

The employment of Fourier Transform Infrared Spectroscopy for discrimination and classification of *Parupeneus barberinoides* fish oil

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ABSTRACT: Goatfish oil (*Parupeneus barberinoides*) is a great of omega-3 source, which had beneficial effect in human health as consequence, these oil could be mixed with by products. Fourier transform infrared (FTIR) spectroscopy combined chemometrics techniques of pattern recognition was used for discrimination and classification of fish head, fish meat, and fish bone of goatfish. Each part of goatfish was scanned using FTIR at mid regions (4000-600 cm⁻¹). The result showed that the partial least square – discriminant analysis (PLS-DA) could be perfectly discrimination and classification of goatfish part by using the whole FTIR spectra as a variable. The variable important in projection (VIP) analysis revealed the vibration at 1710, 721, 1744, 3011, 1116, 1377, and 1032 cm⁻¹ were considered for their significant contribution (VIP value > 1) in discriminating of different parts of goatfish oil. It can be concluded that FTIR spectroscopy combined supervised pattern recognition could be used as a rapid analytical technique for discrimination and classification of fish oil.

KEYWORDS: Chemometrics; FTIR; Marine fish oil; Pattern recognition; *Parupeneus barberinoides*.

1. INTRODUCTION

Goatfish (*Parupeneus barberinoides*) belongs to Mullidae family which widely habits in lagoon and seaward reefs and a commercially important species [1]. Goatfish known as “kuniran” in Indonesia. Goatfish is a tropical benthic invertebrate’s carnivorous fish using a wide range of foraging mode [2]. Environmental disturbance such as coral bleaching will change the benthic habitat. Therefore, goatfish population is used an indicator of coral disturbance [3]. Goatfish containing the essential amino acids such as arginine and lysine that play role in human growth [4]. In addition, it also contains high level omega-3 which plays a role in brain development for children [5]. Based on literature study, publication about nutritional such as fatty acid composition of goatfish is very lacking.

The fatty acids composition of marine fish oil is commonly by using gas chromatography with flame ionization detector (GC-FID) [6]. This method requires the oils sample preparation such as converting of oils sample into fatty acid methyl ester (FAMES) [7]. However, these methods are cost, time, and required the highly skill. In other hand, Fourier Transform Infrared (FTIR) is a rapid, non-destructive, cost effective, and easy technique. The FTIR spectroscopy can be used to assess composition, quality, and lipid oxidation of edible oils [8,9].

Several researches have been reported the using FTIR for food authenticity such as the authentication of raw and cooked freeze-dried rainbow trout (*Oncorhynchus mykiss*) fillets [10], identification of fraud practices of fresh fish with frozen-thawed fish [11], classification and quantification of omega-3 supplement [12], authentication of milkfish [13], discrimination of different species and grades of surimi fish [14]. Thus, the aim of this study was to discrimination and classification different part of goatfish (fish head, fish meat, and fish bone) using FTIR spectroscopy combined pattern recognition namely principal component analysis (PCA) and partial least square – discriminant analysis (PLS-DA).

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2. RESULT AND DISCUSSION

2.1 Fatty acid profile of goatfish oil

The fatty acid profile of 3 goatfish parts (fish meat, fish head, and fish bone) are compiled in Table. Total saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) percentages of the total lipid ranged from 49.80% to 67.40%, from 2.04% to 26.13% and from 0.46% to 24.07%, respectively. Hexadecanoate/palmitate acid the mayor SFA was detected in fish heat and fish bone. Tetradecanoate/myristic acid (7.87%) is the second most important saturated fatty acid. Similarly, Durmus [4] have reported that these fatty acids are mayor fatty acids in marine seafood species. Oleic acid (cis-9-Octadecanoate) was found to be dominated in monounsaturated fatty acid. Meanwhile, docosahexaenoate (DHA), eicosapentaenoate (EPA), and octadecadienoate were found to be dominant in PUFA. Similar results were found by Yi *et al.* [15] and Rincón-Cervera *et al.*, [16] that the polyunsaturated fatty acids in marine fish as dominated by omega-3 such as DHA and EPA.

Table. The fatty acid profile different part of goatfish using gas chromatography mass spectrometry (GC-MS)

No.	Types of fatty acid	Fishbone (%)	Fish heat (%)	Fish meat (%)
1	Butirate	<0.1	<0.1	<0.1
2	Hexanoate	<0.1	<0.1	0.23
3	Octanoate	<0.1	<0.1	<0.1
4	Decanoate	<0.1	<0.1	<0.1
5	Undecanoate	<0.1	<0.1	<0.1
6	Dodecanoate	0.21	0.19	0.20
7	Tridecanoate	<0.1	<0.1	<0.1
8	Tetradecanoate	6.54	7.29	7.87
9	Pentadecanoate	2.93	2.71	1.90
10	Hexadecanoate	51.69	52.66	24.30
11	Heptadecanoate	4.18	1.79	5.72
12	Octadecanoate	1.75	1.88	1.12
13	Eicosanoate	0.1	0.1	0.20
14	Heneicosanoate	<0.1	<0.1	0.35
15	Docosanoate	<0.1	<0.1	0.37
16	Tricosanoate	<0.1	<0.1	4.83
17	Tetracosanoate	<0.1	<0.1	2.71
	Σ SFA	67.4	66.62	49.80
18	cis-9-Tetradecanoate	<0.1	<0.1	0.99
19	cis-10-Pentadecanoate	<0.1	<0.1	0.23
20	cis-9-Hexadecanoate	0.43	0.42	0.41
21	cis-10-heptadecanoate	<0.1	<0.1	2.09
22	cis-9-Octadecanoate	1.03	1.15	10.11
23	trans-9-Octadecanoate	0.98	0.12	9.68
24	cis-11-eicosenoate	<0.1	<0.1	0.43
25	13-Docosenoate	<0.1	<0.1	0.23
26	cis-15-Tetracosanoate	0.37	0.35	1.98
	Σ MUFA	2.81	2.04	26.13
27	9,12-Octadecadienoate	<0.1	<0.1	5.53
28	9,12-Octadecadienoate	0.14	0.09	1.26
29	γ-linolenate (cis-6,9,12)	<0.1	<0.1	0.21
30	α-linolenate (cis-9,12,15)	0.07	<0.1	0.47
31	cis-11,14-Eicosadienoate	<0.1	<0.1	0.20
32	8,11,14-Eicosatrienoate	<0.1	<0.1	<0.1
33	5,8,11,14-Eicosatetraenoate	0.25	0.11	<0.1
34	11,14,17-Eicosatrienoate	<0.1	<0.1	0.21
35	5,8,11,14,17-Eicosapentaenoate	0.15	0.08	1.97
36	cis-13,16-Docasadienoate	<0.1	<0.1	0.23
37	4,7,10,13,16,19-Docosahexaenoate	0.23	0.18	14.00
	Σ PUFA	0.84	0.46	24.07

SFA: Saturated fatty acid,

MUFA: Monounsaturated fatty acid

PUFA: Polyunsaturated fatty acid

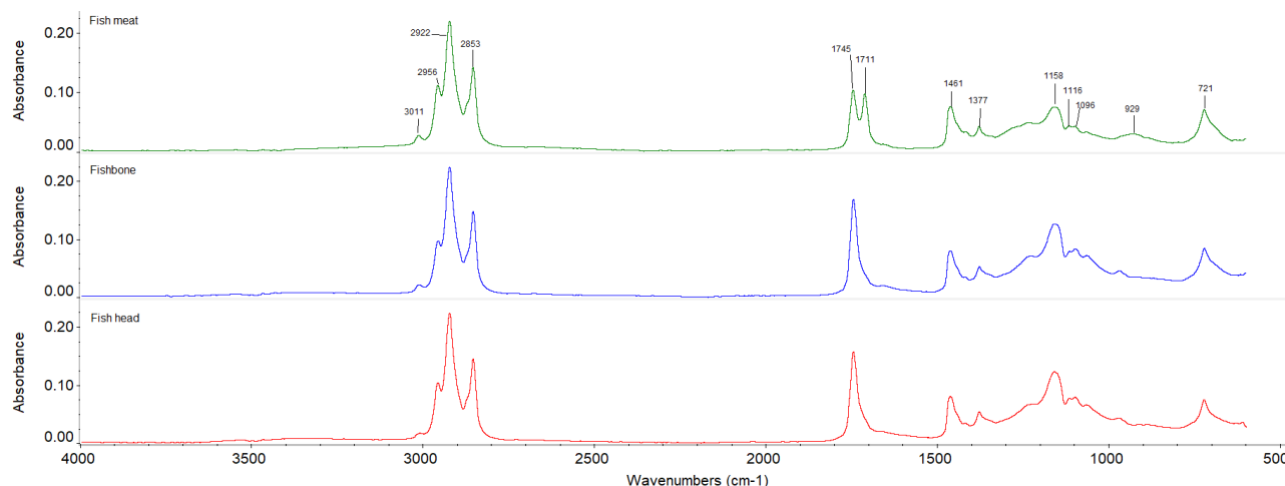


Figure 1. FTIR spectra of different part of goatfish (*Parupeneus barberinoides*) oil at middle infrared region (4000-600 cm^{-1})

2.2 FTIR profile of goatfish oil

FTIR spectroscopy provides fingerprinting spectra specific for fish meat, fish head, and fish bone of goatfish samples. The spectra of fish meat, fish head, and fish bone of goatfish samples measured at the wavenumber ranging from 4000 to 600 cm^{-1} are depicted in Figure 1. The absorption band at 3011 cm^{-1} was corrected to the $=\text{CH}$ stretching vibration, meanwhile asymmetric stretching vibration of $-\text{CH}(\text{CH}_3)$, $-\text{CH}(\text{CH}_2)$, and symmetric stretching vibration of $-\text{CH}(\text{CH}_3)$ was found at 2956, 2922, and 2853 cm^{-1} , respectively. The stretching vibration of carbonyl ($\text{C}=\text{O}$) could be observed at 1745 and 1711 cm^{-1} . The absorption band at 1711 cm^{-1} was specific band for fish meat sample because the other part (fish head and fish bone) was absent [17]. The absorption band at 1461 and 1377 cm^{-1} arise from bending vibration of $-\text{CH}_2-$ and $-\text{CH}_3$, respectively [7]. Other absorption present at 1158, 1116, and 1096 cm^{-1} arise from asymmetric vibration of $\text{C}-\text{C}(=\text{O})-\text{O}$, $\text{O}-\text{C}-\text{C}$, and $\text{C}-\text{O}-\text{C}$. In addition, the absorption at 929 and 721 cm^{-1} were associated with rocking vibration and the out-of-plane deformation of methylene.

2.3 Discrimination using pattern recognition

Two types of pattern recognition technique were used in this study including PCA and PLS-DA. The PCA results shown in Figure 2 were obtained by using absorbance of whole FTIR spectra. Principal component (PC) 1 and PC2 described 98.3 % and 0.9% of the total of variance, respectively. This study revealed the PCA technique could separation of meat fish of goatfish toward other part (fish head and fish bone) but could not clearly discrimination between fish head and fish bone of goatfish sample (partial discrimination). The same result was also reported by Ali and Tukiran [18] that PCA could not be classify of frying oils based on their sources. Therefore in this study, the PLS-DA technique was future used for discrimination of oil from different part of goatfish. PLS-DA using two principal component perfectly discriminated each part of goatfish, fish meat, fish head, and fish bone with clear separation as shown in PLS-DA score plot (Figure 3A). The variable importance in projection (VIP) analysis in PLS-DA model showed that variables of 1710, 721, 1744, 3011, 1116, 1377, and 1032 cm^{-1} were found to be important variables for classification of different part goatfish (Figure 3B). The vibration band at 1710 and 3011 cm^{-1} were significantly high in fish meat of goatfish. In addition, the vibration at 721 and 1744 cm^{-1} were significantly high in fishbone whereas the vibration at 1116, 1377, and 1032 cm^{-1} were found to be high levels in fish head of goatfish. It can be summarized that pattern recognition such as PCA and PLS-DA are promising to be used for discrimination of each part of goatfish based on FTIR spectra data with PLS-DA showing better discrimination result among PCA.

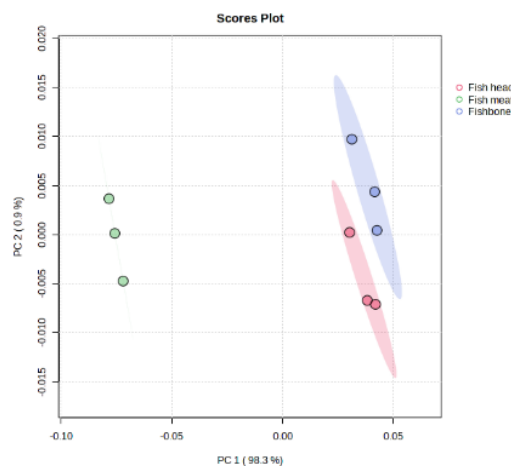
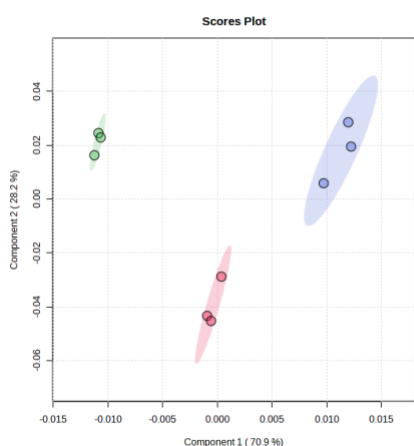
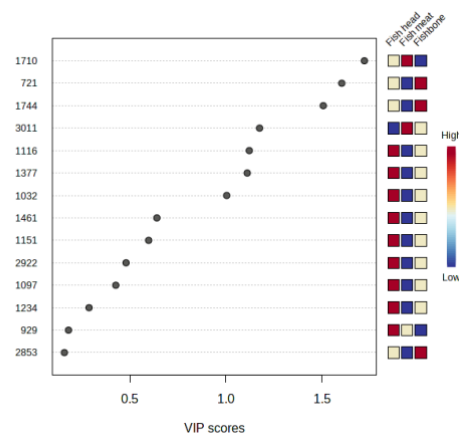


Figure 2. PCA Score plot of fish meat, fish head, and fish bone of goatfish



[A]



[B]

Figure 3. Score plot of PLS-DA [A] and analysis of variable importance for projection (VIP) [B] for discrimination and classification of fish head, fish meat, and fish bone of goatfish

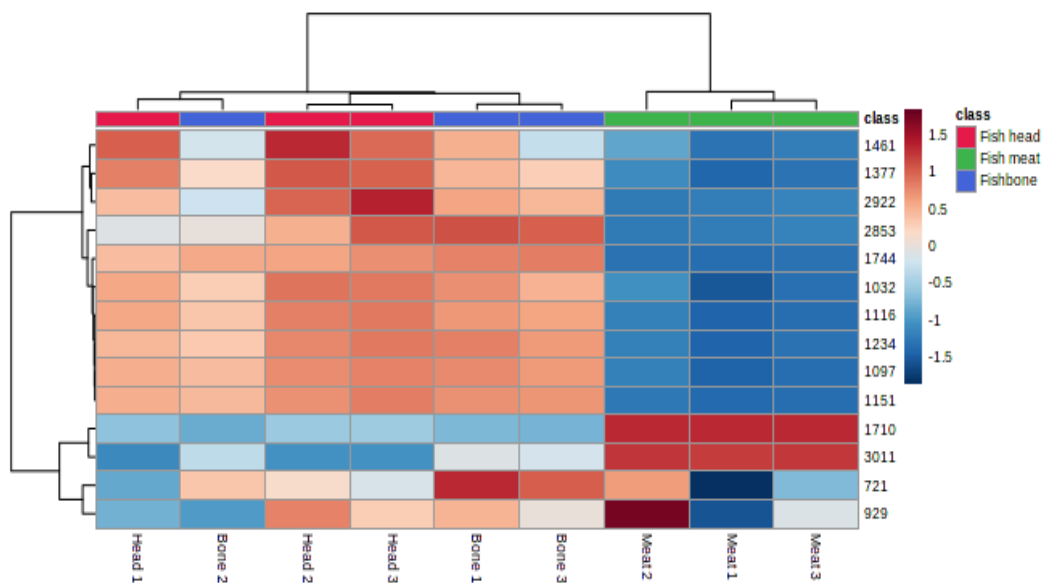


Figure 4. Heatmap analysis

Figure 4 demonstrated the heatmap analysis to observe the distribution each variable. High intensity of vibration at 929 cm^{-1} was extremely high in meatfish oil, meanwhile the high vibration at 721 cm^{-1} was found only in fishbone and vibration at 2922 and 1461 cm^{-1} was highly found in headfish oil. Heatmap analysis is very useful to monitor and identify the distribution of variable that become target for quality control of fish oils.

3. CONCLUSION

FTIR spectroscopy combination with supervised pattern recognition PLS-DA could be used as a rapid analytical technique for authentication of fish oil. This technique also did not involve the use of extensive reagents and solvent, therefore could be considered as a green analytical technique

4. MATERIALS AND METHODS

4.1. Materials

Goatfish species (Figure 5) was collected from a local fisherman Kendari coast, Southeast Sulawesi. The fish sample consists of 5.0 kg with weight range of 200-300 g to preserve the homogeneity and minimize variation possibilities of sample. The fish samples were eviscerated, separated the fish head, fish meat and fish bones, and placed in cooling box, to be sent to the Laboratory of Pharmaceutical Chemistry, Halu Oleo University. Each part of fish samples was dried at temperature of $70\text{--}80^\circ\text{C}$ (for 2×24 hours) using oven (Stuart Scientific), powdered (Philips blender), and then stored at 4°C for future analysis. All solvents and reagents used for analysis were analytical grade and were supplied by E. Merck Darmstadt, Germany.



Figure 5. Goatfish (*Parupeneus barberinoides*)

4.2. Fish oil extraction

Each part of goatfish was extracted using Soxhlet methods according to the methods described by de la Fuente et al. [19] with slight modification. The powdered fish (30 g) were placed in a Soxhlet apparatus and extracted with 450 mL n-hexane for 2 h at temperature of 80°C . After extraction was complete, n-hexane was evaporated at 50°C using a rotary evaporator (Stuart). The oils were collected, weighed, and stored at 4°C . Each fish was extracted in three replicates.

4.3 Fatty acids analysis

The fatty acids analysis of goatfish oil was carried out according to Earlia et al. [20]. Fatty acids were derived to fatty acids methyl esters (FAMES). FAMES of goatfish oil from different part (200 μL) were prepared using an alkali catalyzed (1.0 mL of methanolic potassium hydroxide) and in crew-capped glass test tube. The mixtures were heated at 60°C for 10 min, cooled at room temperature and then added 1.0 mL of boron trifluoride-methanol. The sample mixtures were heated again under the same conditions, when cooled, 2 mL n-hexane was added, shaken and centrifuge at 3000 rpm for 1 min. Finally, the upper n-hexane layer was collected for fatty acid analysis using an Agilent gas chromatography (8890) equipped mass spectrometry detector (MSD, 5977B) and a HP-INNOWAX capillary column (60 m \times 250 μm \times 0.25 μm). The temperature programmed from 150°C to 200°C at rate of $15^\circ\text{C}/\text{min}$, heating until 250°C at rate of 3°C , hold time at 30 min. The temperature of injector was 250°C with a split ratio of 10:1, injection volume was 1 μL . Detector temperature was 260°C . Helium was used as carrier gas (1 mL/min). Peaks characterization of FAME was completed within scan mode using m/z range varied from 15-500 and identification was carried out by comparing the retention time from FAME Mix standards (Supelco, Sigma-Aldrich) and with the spectral database.

4.4 FTIR spectra measurement

The measurement of all goatfish oil samples was performed according the condition as described by Irnawati *et al.* [21]. The measurement of FTIR spectra of all samples were scanned using FTIR spectrophotometer (Thermo Fisher Scientific, Inc.) controlled with Omnic software. The measurements were done in the middle infrared region of 4000-600 cm^{-1} with scanning number of 32 and a resolution of 8 cm^{-1} .

4.5 Chemometrics analysis

Chemometrics technique pattern recognition was performed using MetaboAnalyst 5.0. Patter recognition analysis, unsupervised technique such as principal component analysis (PCA) and supervised technique such as partial least square-discriminant analysis (PLS-DA) were used in this study.

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