Extraction and Characterization of Mackery (Scomber scombrus) Oil for Industrial Use

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Abstract: Fish oils have been recognized as good sources of polyunsaturated fatty acids (PUFA) which are widely used for pharmaceutical purposes and as food supplements. It has been reported that fishes are rich in arachidonic acid (AA) and docosahexaenoic acid (DHA). These fatty acids have been recommended as infant food supplements by health agencies. In this study fish oil from mackery (Scomber scombrus) was extracted using a mechanical screw press system. The total percentage oil yield using screw press extraction was 18.7 %. Characterization of the fish sample was done. From the results of the characterisation of the fish sample, moisture content was found to be 55.7%. The protein content and ash content were 15.6 and 1.5%. Carbohydrates composition estimated in the samples analysed was 0.4% and the lipid content was found to be 26.8% in this study. The analytical properties and microbiological status of the crude and the refined oil were evaluated. It was observed that the crude oil consist of: free fatty acids (FFA) value 2.3 %, peroxide 11.4 meq/kg, saponification value 187 mgKOH/g, iodine value 168 I_2 /100g, refractive index 1.485 and reddish brown colour. The refined oil was also evaluated as follows: free fatty acid value 0.28 %, peroxide 2.5 meq/kg, saponification value 132 mgKOH/g, iodine value 105 I_2 /100g and golden brown colour.The results of the microbiological analysis of fish oil showed that the total viable counts (TVC) was 2.0×10^1 CFU/mL of oil, the total coliforms count was 0.4×10^1 CFU/mL of oil and the faecal coliforms count was 0.25 CFU/mL of oil. These values fall within the acceptable standard values. The refining of the oil brought about a notable improvement in the analytical properties of the oil. Thus, leads to a high quality fish oil in terms of the taste, colour, odours, shelf life and market value. Based on the characteristics of the oil, it could be suitable for applications in pharmaceutical and food industries.
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Introduction

Mackerel are long bodies, rather thick appearing fish known as high sea fish. Fish is one of the most popular food items for human consumption throughout the world. Fish oil is the lipid fraction extracted from fish and fish by products. Fish oils have gained much more importance because of the presence of health beneficial omega-3 fatty acids in them. These polyunsaturated fatty acids (PUFA) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play a crucial role in the prevention of atherosclesis, heart attack, depression, stroke, diabetes, obesity, premature ageing, hypertension, cancer and improve the vision power and memory (Chin and Dart, 1995).

Scombroids (mackerel) and *clupeids* (herring) provide the largest single source of raw material for production of fish oil and fishmeal. They are regarded as fatty species, having fat content well distributed throughout the body. Generally, fish oils are more complex than land-animal oils or vegetable oils due to long – chain unsaturated fatty acids (Hall, 1992). Fish oil is considered as liquid oil, but, in fact contains triglycerides of intermediate melting point for the oils to be partially solid at 20°C. Fish oils are unique in the variety of fatty acids of which they composed and their degree of un-saturation (Ackman etal, 1982). Refined fish oils are rich in polyunsaturated fatty acids of the linolenic acid family. Current medical research suggests that these fatty acids might have a unique role to play in prevention of coronary artery disease and the growth of different types of cancers. The oil is industrially used in leather tanning, production of soap and glycerol, and other products. Presently, the production of fish oil is becoming more demanding, as there is a sizeable and growing world market demand for high quality fish oils. In other to meet, the demand of the society there is the need to locate new oil fish and further research to know their characteristics and usefulness.

The project is aimed at the mechanical extraction of the oil from mackerel (*scomber scombrus*) in relation with heating time of pre-treatment as the only optimising parameters, while keeping other parameters (e.g. particle sizes, temperature etc.) constant. It also refines the extracted oil and carries out analytical test of the oil to ascertain some of its physical and chemical properties. These include the appearance, refractive index, iodine value, saponification value, free fatty acid value, peroxide value.

Materials And Methods

Selection, Preparation and Pre-treatment of Test Materials

The fish (Atlantic mackery) were bought fresh from the market. Prior to analysis, the internal organs of the fish were removed and the fish was washed to remove the residual blood. The fish was cut into small pieces and was heated to 95-100 °C for approximately 10-20 minutes. This process coagulates the proteins and disrupts the cell membranes thus allowing leakage out of bound water and oil.

Characterisation of fish sample (Proximal analysis)

The proximate analysis of the samples was carried out according to the procedures of the Association of Official Analytical Chemists (AOAC, 2002). Crude protein content was determined by the Protein automatic method (with a nitrogen conversion factor of 6.25) using an automatic Kjeldahl system. Moisture was determined by using the air oven until a constant weight is reached (at 102 °C -105 °C for 12 h). Ash was determined by incineration in a muffle furnace at 550 °C for 4 h. Lipid content was determined according to the Bligh and Dyer method (Bligh and Dyer, 1959). The percentage of carbohydrate was calculated by simply subtracting the total percentage of protein, fat, moisture and ash from 100. The experiments were replicated three times.

Protein content (Protein automatic method): The method is a version of the original Kjeldahl method. About 5 g of minced fish sample was mixed with potassium sulphate (K_2SO_4) and a little of copper sulphate ($CuSO_4$) as a catalyst and digested in a long necked Kjeldhal bottles with concentrated sulphuric acid for approximately 2 hours (one hour after contents are clear). Distilled water was then added. The Kjeldahl bottle was placed in Kjeltec auto sampler 1035/30 system where the ammonia was distilled into boric acid and the acid was simultaneously titrated with diluted sulphuric acid (ISO,1979)[1]. A complete blank determination using only a piece of filter paper instead of the fish was carried through.

Nitrogen content is calculated by:

$$N(\%) = \frac{0.7(V_1 - V_0)}{M} \text{ (ISO,1979)}^1. \quad 1$$

Where;

V_1= Mean volume in ml of 0.1 M sulphuric required for fish,

V_0= Mean volume in ml of 0.1 M sulphuric required for blank,

M = Weight in grams of the portion taken of the sample.

Protein content is calculated as N x 6.25. Results are expressed as percentage of wet weight.

Water content: Water was determined by drying in an oven and measuring the weight loss. About 5 g of minced fish sample was weighed accurately in a clean and dry (pre weighed) metal dish, with a lid. The sample was heated in a heating oven at 102 °C -105 °C for 12 hours. Then the lid was placed on the dish which is cooled in a desiccator and weighed. Moisture loss corresponds to the weight loss (ISO,1979)[2].

Results were expressed as percentage of wet weight.

$$\%\ Moisture = \frac{W_1 - W_2}{W_1 - W_o} \times 100\ \% \quad 2$$

Where;

W_0 = Weight of cylinder,

W_1 = Weight of cylinder and fresh fish sample,

W_2 = Weight of cylinder and dried fish sample.

Lipid content: Total lipid content was determined using methanol/chloroform extraction (Bligh and Dyer, 1959). A representative sample of fish tissue (50g) was homogenized in a blender for 2 minutes with a mixture of methanol (100 ml) and chloroform (50 ml). Then 50 ml of chloroform was added to the mixture. After blending for an additional 30 seconds, distilled water (50 ml) was added. The homogenate was stirred with a glass rod and filtered through a Whatman no.1 filter paper on a Buchner funnel under vacuum suction. 20 ml chloroform was used to rinse the remainder. The filtrate was allowed to settle to separate into the organic and aqueous layers. The chloroform layer containing the lipids was transferred into another beaker and 3 g of anhydrous sodium sulphate was added to remove any remaining water. The mixture was filtered through a Whatman no. 1 filter paper and chloroform was used to rinse the remainder. Finally, a known amount of Butylated hydroxytoluene (BHT) of about 0.02 g was added to the lipid solution as an antioxidant (Kinsella et al, 1977). The solution was then evaporated to a constant weight in a tared 100 ml round-bottom flask with a rotary evaporator at 40 °C. Results were expressed as grams of lipid per kilogram of samples.

$$\text{Fat content (\%)} = \frac{\text{Final weight of flask contents in grams}}{\text{Weight of sample}} \times 100\ \% \quad 3$$

Mineral content: Minerals, the inorganic components of fish, often collectively called ash. Measurement of ash is sometimes a useful indicator of the amount of leaching of soluble constituents of fish resulting from contact with water or melting ice.

The method of the Analytical Methods Committee (AMC) of the Royal Society of Chemistry in 1979 in which the material is heated to a high temperature to drive off all water and volatile substances, and to decompose all organic matter would be used (Kinsella et al, 1977).

A platinum dish was heated in a furnace at 550-600°C for 20 minutes; removed and cooled in a

desiccator. The dish was weighed accurately and about 5 g of the prepared sample was added, spread out evenly, and then reweighed accurately. By pipette, exactly 1 ml of magnesium acetate solution (25 g of anhydrous magnesium acetate made up to 100 ml in water) was added to the dish, distributing it as evenly as possible over the material. The portion was Dried and charred by heating carefully, then heated at 550-600°C in the furnace for 3 hours. It was cooled in a desiccator and reweighed. The sample was reheated for 30 minutes, cooled and reweighed, and continued until successive weighing agreed. A blank determination was carried through regularly, using magnesium acetate solution only, and subtracted from the measured weight of ash.

$$Ash\ content\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ portion} \times 100 \quad 4$$

Carbohydrate Content: The percentage of carbohydrate was calculated by simply subtracting the total percentage of protein, fat, moisture and ash from 100. The following equation was used to determine the amount of carbohydrate:

$$\text{Carbohydrate } content\ (\%) = 100 - \%\ \text{of (Protein} + \text{Fat} + \text{Moisture} + \text{Ash)} \quad 5$$

Oil Extraction

The oil was extracted in hot (70º C approximately) with a screw press and clarification was done to separate the oil from its entrapped impurities. The fluid extracted out of the press was a mixture of fish oil, water, cell debris, and non-oily solids. The fluid was allowed to stand undisturbed to settle by gravity so that the oil, being lighter than water, will separate and rise to the top. The clear oil was decanted into a reception container, sieved and heated to remove moisture in the oil.

Characterisation of mackerel oils

Yield of oil extraction

The yield of recovered oil was calculated as:

$$Yield\ (\%) = \frac{Weight\ of\ extracted\ oil\ (g)}{Weight\ of\ oil\ in\ the\ initial\ sample\ (g)} \times 100 \quad 6$$

The weight of oil in the initial sample was determined using the oil content as

measured by the proximate analysis (Horwitz, 2000).

Physicochemical Properties of Fish Oil

The physicochemical properties of the oil are determined to assess its quality and purity. A number of physical and chemical 'constants' have been established for these purposes. These include free fatty acid content, saponification value, iodine value, peroxide value, anisidine value, viscosity and moisture content. Six oil quality indices would be used to determine the quality of the extracted oil. They are the colour, refractive index, free fatty acid content, saponification value, iodine value and peroxide value. The experiments were replicated three times.

Colour: The Colour of test oils were measured by AOCS (1992) official methods as absorbance of 50 % (v/v) solution of sample with n-hexane (1:1), then filtered through 0.45 \m Millipore membrane filters. The filtered samples were placed in vacuum dissector to remove n- hexane and the absorbance were measured at 420 nm with the help of Shimadzu UV-160 Spectrophotometer.

Refractive index: Abbey refractometer was used in determining the refractive index of the oil. The measuring prism surface was cleaned with solvent and distilled water, and then wiped with a clean towel after which the mode selector was regulated to the desired mode position. A drop of oil was dropped on the prism surface using a glass dropper and covered. The illumination arm was then positioned so that the exposed face of the upper prism will be fully illuminated. The refractometer was used through the eyepiece, the dark position viewed was adjusted to be in line with the cross line. At no parallax error, the pointer to the scale pointed in the refractive index, the reading was then taken. This measurement represents the refractive index of the oil sample.

Free fatty acids (FFA): Free fatty acids (FFA) value was determined according to the method describe in AOCS method (AOCS, 1992). An amount of 5 g oil sample was mixed with 75 ml of 95 % neutral ethyl alcohol and swirled. Phenolphthalein was added as indicator. The solution was titrated with 0.1 N sodium hydroxide until pinkish colour was observed at end point. FFA concentration in fats and oils is calculated as percentage oleic acid. The expression as given in AOCS Official Method by AOCS, (1992) as:

$$\%\text{FFA as oleic acid} = \frac{\text{alkali volume (mL)} \times \text{alkali normality} \times 28.2}{Sample\ weight\ (g)} \quad 7$$

Saponification Value (Sv): The saponification value (SV) of the fish oil was determined following procedures described in AOCS method (AOCS, 1992). Oil sample (1 g) was dissolved in 12.5 ml of 0.5 N ethanolic potassium hydroxide. The mixture was refluxed for 30 minutes until oil droplets disappear and was left to cool to room temperature. Phenolphthalein indicator was then added and the hot soap solution was titrated with 0.5 N HCl until the pink colour disappears. A blank titration was also carried out in the same manner except that no oil was added. Saponification value was calculated using the formula:

$$Saponification\ value\ (SV) = \frac{56.1\ (a-b) \times N}{W} \quad 8$$

Where;

a = Volume (ml) of 0.5 mol/l hydrochloric acid consumed in the blank test,

b = Volume (ml) of 0.5 mol/l hydrochloric acid consumed in the test,

N = Normality of hydrochloric acid,

W = Weight of oil sample, g.

Iodine Value (IV): About 0.1g sample was delivered to a 300 mL conical flask with ground-in stopper and mixed with 20.0mL carbon tetrachloride and sealed. It was dissolved in an ultrasonic washing machine. 25.0mL Hanus solution was added and sealed. It was shook for one minute, Kept sealed and left in a dark room (about 20°C) for 30 minutes. 10.0mL of 15% potassium iodide and 100 mL water were added, sealed and Shook for 30 seconds. The mixture was titrated with 0.1mol/L sodium thiosulfate to obtain iodine value. Likewise, blank test was performed to obtain blank level (AOCS, 1992).

$$Iodine\ value\ (IV) = \frac{127\ (a-b)\times N}{10\ W} \quad 9$$

Where;

a =Volume (ml) of 0.1 mol/l sodium thiosulfate consumed in the blank

test,

b = Volume (ml) of 0.5 mol/l sodium thiosulfate consumed in the test,

N = Normality of sodium thiosulfate,

W = Weight of sample.

Iodine Number : Number of iodine (g) absorbed by 100 g of oil.

Peroxide values (pv): The Peroxide values (PV) of fish oil was determined according to AOCS method (AOCS, 1992). Oil sample (5 g) was weighed into a 200 ml conical flask and mixed with 300 ml of glacial acetic acid and chloroform (3:1) and mixed thoroughly by swirling the flask. Saturated potassium iodide (0.5 ml) was then added and the mixture was left in the dark for 1 minute with occasional swirling, followed with further addition of 30 ml distilled water. The mixture was titrated with 0.1 N sodium thiosulphate solution with 1 ml of 1.0 % soluble starch as indicator until the blue colour disappears. A blank sample titration was also carried out in the same manner but with no oil added.

$$Peroxide\ value = \frac{(a-b)\times 10}{Weight\ (g) of\ the\ sample} \quad 10$$

Where; a =Volume (ml) of 0.1 mol/l sodium thiosulfate consumed in the blank

test,

b = Volume (ml) of 0.1 mol/l sodium thiosulfate consumed in the test.

Microbiological Analysis of the Fish Oil

Total Viable Count (TVC) Using the pour-plate method

Iron agar (IA) containing sodium thiosulfate and L-cysteine was used for determination of total psychrotrophic bacteria and H_2S – producing organisms. Bacteria able to produce H_2S form black colonies when S^{-2} reacts with Fe^{+2} in the medium (FeS). The samples were fish oil, 1 mL of oil sample was first dissolved in 9 mL of distilled water resulting in 1/10 dilution and diluted further using three tubes with 9ml buffer from 1/100, 1/1000 and 1/10000 dilution. One plate was used for each dilution. Of each dilution, used 0.1 ml was transferred with pipettes to the plates. Melted 45°C iron agar was poured on the plate and the content was mixed and allowed to solidify. After solidification the plates were covered with overlay of iron agar and incubated at 37°C for 24-48 hours. Count was done using hand lens. The colony forming unit (CFU/mL) was calculated according to Vanderzant and Splittstoesser, (1992) from the equation;

$$Cfu = \frac{N}{VD} \quad 11$$

Where; N = number of colonies, V= volume of diluents, D = dilution factor.

Total coliforms and Faecal Coliforms counts

Faecal coliforms ferment lactose and produce acid and gas. Lauryl sulphate tryptose (LST) broth was used as a pre-enrichment media. Brilliant green lactose bile (BGLB) broth was used for total coliforms and Escherichia coli (EC) broth for faecal coliforms. All media contained lactose. Three tubes of LST media used for each dilution, and each dilution was transferred with pipettes to tubes, for first three tubes using 10 ml from 1/10 dilution and then from other six tubes using 1ml from 1/100 and 1/1000 dilution and were incubated at 35°C for 48 hours. After primary incubation one loopful of positive tubes (gas formation tubes) were transferred to BGLB media for total coliforms (incubation at 35°C for 48 hours) and EC broth for faecal coliforms (incubated at 44.5°C for 24 hours). Results were expressed as CFU/mL (colony forming units/mL).

Results And Discussions

Characterization of the Fish Sample

Results of the characterization of the fish samples are presented in Table 1. Proximate composition included moisture, lipid, protein, ash and carbohydrate contents. From Table 1, the moisture content was found to be 55.7 %. The protein content and the ash content were 15.6 and 1.5 %. Carbo-hydrates composition estimated in the samples analysed was 0.4 % and the lipid content was found to be 26.8 % in this study. According to Ackman (1989), fish can be grouped into four categories according to their fat content as lean fish (<2%), low (2-4%), medium (4-8%) and high fat (>8%). In terms of the lipid content, fish species examined can be considered to be in the high fat fish category.

Table 1: Characterisation of Fish Sample

Parameter		Experimental Value
Water content		55.7 %
Protein content		15.6 %
Oil content		26.8 %
Ash content		1.5 %
Carbohydrates		0.4 %

Characterisation of mackerel oils

Figure 1 is the photograph of the extracted fish oil in plastic bottles and Table 2 shows the Characterisation of mackerel oils. The refractive index of the oil was found to be 1.485 which falls between the standard values .The appearance of the oil was reddish brown due to the prolonged heating period method, which often oxidizes the product (i.e. the oil) and produces a reddish colour (Hall, 1992). The cake produced was of good palatable quality with good appearance and rancid free flavour and therefore suitable for feed formulation for livestock.

In order to determine the stability and quality of fish oil extracts, some quality assessment was conducted. It was observed that all the results obtained were tolerable to the standard values. These results are shown in Table 2. The level of FFA gives an indication of the oxidation state of the oil. FFA are very susceptible to oxidation leading to the deterioration of the taste of the oil. The value of free fatty acids (FFA) in extracted fish oil was found to be 2.3 %, which is within the standard value of 2 % to 5 % for fish oil. The iodine value was found to be 168 I_2/100g which is within the standard value of between 160 to 190 I_2 /100g of the sample. Bimbo (1998) has reported that peroxide value (PV) of crude fish oil was between 3 and 20 meq/kg. In this study, the PV was found to be 11.4 meq/kg, which is well below acceptable limit of 20 meq O2/kg oil. This indicated that the fish oil extracted had low lipid oxidation rate. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by alkali treatment. The SV of fish oil obtained in this study was within (187 mg KOH/g) the standard value range for fish oil (165-195 mg KOH/g), given by Bimbo (1998).

The free fatty acid value of the refined oil was found to be 0.28 %, which shows a considerable reduction from that of crude oil. The peroxide value of the refined oil was also found to be 2.50 mEq/kg. These reductions in acid and peroxide value imply that there is an improvement in the quality of the oil, as it reduces the susceptibility of the oil to rancidity and improve stability. The saponification value of the oil was reduced to 132 mgKOH/g in the refined oil. This reduction in the saponification value equals the calorific and weight loss by the oil. The Iodine value of the refined oil was found to be 105 I_2/100g of the sample, which implies that few of the double bonds in the oil has been saturated, hence, giving the oil wider applications. In addition, the colour of the oil was observed to change from reddish brown to golden brown, which indicated the significance of the percentage pigments removed. Hence, the refining procedures contributed to the physical improvement of the refined oil.

According to Middlebrooks et al (1988), mackerel stored at 0, 15, and 30°C have counts of about 10^6 CFU/g of flesh. The results of the microbiological analysis of fish oil showed that the total viable count was 2.0×10^1 CFU/mL of oil, total coliforms count was 0.4×10^1 CFU/mL of oil and the faecal coliforms count was 0.25 CFU/mL of oil. The reduction in the coliforms counts is as a result the use of heat in the oil extraction process.

Fig. 1: Extracted Fish Oil in Plastic Bottles

Table 2: Characterisation of Mackerel Oils

Parameter	Unit	Test Value	
		Crude Oil	Refined Oil
Yield of oil extraction	%	18.7	
Physicochemical properties			
Colour		Reddish Brown	Golden brown
Refractive Index		1.485	-
Free Fatty Acid Value	%	2.3	0.28
Saponification Value	mgKOH/g	187	132
Iodine Value	Iodine/100g	168	105
Peroxide Value	$mEqO_2/kg$	11.4	2.5
Microbiological Analysis			
Total Viable Count (TVC)	CFU/mL	-	2.0×10^1
Total Coliforms Count	CFU/mL	-	0.4×10^1
Faecal Coliforms Count	CFU/mL	-	0.25

Conclusion

The characterization of fish which was carried out had moisture content, protein content, ash content, carbohydrate content and lipid content with mean values of 55.7 %, 15.6, 1.5 %, 0.4 % and 26.8 % respectively. The total percentage oil yield from mackerel (*scomber scrombrus*) using mechanical screw press extraction was 18.7 %. The oil was evaluated and some of its physical, chemicals and microbiological properties were determined. All the analytical results obtained were tolerable to the standard values. The refining of the oil brought about great improvement in the analytical properties of the oil. These properties were in conformity with standard values. All these resulted in improving the quality of the fish oil in terms of the taste, colour, odours, shelf life and market value. Based on the characteristics of the oil, it could be suitable for applications in pharmaceutical and food industries.

References

1. Ackman, R.G. (1989). Nutritional composition of fats in sea foods. Prog. Food Nutr. Sci, 13: 161-289.
2. Ackman R. G., Barlow S. M., and Stansby M. E. (1982). *Fatty acid composition of fish oils, Nutritional evaluation of long-chain fatty acids in fish oil*, Academic Press, Pp. 25-80.
3. AOAC (2002). Official methods of analysis of the Association of Official Analytical Chemists (14th ed.), Arlington.
4. AOCS (1992). Official methods and recommended practices of the American Oil Chemists Society (4th ed.), Champaign, American Oil Chemists' Society, USA.
5. Bimbo, A.P. (1998): Guidelines for characterizing food-grade fish oil. *Inform*, 9, 473-483.
6. Bligh, E.G. and Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911-917.
7. Chin, J. P. F. and A.M. Dart (1995). How does fish oil affect vascular function clinic. Experi. Pharmac., 22:71-81.
8. Hall G. M. (1992). *Fish Process Technology*, Food Engineering and Biotechnology Group, University of Technology, Loughborough, p. 4-7, 172-181.
9. Horwitz, W. (2000). Official Methods of Analysis of Association of Official Analytical Chemist (AOAC) International. AOAC International, Maryland-Gaithersburg, USA.
10. International Standard Organization, ISO, (1979)[1]. Animal feeding stuff - determination of nitrogen content and calculation of crude protein, 1st edition, Switzerland. pp. 1-4.
11. International Standard Organization, ISO, (1979)[2]. Animal feeding stuff- determination of moisture content, 1st edition, Switzerland. pp. 1-3.
12. Kinsella, J.E., Shimp, J.L., Mai J. and Weihrauch, J., (1977). Fatty Acid Content and Composition of Freshwater Finfish, Journal of American Oil Chemist Society, 54: 424-429.
13. Middlebrooks, B. L.; Toom, P. M.; Douglas, W. L.; Harrison, R. E.; McDowell, S. (1988). Effects of Storage Time and Temperature on the Microflora and Amine Development in Spanish Mackerel(Scomberomorusmaculatus).J.Food Sci. 53,1024-1029.
14. Vanderzant, C. and Splittstoesser, D. (1992). Compendium of Methods for the microbiological examination of Foods, pp: 153 – 156. American Public Health Association.

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