muscle fish species, hoki and yellowfin sole.

Key words: fish oil, fish processing by-products, fatty acid, PUFA

INTRODUCTION

Every year in Korea, over 2,000 tons of fish and shellfish are harvested, and only about 50～60% of the total catch is used for human consumption. Approximately 45% of the fish tissue remains after processing, including offals, fins, skin, internal organs, head, bones, etc., which are not used as foods. Some of these fish processing by-products are utilized, but huge amounts are still discarded as wastes. In particular, fish processing by-products contain valuable protein and lipid fractions, as well as vitamins and minerals.

Fish oils have well documented beneficial health effects. They are readily available sources of long-chain polyunsaturated fatty acid (PUFA), especially the n-3 series consisting mainly of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the n-6 series including arachidonic acid (AA) and γ-linolenic acid (GLA). These PUFAs have many physiological functions, and are used for various purposes. For example EPA has been used for the treatment of arteriosclerosis and hyperlipemia since 1990 in Japan. DHA plays a role in the prevention of a number of diseases in humans, including cardiovascular disease (1). For these reasons, tuna oil containing DHA has been used as a food material, an ingredient in infant formulas, and as a health food (2).

From previous studies, fishes are known to be excellent sources of high quality protein, and contain sufficient amounts of most of the essential amino acids (3). Fishes are also high in PUFA, EPA, and DHA, and the effects of these fatty acids are well documented in numerous investigations (4,5). The most important fish in Korea are sardines, herring, anchovies, mackerel, and skipjack. These fishes are among the best sources of PUFA. However, the fish oil contents and fatty acid compositions of these fishes are not constant (6), and great variations are reported, in terms of fat content and fatty acid composition, for various marine organisms (7-9).

PUFA can be obtained from marine organisms like fish, and the primary commercial source of PUFA is fish oil. Fish processing by-product comprises ～50% of the fish body weight (10). Such by-products are used to produce fishmeal, with fish oil as a by-product, or as fertilizers. Fish oil content depends on the fish species and tissue. Therefore, fish processing by-products are important sources of fish oil that could also serve as good sources of PUFA, while adding value to the by-products.

In this study, we extracted fish oil from fish processing by-products of four species, and analyzed their fish

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oil amounts and fatty acid compositions to evaluate their potential for use as bioactive substances.

MATERIALS AND METHODS

Materials

Approximately 40 kg of hoki (*Johnius belengeri*) and yellowfin sole (*Limanda aspera*) by-products, respectively, were obtained from Daerim Co. (Busan, Korea). The mackerel (*Scomber japonicus*) and horse mackerel (*Trachurus japonicus*) were purchased from a local fish market (Busan, Korea). The inedible portions (head, skin, and internal organs) of the hoki, yellowfin sole, mackerel, and horse mackerel were collected, homogenized, and stored at -80°C until use.

Fatty acid standards and BF_3/methanol were purchased from Sigma (St. Louis, MO, USA). Silica gel (70～230 mesh) was purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

Proximate composition analysis

The moisture, lipid, protein, and ash contents of the by-products were determined by the AOAC methods with some modifications (11). The experiments were performed in triplicate and values are expressed as mean ±standard deviation.

Total lipid extraction

Total lipids were extracted from the fish processing by-products using the method of Bligh and Dyer (12). Briefly, the extraction procedure was as follows: 100 g of sample was homogenized to raw fish waste with a mixture of 100 mL of chloroform and 200 mL of methanol for 2 min to obtain a monophasic system. To this monophasic ternary system, 100 mL of chloroform was added and the mixture was blended for 30 s. Next, 100 mL of water was added and blending was continued for 30 s. The homogenate was filtered through Whatman No. 1 filter paper, and the filtrate was collected in a separatory funnel. After separation of the filtrate into two layers, the volume of the chloroform layer was measured.

Chemical properties of extracted lipids

Acid values (AV) and peroxide values (POV) of the extracted fish oils were determined using AOAC methods (13).

Separation of neutral lipid, glycolipid, and phospholipid

The total lipid was separated into neutral lipid, glycolipid, and phospholipid through a glass column (ID 20×300 mm) packed with a slurry of activated silica gel (70～230 mesh; Merck, Germany) in chloroform according to the method of Rouser et al. (14). The eluents for the neutral lipids, glycolipids, and phospholipids were chloroform, acetone, and methyl alcohol, respectively. The solvents were evaporated using a rotary evaporator, and the percentage of each fraction was determined gravimetrically.

Thin-layer chromatography

The various lipid classes were separated and fractionated by thin-layer chromatography (TLC, 0.25 mm silica Gel 60 F_{254}, Merck, Co., Ltd.). The lipids were fractionated using hexane/diethyl ether/acetic acid (80:20:2, v/v/v). The developed TLC plates were sprayed with a 95% sulfuric acid solution and then heated at 110°C for 20 min. The neutral lipid standards consisted of monoacylglycerol, 1,2-diacylglycerol, 1,3-diacylglycerol, cholesterol, fatty acid, triacylglycerol, cholesterol ester, and wax.

Fatty acid analysis

The total lipid was saponified with 0.5 M-NaOH in methanol for 5 min at 100°C. The fatty acids were methylated with 14% BF_3 in methanol for 30 min at 100°C, and measured using a GC-MS (Shimadzu QP-5050A, Kyoto, Japan) equipped with a HP-INNOWax capillary column (ID 0.25 mm×30 m). Nitrogen was used as the carrier gas with a 0.5 mL/min flow rate and 30:1 split ratio. The initial temperature was 210°C. The temperature was then increased from 1°C/min to 240°C, where it was held for 17 min. The injector and detector temperatures were 250 and 300°C, respectively. A 1 μL sample of fatty acid methyl esters was injected into the GC column. The fatty acids were identified by comparing their retention times with those of standards known fatty acid composition.

RESULTS AND DISCUSSION

Proximate compositions of the fish processing by-products

The proximate compositions of white muscle fish species (hoki and yellowfin sole) dark muscle species (mackerel and horse mackerel) were determined (Table 1). The protein contents for hoki, yellowfin sole, mack-

Table 1. Proximate compositions of fish by-products (%)

	Hoki	Yellowfin sole	Mackerel	Horse mackerel
Moisture	78.1±3.5	75.6±3.0	68.1±2.1	69.3±1.9
Ash	1.2±0.1	1.2±0.2	1.3±0.1	1.6±0.2
Protein	15.2±0.8	13.8±0.5	17.2±0.3	18.8±0.3
Lipid	5.5±0.2	9.4±0.3	13.4±0.1	10.3±0.2

Values are means±standard deviations of triplicate determinations.

Table 2. Chemical properties of fish oil extracted from various fish by-products

	Hoki	Yellowfin sole	Mackerel	Horse mackerel
Acid value (mg/g)	1.57±0.05	4.79±0.12	1.62±0.09	1.86±0.13
Peroxide value (meq/kg)	1.28±0.10	9.66±0.21	8.39±0.13	9.23±0.25

Values are means±standard deviations of triplicate determinations.

erel, and horse mackerel were 15.2, 13.8, 17.2, and 18.8%, respectively. Ash contents were between 1.2 and 1.6%, while moisture contents varied from 68.1 to 78.1%. The corresponding lipid contents of the above fish by-products were 5.5, 9.4, 13.4, and 10.3%, respectively. According to these results, the lipid contents of the dark muscle fish, mackerel and horse mackerel, were greater than those of hoki and yellowfin sole, the white muscle fish.

Chemical properties of the fish oil

Most fish processing by-products are perishable. However, they are important marine sources of oil, since fish oil is an excellent source of DHA and EPA as compared to other sources such as seed oils.

The AVs and POVs of the fish oils are shown in Table 2. The AVs of hoki, yellowfin sole, mackerel, and horse mackerel were 1.57, 4.79, 1.62, and 1.86 mg/g, respectively. The POVs of the fish oil from the above species were 1.28, 9.66, 8.39, and 9.23 meq/kg, respectively. POV is indicative of the onset of auto-oxidation. The AV and POV results were considered typical for crude oil extracts. Zuta et al. (15) reported that the AVs and POVs of fish oil from mackerel by-products were 4～5 mg/g and 3.3～3.6 meq/kg, respectively. Chantachum et al. (16) reported that oils prepared from precooked tuna heads had extremely high POVs (>200 meq/kg), as compared to the POV of approximately 25 meq/kg in oil from non-precooked samples. There was marked variation in these indicators between the different batches of fish, primarily as a result of the non-homogeneous nature of the fish processing waste, arising from the fact that the tissues were generated from different batches of fish and catches from different geographic locations with varying physiological states (15). Thus, it is important to choose suitable materials for producing PUFA, emphasizing the need for preliminary screening to assess for batches with suitable baseline characteristics. In this work, the AV of the mackerel oil was slightly lower than that reported by Zuta et al. (15). For the effective utilization of fish processing by-products, freezing by-products is beneficial in order to retard fish oil oxidation.

Composition of lipid classes

The neutral lipid, glycolipid, and phospholipid contents of the extracted fish oils of the by-products are shown in Table 3. The total lipids of the hoki, yellowfin sole, mackerel, and horse mackerel were 6.21, 10.43, 12.81, and 10.06%, respectively. Based on the total lipids, the neutral lipid contents were 77.38, 77.46, 87.21, and 86.79%, respectively. Glycolipid and phospholipid contents of the hoki, yellowfin sole, mackerel and, horse mackerel fish oils were 9.12, 9.45, 8.93, and 9.29%, and 7.41, 6.07, 3.91, and 3.48%, respectively. The glycolipid and phospholipid contents of the hoki and yellowfin sole fish oils were greater than those of mackerel and horse mackerel. Copeman et al. (17) reported that the phospholipid contents of cod flesh oil and cod liver oil were 54.9% and 12.3%, respectively. Passi et al. (18) reported that sardine oil contained 40.8% triacylglycerol and 18.4% phospholipid. Shrimp contained 1.8% to 2.6% lipid, and their oil was dominated by triacylglycerols at 47.8%, and also contained 17.8% phospholipid (18).

The qualitative composition of the neutral lipid fraction by TLC is shown in Fig. 1. Triacylglycerol was the major component in all the samples; 1,3- and 1,2-diacylglycerols, free fatty acids, free sterols, and sterol esters were the minor components. Lean fish store about 50～80% of their lipid as triacylglycerols in the liver, and this fat is a good source of lipid soluble vitamins (19). On the other hand, Okland et al. (20) reported that phospholipid was dominant in fish oil from the muscle of deep-sea fish such as roughhead grenadier, mora, portuguese dogfish, black dogfish, and leafscale gulper

Table 3. Lipid class compositions of fish oil extracted from various fish by-products (%)

	Hoki	Yellowfin sole	Mackerel	Horse mackerel
Neutral lipid[1)]	77.38±4.14	77.46±3.25	87.21±6.50	86.79±5.21
Glycolipid[1)]	9.12±0.42	9.45±0.55	8.93±0.43	9.29±0.14
Phospholipid[1)]	7.41±0.30	6.07±0.20	3.91±0.12	3.48±0.15
Total lipid[2)]	6.21±0.52	10.43±0.25	12.81±0.34	10.06±0.39

[1)]Percentage of lipid weight to total lipid. [2)]Percentage of lipid weight to weight of inedible parts.
Values are means±standard deviations of triplicate determinations.

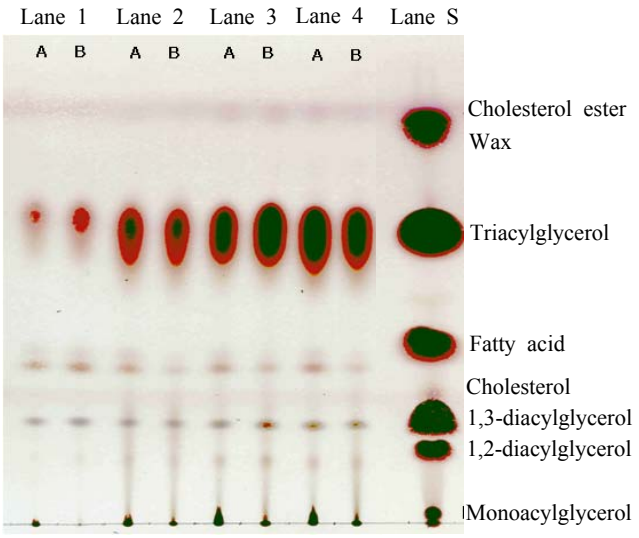

Fig. 1. Lipid composition of fish oil extracted from 4 species of fish by-products by TLC. Lane A: lipid before purification with silica column, Lane B: lipid after purification. Lane 1: hoki, Lane 2: yellowfin sole, Lane 3: mackerel, Lane 4: horse mackerel, Lane S: standard mixtures.

shark; and varied between 65 and 94% of the total lipid, while triacylglycerols were between 0.88 and 5.48% of the total lipid. Remme et al. (21) further reported that triacylglycerols and phospholipids were the two dominant lipid fractions of fish oil prepared from 5 different deep-sea fish eggs, varying from 35.6 to 55.4%, and 34.0 to 41.4%, respectively. The compositions of fish oils extracted from various fish are influenced by species, season, geographical regions, age, and maturity (22). In this work, the lipid classes of the fish oils that were extracted from the 4 species tested were considered typical.

Fatty acid compositions

The fish oil fatty acid compositions for the hoki, yellowfin sole, mackerel, and horse mackerel are shown in Table 4. The results demonstrate that significant portions of their fatty acids were unsaturated, as the lipid fractions contained 39.0～52.8% unsaturated fatty acids. Unsaturated fatty acid content was greater in the hoki, mackerel, and horse mackerel than saturated fatty acid content, but saturated fatty acid was higher in yellowfin sole. The saturated fatty acid contents ranged from 44.0 to 58.2%. The fatty acid composition of hoki was dominated by the saturated fatty acid, palmitic acid C16:0 (22.3%), the monosaturated fatty acid oleic acid C18:1 (18.9%), and saturated fatty acid stearic acid 18:0 (12.2%). The major fatty acids of yellowfin sole were palmitic acid C16:0 (33.8%) myristic acid 14:0 (20.9%), and the monounsaturated fatty acid, palmitoleic acid C16:1 (15.2%). The predominant fatty acids in mackerel

Table 4. Fatty acid compositions of fish oils extracted from various fish by-products (Area %)

Fatty acid	White muscle		Dark muscle	
	Hoki	Yellowfin sole	Mackerel	Horse mackerel
Saturated fatty acid				
C12:0	0.5	0.7	0.8	0.6
C13:0	0.2	0.2	1.3	0.3
C14:0	8.5	20.9	6.7	7.7
C15:0	–	–	3.2	3.5
C16:0	22.3	33.8	17.2	18.4
C18:0	12.2	2.6	17.5	16.9
C20:0	0.4	–	1.4	0.6
C24:0	–	–	0.2	0.2
∑SAFA	44.0	58.2	48.3	48.0
Monounsaturated fatty acid				
C14:1n-5	0.2	0.1	0.2	0.1
C15:1	–	0.3	0.3	0.2
C16:1n-7	6.4	15.2	4.2	5.4
C17:1n-9	1.7	0.8	1.7	1.3
C18:1n-9	18.9	14.2	14.9	14.9
C20:1n-9	10.5	1.7	2.9	1.2
∑MUFA	37.6	32.4	24.2	23.1
Polyunsaturated fatty acid				
C18:2n-6	2.5	0.8	2.6	3.4
C18:3n-6	0.8	0.3	2.3	3.5
C18:3n-3	1.0	1.2	2.6	4.8
C20:2n-6	0.3	0.1	0.7	0.3
C20:3n-6	0.2	–	0.5	0.2
C20:3n-3	1.2	–	0.4	0.3
C20:4n-6	1.2	0.3	5.4	1.8
C20:5n-3	3.0	0.2	4.7	4.1
C22:6n-3	5.0	3.7	8.9	9.5
∑PUFA	15.2	6.6	28.1	27.9

and horse mackerel included the major saturated fatty acids palmitic acid C16:0, stearic acid 18:0 and the monosaturated fatty acid oleic acid C18:1 at 17.2%, 17.5%, 14.9%, and 18.4%, 16.9%, 14.9%, respectively. The major saturated fatty acid of the 4 fish species was palmitic acid C16:0. Zuta et al. (15) reported that palmitic acid was a dominant fatty acid (15.3～19.2%) in the muscle, viscera, and skin of mackerel. Chantachum et al. (16) also reported that palmitic acid (30.3%) was the highest fatty acid in fish oil extracted from tuna heads.

PUFAs are considered to be of major importance in terms of human health. The PUFA contents ranged from 6.6 to 28.1%, with docosahexaenoic acid (22:6n-3, 3.7～9.5%) being dominant. The EPA (20:5n-3) contents were in the range of 3.0～4.7%, except for yellowfin sole (0.2%). In particular, the DHA and EPA contents of the dark muscle fish, mackerel and horse mackerel, were greater than those of the white muscle fish, hoki and yellowfin sole.

The PUFA and essential fatty acid (EFA) contents of the dark muscle fish, mackerel and horse mackerel, were

greater than those of the white muscle fish, hoki and yellowfin sole; and they contained low amounts of monounsaturated fatty acids. A similar trend was observed for mackerel and catfish wastes in an earlier study (23).

The reported PUFA content of tropical freshwater fish is between 5.8 and 29.8% (24). Others have also shown that freshwater fish have less PUFA than tropical saltwater fish (25). This difference can be attributed to the fact that freshwater fish feed largely on vegetation and plant materials, whereas marine fish feed mainly on microalgae, microorganism, and zooplankton, which are rich in PUFAs. Cod liver oil is an excellent source of several types of PUFA, and it is widely used for the isolation and purification of PUFA (26). The purification process of PUFA is necessary to remove lipophilic contaminants, including heavy metals and pesticides, and these may be found at elevated levels in the lipid of fish processing by-products. Still, the oil from by-products can be recovered and converted into edible oil. Based on this study, mackerel and horse mackerel processing by-products would be better sources of marine PUFA than either hoki or yellowfin sole.

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REFERENCES

1. Garg ML, Bosscheietor BB, Coulander CDL. 1985. The inverse relation between fish composition and 20-year mortality from coronary heart disease. *New Engl J Med* 312: 1205-1209.
2. Maruyama K, Nishikawa M. 1995. Physiological function of a fish oil component and its application to foods. *Food Chemicals* 4: 31-37.
3. Barakat SMM, Kawai Y, Yamazaki K, Miyashita K, Suzuki T. 2007. Effect of treatment with electrolyzed NaCl solutions and essential oil compounds on the proximate composition, amino acid and fatty acid composition of carp fillets. *Food Chem* 101: 1492-1498.
4. Horrocks LA, Yeo YK. 1999. Health benefits of docosahexaenoic acid. *Pharmaceutical Research* 40: 211-225.
5. Leaf A, Kang JX, Xiao YF, Billman GE, Voskuyl RA. 1999. The antiarrhythmic and anticonvulsant effects of dietary n-3 fatty acids. *J Membrane Biology* 172: 1-11.
6. Zlatanos S, Laskarids K. 2007. Seasonal variation in the fatty acid composition of three Mediterranean fish-sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). *Food Chem* 103: 725-728.
7. Huynh MD, Kitts DD, Hu C, Trites AW. 2007. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*. *Comp Biochem Physiol B Biochem Mol Biol* 146: 504-511.
8. Toppe J, Albrektsen S, Hope B, Aksnes A. 2007. Chemical composition, mineral content and amino acid and lipid profiles in bones from various fish species. *Comp Biochem Physiol B Biochem Mol Biol* 146: 395-401.
9. Robin JH, Regost C, Arzel J, Kaushik SJ. 2003. Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis. *Aquaculture* 225: 283-293.
10. Babbit KJ. 1990. Intrinsic quality and species of North Pacific fish. In *Making Profits Out of Seafood Wastes.* Keller S, ed. Proceedings of International Conference on Fish By-Products, Anchorage, AK, April 25-27, University of Alaska Sea Grant, Fairbanks. p 39-43.
11. AOAC. 1990. *Official Methods of Analysis.* 14th ed. Association of Official Analytical Chemists, Arlington, VA.
12. Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37: 911-917.
13. AOCS. 1999. *Official Methods and Recommended Practices of the American Oil Chemists' Society.* 5th ed. Firestone D, ed. American Oil Chemists' Society, Campaign, IL.
14. Rouser G, Kritchevsky D, Simon G, Nelson GJ. 1967. Quantitative analysis of brain and spinach leaf lipids employing silicic acid column chromatography and acetone for elution of glycolipids. *Lipids* 2: 37-42.
15. Zuta CP, Simpson BK, Chan HM, Phillips L. 2003. Concentrating PUFA from mackerel processing waste. *J Am Oil Chem Soc* 80: 933-936.
16. Chantachum S, Benjakul S, Sriwirat N. 2000. Separation and quality of fish oil from precooked and non-precooked tuna heads. *Food Chem* 69: 289-294.
17. Copeman L, Parrish CC. 2004. Lipids classes, fatty acids, and sterols in seafood from Gilbert Bay, southern Labrador. *J Agric Food Chem* 52: 4872-4881.
18. Passi S, Cataudella S, Marco PD, Simone FD, Rastrelli L. 2002. Fatty acid composition and antioxidant levels in muscle tissue of different Mediterranean marine species of fish and shellfish. *J Agric Food Chem* 50: 7314-7322.
19. Jacquot R. 1961. Organic constituents of fish and other aquatic animal foods. In *Fish as Food.* Borgstrom G, ed. Academic Press, New York. Vol 1, p 146-209.
20. Okland HMW, Stoknes IS, Remme JF, Kjerstad M, Synnes M. 2005. Proximate composition, fatty acid and lipid class composition of the muscle from deep-sea teleosts and elasmobranches. *Comp Biochem Physiol B Biochem Mol Biol* 140: 437-443.
21. Remme JF, Synnes M, Stoknes IS. 2005. Chemical characterisation of eggs from deep-sea sharks. *Comp Biochem Physiol B Biochem Mol Biol* 141: 140-146.
22. Piggott GM, Tucker BW. 1990. *Effects of Technology on Nutrition.* Marcel Dekker, NewYork.
23. Sathivel S, Prinyawiwakul W, Grimm CC, King JM, Lloyd S. 2002. Composition of crude oil recovered from catfish viscera. *J Am Oil Chem Soc* 79: 989-992.
24. Zenebe T, Ahlgren G, Boberg M. 1998. Fatty acid content of some freshwater fish of commercial importance from tropical lakes in the Ethiopian rift valley. *J Fish Biol* 53: 987-1005.
25. Vileg P, Body DB. 1988. Lipid contents and fatty acid composition of some New Zealand freshwater finfish and marine finfish, shellfish and roes. *New Zealand J Marine Freshwater Res* 22: 151-157.
26. Guerrero JLG, Belarbi EH. 2001. Purification process for cod liver oil polyunsaturated fatty acids. *J Am Oil Chem Soc* 78: 477-484.

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