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Research Article

ANALYSIS OF ALPHA-LINOLENIC ACID AND DOCOSAHEXAENOIC ACID IN MACKEREL FISH OIL (*RASTRELLIGER KANAGURTA*) USING GAS CHROMATOGRAPHY

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ABSTRACT

Objective: This study aimed to obtain the levels of alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA) in mackerel fish oil by pressing and extraction with solvent methods.

Methods: The optimum conditions were determined, and validation methods were performed for a mixture of ALA and DHA to obtain a valid method for the determination of the levels of ALA and DHA in mackerel fish oil. Derivatization was performed by the Lepage esterification method using methanol:toluene 4:1 (v/v) and an acetyl chloride catalyst. Gas chromatography with the Shimadzu GC-17A with a DB-5 column and flame ionization detector was used to analyze samples at a column temperature of 200°C with an increase of 2°C/min up to 230°C (maintained for 20 min). Injector and detector temperatures of 250°C were used with a flow rate of 1.00 mL/min.

Results: The retention time of ALA and DHA was 11.440 min and 22.337 min with a T_f of 0.949 and 1.006, respectively. The validation results fulfilled the acceptance criteria with r values of 0.99953 and 0.99934, respectively. Total levels of ALA and DHA in mackerel fish oil were 0.39521% by pressing and 0.33014% by extraction with solvents.

Conclusion: This method could be used as an alternative method to analyze ALA and DHA level in fish oil.

Keywords: Alpha-linolenic acid, Docosahexaenoic acid, Extraction with solvents, Gas chromatography, Optimization, Pressing, Validation.

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INTRODUCTION

Indonesia has the potential for sea fishery development with 6.5 million tons per year of marine fish production, but the utilization of marine fish is still limited to only food. Research is needed to improve the economic value of marine fish by producing fish products other than food, including fish oil [1-4]. Fish oil, which is rich in omega-3, has become one of the many nutraceutical items used by people worldwide [5].

Omega-3 is a polyunsaturated fatty acid that has many double bonds. Omega-3 is an essential fatty acid that cannot be formed by the body and must be supplied directly from the diet. The omega-3 precursor is included in the process of elongation and desaturation, resulting in three forms of omega-3 fatty acids, including alpha-linolenic acid (ALA) (C18: 3, n-3), eicosapentaenoic acid (C20: 5, n-3), and docosahexaenoic acid (DHA) (C22: 6, n-3) [3]. Omega-3 is useful for reducing the risk of heart disease and arteriosclerosis, preventing mental retardation, lowering cholesterol, and maintaining eye health as well as for brain development [6,7].

According to the Ministry of Health of Indonesia, omega-3 can be found in tuna, salmon, mackerel, pomfret, flying fish, and lemuru. The highest omega-3 content is found in salmon, which is imported at high prices; however, omega-3 fatty acids can also be found in local fish species, namely mackerel (*Rastrelliger kanagurta*), which can be easily obtained at a more affordable price and is the target of traditional fishermen in Java, Indonesia [8-10].

Various methods of fish oil extraction are used, such as boiling with water first and then pressing or extracting using a solvent. The crude oil obtained is then subjected to the hydrolysis of fatty acids to purify fatty acids from free fatty acids, phosphatides, metal ions, dyestuffs, protein carbohydrates, oxidation byproducts, and solids [11]. Fatty acids can be detected using gas chromatography and high-performance liquid chromatography. Both methods use the derivatization of fatty acids turned into their ester form.

In this study, we boiled a sample of mackerel fish and pressed and extracted oil using a solvent. The resulting fish oil was then hydrolyzed by addition of an aqueous alkaline solution and concentrated HCl, which aims to form a fatty acid. Analysis of ALA and DHA was performed by gas chromatography using fatty acid derivatization and turned into an ester form.

MATERIALS AND METHODS

Equipment

Shimadzu GC-17A gas chromatography equipped with a flame ionization detector, capillary column 30 m×0.32 mm, 0.25-µm film thickness with DB-5 stationary phase, helium carrier gas; Class GC Solution data processor; 5-µl Microsyringe (Hamilton Co., Nevada); Centrifugator (NF 400R); Vortex (Thermo Scientific); Heat resistant reaction tubes with Teflon (Iwaki Pyrex); Glass blender; Vacuum pump; Stainless steel pan; Pressing tools; Whatman No.1 filter paper; Splitter; Buchner funnel; Rotary evaporator (IKA® Lab.); Analytical balance; Hotplate (IKA® C-MAG HS 7); Wardrobe; Freezer; and Micropipettes and glass tools are commonly used in quantitative analysis.

Materials

Fresh mackerel fish (Place of Muara Baru Fish Auction, North Jakarta); DHA oil (Huatai Biopharm, Inc.); Flaxseed oil (Jilin Baili Biotech., Co., Ltd.); Methanol p.a (Merck); Toluene p.a (Merck); Acetyl chloride p.a (Merck); Chloroform p.a (Merck); Ethanol 96% p.a (Merck); Hexane p.a (Merck); Potassium carbonate (Merck); NaOH (Sigma-Aldrich); Concentrated HCl (Merck); Nitrogen (UHP and HP); and helium (UHP) were used.

Working process

Preparation of potassium carbonate solution 6%

Potassium carbonate (6%) was prepared by dissolving 6 g of K_2CO_3 in Aqua Dest water to obtain a final volume of 100 mL.

Preparation of main solution DHA oil and flaxseed oil

DHA oil (242.72 mg) and flaxseed oil (199.72 mg) (equivalent to 100 mg DHA and 100 mg ALA) were each dissolved in hexane to obtain 100-mL standard solutions of DHA and ALA with concentrations of 1000 ppm each.

Lepage esterification method

Abaout 2.0 mL of solution was put into a Teflon-covered reaction tube and drained using nitrogen gas. Then, 0.40 mL of toluene and 1.6 mL of methanol were added followed by vortexing. Of note, 0.2 mL of acetyl chloride was slowly added to the test tube while shaking. The tube was tightly closed and then heated in the oven at 100°C for 60 min. The tube was cooled in water, and then 5.0 mL of 6% potassium carbonate solution was slowly added. The mixture was vortexed and centrifugated at 3000 rpm for 5 min. The toluene layer containing methyl esters was used for the analysis.

Determination of optimum analysis conditions

About 1.0 mL of flaxseed oil and DHA oil (1000 ppm) were each put into a 10.0-mL measuring flask. Hexane was added to each to obtain a 10-mL solution with a 100 ppm concentration. 1.0 mL of each solution was esterified using the Lepage method. The upper layer containing a methyl ester mixture of ALA and DHA (100 ppm) was injected in gas chromatography with an injection volume of 1 µL.

Optimum analysis conditions were determined with temperature programming with an initial temperature variation column at 160°C, 180°C, and 200°C and flow rate variations of 0.8, 1.0, and 1.2 mL/min. The starting temperature was raised 2°C/min to 230°C (maintained for 20 min), the injector temperature was 250°C, and the detector temperature was 250°C. The results were selected with relatively short retention times (tR), the largest number of theoretical (N) plates, the smallest height equivalent to theoretical plate (HETP), the smallest follow-up factor (Tf), and the best separation with a resolution value of 1.5 or more.

Test system compatibility

About 1.0 mL of flaxseed oil and 1.0 mL of DHA oil (1000 ppm) were each added to 10.0-mL measuring flasks. Hexane was added to each to obtain a 10-mL solution with a 100 ppm concentration. 1.0 mL of each solution was esterified using the Lepage method. The upper layer containing a methyl ester mixture of ALA and DHA (100 ppm) was injected in gas chromatography with an injection volume of 1 µL under selected analysis conditions. Injection was performed in 6 times in a row, then tR, number of theoretical plate (N), precision coefficient of variation (CV), HETP, and Tf were recorded.

Validation methods

Linearity test, calculation of limit of detection (LOD), and limit of quantitation (LOQ)

About 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mL of flaxseed oil and DHA oil (1000 ppm) were put into seven 10.0-mL measuring flasks, allowing the volume of each to be measured by the measuring flask using hexane p.a, to obtain standard solutions at 40, 60, 80, 100, 120, 140, and 160 ppm. In total, 1.0 mL of each solution was esterified using the Lepage method. The upper layer containing a methyl ester mixture of ALA and DHA was injected into gas chromatography with an injection volume of 1 µL under selected analysis conditions. The peak area was recorded and used to calculate the linear regression equation and the correlation coefficient. From the calibration curve, the LOD and quantity limits were calculated.

Test accuracy and precision

A simulation method was performed, which was made of blanco sample (fish pellet) containing a standard amount of ALA and DHA standard (80%, 100%, and 120%) to obtain a solution with a certain concentration. In total, 1.0 mL of each solution was esterified using the Lepage method. The upper layer containing a methyl ester mixture of ALA and DHA was injected in gas chromatography with an injection volume of 1 µL at the selected analysis conditions 6 consecutive times. The peak area was recorded. Recovery (UPK) was calculated to test accuracy. The standard deviation (SD) and relative SD or CV were calculated to determine precision. The solution was said to meet the accuracy test if the percentage of UPK was in the range 98%–102% and meet the precision test if the relative SD value or the CV was not >2.0%.

Oil extraction from mackerel through pressing

The scales and any dirt were removed from the fresh mackerel fish. A total of 100 g of fish samples were boiled until cooked with 500 mL of water in a double-dip pan. The ripe fish was separated from the boiling water. The rest of the boiling liquid was decanted to see layers of oil and water. The ripe fish was then pressed to separate between the oil and the meat pulp, and then the oil and water layers were separated using a separating funnel. Oil from the pressing and oil from the remaining boiling water were combined.

Extraction of oil from mackerel using a solvent

The scales and any dirt were removed from the fresh mackerel fish. A total of 100 g of fish samples were boiled until cooked with 500 mL of water in a double-dip pan. The ripe fish was separated from the boiling water. The rest of the boiling liquid was decanted to see layers of oil and water. The fish oil that was filtrated from the boiling water was combined with the mature fish and then homogenized in a blender for 2 min with a mixture of 200 mL of methanol and 100 mL of chloroform. Of note, 100 mL of chloroform was added to the mixture, and after blending for 30 s, 100 mL of water was added and blended for 30 s. The homogenate was stirred with a glass rod and filtered with Whatman No.1 filter paper using a Buchner funnel under a vacuum pump. A total of 20 mL of chloroform was used to rinse any remaining material. The filtrate layer was rested to separate between the chloroform and methanol layers. The oil-containing chloroform layer was then evaporated using a rotary evaporator at 40°C.

Fatty acid hydrolysis

The fish oil obtained from pressing and extraction using the solvent was then hydrolyzed in as much as 1.1 g of fish oil produced mixed with 20 mL of NaOH solution in dilute alcohol (5 g of NaOH dissolved in 10 mL of Aqua Dest and 50 mL of 96% ethanol) while heated and stirred constantly at 50°C for 30 min. Then, 50 mL of hexane was added. The liquid fraction containing the fatty acid that was saponified was separated and concentrated HCl was added until the pH of the solution reached 1. The fraction containing free fatty acid was then added to 30 mL hexane, and then the fraction of free fatty acid that was dissolved in hexane was separated. Then, the mixture was evaporated with a rotary evaporator at 40°C.

Qualitative and quantitative analysis of ALA and DHA in fish oil samples

Fatty acid samples obtained from fish oil were weighed, and then dissolved in hexane p.a to obtain a 100-ppm concentration, and then esterificated using the Lepage method. The toluene layer containing methyl esters of fatty acids was then injected in gas chromatography with an injection volume of 1 µL under the selected analysis conditions 3 times in a row. The obtained chromatogram was used for:

a. Qualitative analysis, that is, the tR obtained was recorded and compared to the standard tR. The comparison data obtained were used as the basis for the identification of ALA and DHA used.
b. Quantitative analysis, that is, peak area obtained was recorded, and then the level was calculated based on the linear regression equation.

RESULTS AND DISCUSSION

Determination of optimum analysis conditions

ALA and DHA are polyunsaturated fatty acids with carboxylic groups. To analyze, ALA in flaxseed oil and DHA in DHA oil by gas chromatography, ALA and DHA first need to be derivatized using the Lepage method to transform the carboxyl group derivative into an ester form. This derivatization aims to lower its boiling point, so it can easily evaporate at the analytical temperature [12-15].

In this study, the injector temperature was 250°C and the detector temperature was 250°C. The injector temperature should be set higher than the maximum column temperature so that all samples can be volatile after the sample was injected into the gas chromatographic apparatus, and the detector temperature should be higher than the boiling point of the compound being analyzed. The detector used was flame ionization, where the temperature of the detector should be above 100°C to prevent the condensation of water vapor, which may cause the dampening of the flame ionization detector or may result in a decrease in its sensitivity.

Optimization was carried out with variations in the initial column temperature and gas carrier flow rate. The initial temperature of the column was varied among 160°C, 180°C, and 200°C, and the flow rate variations used were 0.80, 1.00, and 1.20 mL/min. At the initial temperature, the column was raised 2°C/min–230°C and maintained for 20 min. The result of optimum analysis conditions of a mixture of ALA and DHA at the initial temperature of the column was 200°C with a flow rate of 1.00 mL/min (Tables 1 and 2).

Test system compatibility

The equality test was performed to ensure the effectiveness of the operational system and to provide results that were appropriate for analysis before performing the analytical method. The system equation test was performed 6 times in a row using optimal analysis that has been done before for the mixture of ALA and DHA. The coefficient values generated for the mixture of ALA and DHA were 0.3253% and 0.2744%, respectively. The CV was <2.0% (Table 3 and 4).

Validation of analytical methods

Selectivity test

A selectivity test was used to see the possibility of interference around the tR of methyl ester fatty acid. The test results showed that this method was selective because there was no interference with tR of ALA and DHA (Fig. 1).

Test linearity

The linearity test was performed by making the calibration curve using seven points of different concentrations of ALA and DHA mixes such as 40, 60, 80, 100, 140, 180, and 200 ppm. ALA and DHA had regression equations of y=1049.2643x+17278 and y=558.405x–542.964, respectively. ALA and DHA gave linear results with correlation coefficient (r) values of 0.99953 and 0.99934, respectively. The results could be declared valid because the correlation coefficient was close to 1 or r >0.9990 (Figs. 2 and 3).

Determination of LOD and LOQ

The linearity tests for ALA and DHA gave detection limit values of 4.342 µg/mL and 5.102 µg/mL, respectively. The quantitative limit values for ALA and DHA gave results of 14.474 µg/mL and 17.007 µg/mL, respectively.

Test accuracy and precision

In this experiment, the concentration of ALA and DHA was 80, 100, and 120 µg/mL. At each concentration, they were injected 6 times into the gas chromatographic device under selected analysis conditions. Based on the results of the analysis, the values of recovery (UPK) for ALA and DHA were approximately 100.49%–101.98% and 100.36%–101.99%, respectively. The CV values for ALA and DHA were approximately 0.26%–0.40% and 0.25%–0.93%, respectively. These results indicated that the analytical methods of ALA and DHA mixtures used were accurate and precise (Tables 5 and 6).

Fish oil extraction

Each 100 g sample of mackerel fish was boiled in 500 mL of water using a high-pressure double-bottomed pot that aimed to coagulate the protein in the fish cell wall and to break the cell wall so that the

Table 1: Relationship between retention time, peak area, number of theoretical plates, column efficiency, resolution, and methylated ALA chromatogram coupling factors to alterations in initial temperature column and following gas flow rate

Initial temperature column (°C)	160			180			200		
Flow rate (mL/min)	0.8	1.0	1.2	0.8	1.0	1.2	0.8	1.0	1.2
Retention time (min)	28.121	25.445	23.502	19.397	17.117	15.516	12.833	11.440	9.829
Peak area (µV/s)	46363	54704	60035	67275	69513	75451	100130	120186	90614
Theoretical plates (N Plates)	559753.108	499392.584	328604.424	209686.955	186531.861	156382.125	112801.434	93161.231	94735.598
HETP (CM/plates)	0.005359	0.006007	0.009129	0.014307	0.016083	0.019183	0.035294	0.032202	0.031667
Resolution (R)	47.067	47.319	41.935	37.934	44.535	35.614	30.307	67.548	27.989
Tailing factors (Tf)	0.860	0.792	0.907	0.854	0.883	0.897	0.821	0.949	1.017

ALA: Alpha-Linolenic Acid, HETP: Height equivalent to theoretical plate

Table 2: Relationship between retention time, peak area, the number of theoretical plates, column efficiency, resolution, and methylated DHA chromatogram coupling factors to changes in initial temperature column and carrier gas flow rate

Initial temperature column (C)	160			180			200		
Flow rate (mL/min)	0.8	1.0	1.2	0.8	1.0	1.2	0.8	1.0	1.2
Retention time (min)	44.600	40.418	37.747	34.193	30.272	27.755	25.149	22.337	19.349
Peak area (µV/s)	31217	35183	38313	41884	43512	46359	48699	57601	51870
Theoretical plates (N Plates)	518038.540	548379.233	557583.188	290964.050	325343.500	293259.679	187371.958	181673.200	157421.722
HETP (CM/plates)	0.005791	0.005471	0.005380	0.010310	0.009221	0.010229	0.016010	0.016513	0.019057
Resolution (R)	74.316	70.272	16.242	40.836	43.112	37.443	40.120	60.781	59.137
Following factors (Tr)	0.858	0.837	0.917	1.054	0.958	0.893	0.916	1.006	0.898

DHA: Docosahexaenoic acid, HETP: Height equivalent to theoretical plate

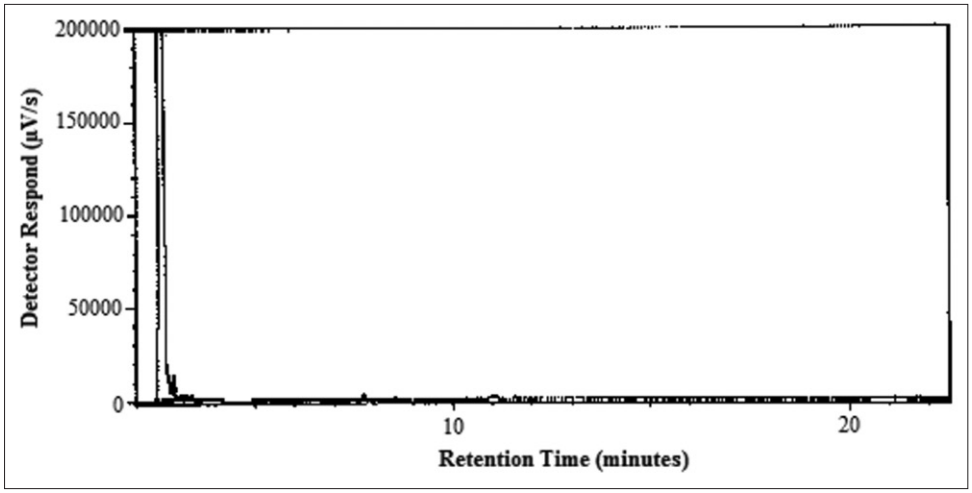

Fig. 1: Results of esterification chromatograms of blanco sample without addition of standard alpha-linolenic acid and docosahexaenoic acid

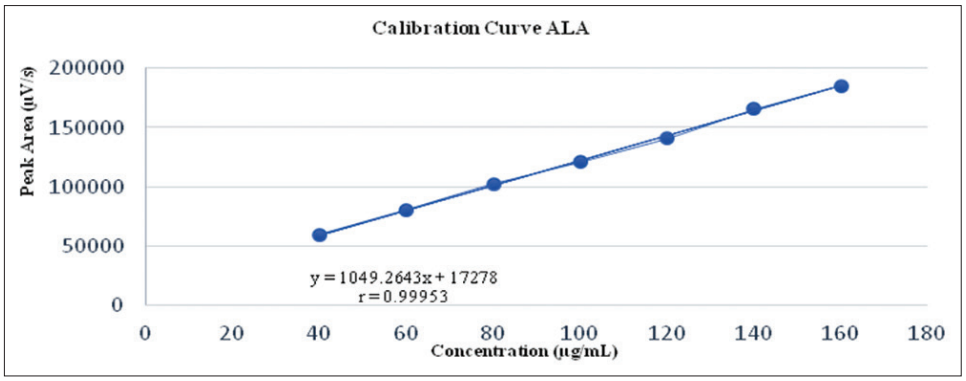

Fig. 2: Alpha-linolenic acid calibration curve

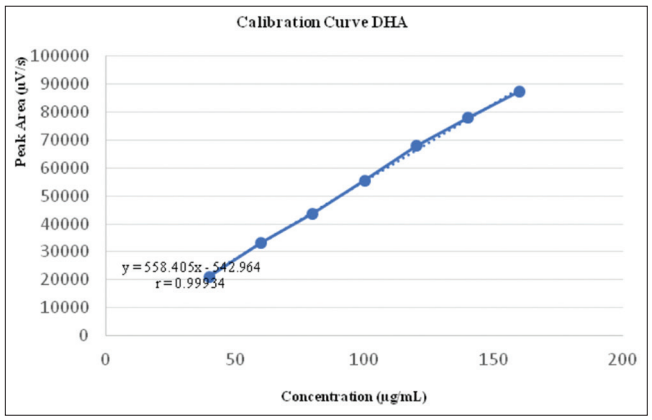

Fig. 3: Docosahexaenoic acid calibration curve

Fig. 4: Mackerel fish oil. Sample A, pressed fish oil; Sample B, fish oil extracted with solvent

oil inside could easily get out. Then, the extraction of fish oil was done by pressing and extraction by the solvent. The crude oil produced by pressing was thick and pale yellow with a fishy odor and a density of 0.8418 g/mL. The possessed crude oil extracted using a solvent was liquid and golden yellow with a rancid odor and a density of 1.3671 g/mL (Fig. 4).

The crude oil produced from each method of pressing and extraction using a solvent was then hydrolyzed using a saponification method using a dilute NaOH solution. The use of NaOH aimed to purify fish oil into its fatty acid form. Each of the 1.1 g of fish oil used from each pressing and extraction was done using a solvent. The resulting fatty acids were 25.7 mg and 48.9 mg, respectively.

Qualitative and quantitative analysis of ALA and DHA in the sample of mackerel fish oil

Qualitative analysis

In the sample of pressed fish oil (Sample A), there were ALA and DHA with tR of approximately 11.077 min and 21.735 min, respectively. In

Table 3: ALA system compatibility test results

Concentration (ppm)	Peak area (μV/s)	Retention time (min)	Theoretical plates (N plates)	Resolution (R)	Following factors (Tr)	HETP (CM/plates)	Mean±SD	CV (%)
100	120608	11.193	96931.244	28.648	0.700	0.03094	120817±392.989	0.3253
	120322	11.184	97017.577	28.657	0.885	0.03092		
	120754	11.198	96816.792	28.638	0.701	0.03098		
	121419	11.166	96560.038	28.616	0.887	0.03106		
	121127	11.437	109274.977	24.432	0.985	0.02745		
	120672	11.462	89624.480	24.859	0.862	0.03347		

ALA: Alpha-linolenic acid, HETP: Height equivalent to theoretical plate, SD: Standard deviation, , CV: Coefficient of variation

Table 4: DHA system compatibility test results

Concentration (ppm)	Peak area (μV/s)	Retention time (min)	Theoritical plates (N plates)	Resolution (R)	Following factors (Tr)	HETP (CM/plates)	Mean±SD	CV (%)
100	57854	21.892	148242.214	58.097	1.021	0.02023	57782±158.579	0.2744
	57563	21.875	168272.707	71.603	0.887	0.01782		
	57704	21.892	148501.007	58.160	1.024	0.02020		
	58036	21.895	167293.862	71.603	1.829	0.01793		
	57739	22.328	174386.955	59.759	0.937	0.01720		
	57794	22.377	157646.650	57.815	1.073	0.01902		

DHA: Docosahexaenoic acid, HETP: Height equivalent to theoretical plate, SD: Standard deviation, CV: Coefficient of variation

Table 5: ALA accuracy and precision test results

Concentration ppm (%)	Peak area from analysis result (μV/s)	Concentration from determination results (ppm)	UPK (%)	Mean±SD (%)	CV (%)
80	105,047	83.64813	102.84	101.99±0.95291	0.93
	104,047	82.69508	101.67		
	104,441	83.07059	102.13		
	104,752	83.36698	102.49		
	102,823	81.52855	100.23		
	104,826	83.43751	102.58		
100	122,033	99.83662	101.09	100.82±0.25562	0.25
	121,748	99.56500	100.81		
	121,752	99.56881	100.82		
	122,079	99.88046	101.13		
	121,483	99.31244	100.56		
	121,449	99.28004	100.52		
120	141,268	118.16851	100.21	100.36±0.30835	0.31
	141,210	118.11323	100.16		
	141,103	118.01126	100.08		
	142,066	118.92904	100.86		
	141,770	118.64694	100.62		
	141,286	118.18566	100.23		

ALA: Alpha-linolenic acid, SD: Standard deviation, CV: Coefficient of variation

Table 6: DHA accuracy and precision test results

Concentration ppm (%)	Peak area from analysis result (μV/s)	Concentration from determination results (ppm)	UPK (%)	Mean±SD (%)	CV (%)
80	44,585	80.81583	101.99	101.98±0.40748	0.40
	44,309	80.32156	101.36		
	44,658	80.94656	102.16		
	44,633	80.90179	102.10		
	44,837	81.26712	102.56		
	44,469	80.60810	101.73		
100	55,875	101.03413	100.61	100.49±0.2756	0.27
	55,790	100.88191	100.46		
	55,839	100.96966	100.54		
	55,677	100.67955	100.26		
	55,620	100.57747	100.15		
	56,054	101.35469	100.93		
120	68,349	123.37276	100.68	100.58±0.31591	0.26
	68,570	123.76853	101.00		
	68,109	122.94296	100.33		
	68,476	123.60019	100.86		
	68,183	123.07548	100.44		
	68,017	122.77821	100.19		

DHA: Docosahexaenoic acid, SD: Standard deviation, CV: Coefficient of variation

Table 7: Determination of levels of ALA on fatty acid samples of mackerel oil

Sample type	Sample of fatty acid weight (g)	Peak area from analysis (µV/s)	Total concentration measured (µg/mL)	Mean (µg/mL)	ALA levels (%)	Mean (%)
A	0.0257	34467	16.38195	15.97468	0.18153	0.18153
		32749	14.74462		0.16755	
		34903	16.79748		0.19088	
B	0.0489	17022	0.24398	0.86060	0.00277	0.00978
		18552	1.21418		0.01379	
		18457	1.12364		0.01277	

ALA: Alpha-linolenic acid

Table 8: Determination of levels of DHA on fatty acid samples of mackerel oil

Sample type	Sample of fatty acid weight (grams)	Peak area from analysis (µV/s)	Total concentration measured (µg/mL)	Mean (µg/mL)	DHA levels (%)	Mean (%)
A	0.0257	10184	19.21001	18.80349	0.21830	0.21368
		9286	17.60185		0.20002	
		10401	19.59861		0.22271	
B	0.0489	14421	26.79769	28.19154	0.30452	0.32036
		15340	28.44345		0.32322	
		15837	29.33348		0.33333	

DHA: Docosahexaenoic acid

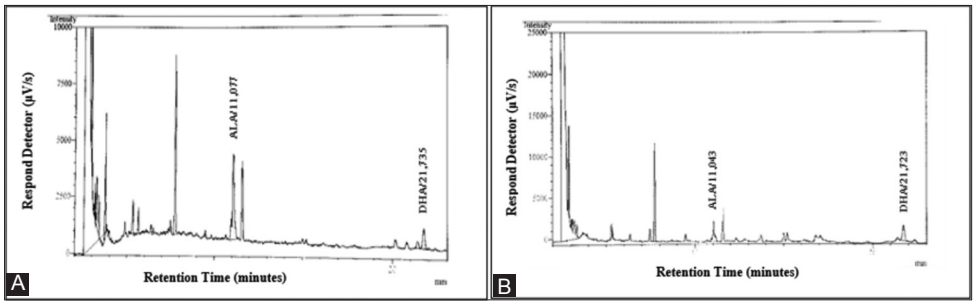

Fig. 5: Sample Chromatogram A (pressed result) and Sample B (extraction with solvent) The analysis was carried out at an initial temperature column of 200°C with an increase in temperature of 2°C/min to 230°C (maintained for 20 min). The injector temperature was 250°C, the detector temperature was 250°C, and the following gas flow rate (He) was 1.00 mL/min

the sample of fish oil extracted using solvent (Sample B), there were ALA and DHA with tR of approximately 11.043 min and 21.723 min, respectively (Fig. 5).

Quantitative analysis

Determination levels of ALA and DHA in mackerel fish oils that were pressed and extracted using solvent were calculated based on the linear regression equation. Based on the results obtained, fatty acids in fish oil samples from the pressing (Sample A) contained ALA and DHA with average levels of 0.18153% and 0.21368%, respectively. Fatty acids in fish oil extracted using a solvent (Sample B) contained ALA and DHA with average contents of 0.00978% and 0.32036%, respectively (Tables 7 and 8).

CONCLUSION

The optimum conditions for the analysis of ALA and DHA mixtures by gas chromatography were at an initial column temperature of 200°C with an increase in temperature of 2°C/min to 230°C (maintained for 20 min). The injector temperature was 250°C, the detector temperature was 250°C, and the following gas flow rate (He) was set to 1.00 mL/min. The tR for ALA and DHA were 11.440 min and 22.337 min, respectively. The analysis method was validated. The analysis showed that fatty acid in fish oil from pressing results contained amounts of ALA and DHA equal to 0.39521% and fatty acid samples in fish oil extracted using solvent contained amounts of ALA and DHA equal to 0.33014%.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest in this research.

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