Journal of Research in Pharmacy

Research Article

www.jrespharm.com

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

Irnawati IRNAWATI[1] *, Asriullah JABBAR[1], Muhammad Ilyas YUSUF[1], Abdul ROHMAN[2], Sakinah ALFADİLLAH [1]

1 Study Program of Pharmacy, Faculty of Pharmacy, Halu Oleo University, Kendari, 93232, Indonesia.
2 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
* Corresponding Author. E-mail: irnawati.vhina@gmail.com (Irnawati IRNAWATI); Tel. +62-852-417 738 82.

Received: 26 October 2023/ Revised: 20 December 2023 / Accepted: 20 December 2023

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 trasform 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).

How to cite this article: Irnawati, Jabbar A, Yusuf MI, Rohman A, Alfadillah S. The employment of Fourier Transform Infrared Spectroscopy for discrimination and classification of Parupeneus barberinoides fish oil. J Res Pharm. 2024; 28(5): 1465-1471.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.824

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
	∑ 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
	∑ 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
∑ PUFA		**0.84**	**0.46**	**24.07**

SFA: Saturated fatty acid,
MUFA: Monounsaturated fatty acid
PUFA: Polyunsaturated fatty acid

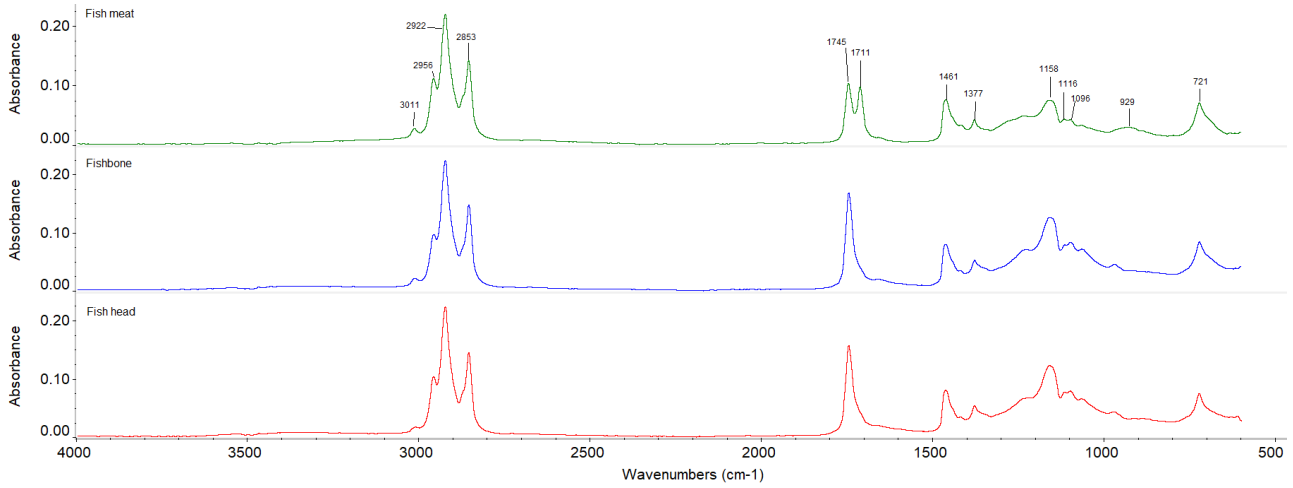


Figure 1. FITR spectra of different part of goatfish (*Parupeneus barberinoides*) oil at midle infrared region (4000-600 cm^{-1})

2.2 FTIR profile of goatfish oil

FTIR spectroscopy provides fingerprinting spectra specific for fish meat, fist head, and fish bone of goatfish samples. The spectra of fish meat, fist 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 =CH streching vibration, meanwhile asymmetric streching vibration of -$CH(CH_3)$, -$CH(CH_2)$, and symmetric streching vibration of -$CH(CH_3)$ was found at 2956, 2922, and 2853 cm^{-1}, respectively. The streching vibration of carbonyl (C=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 -CH_2- and -CH_3, respectively [7]. Othe absorption present at 1158, 1116, and 1096 cm^{-1} arise from asymetric vibration of C-C(=O)-O, O-C-C, and C-O-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 type of patter 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 tecnique was future used for dicrimination 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 goadfish (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 dan 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 patter 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.

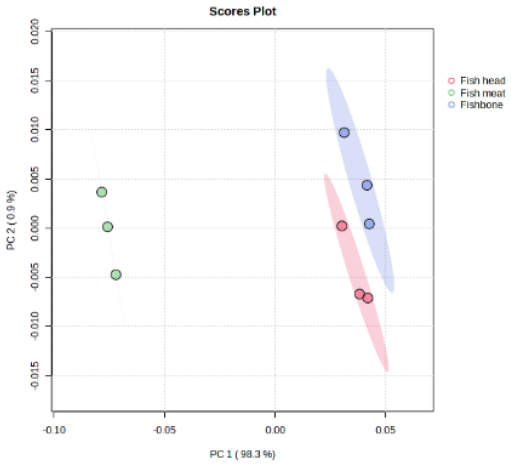
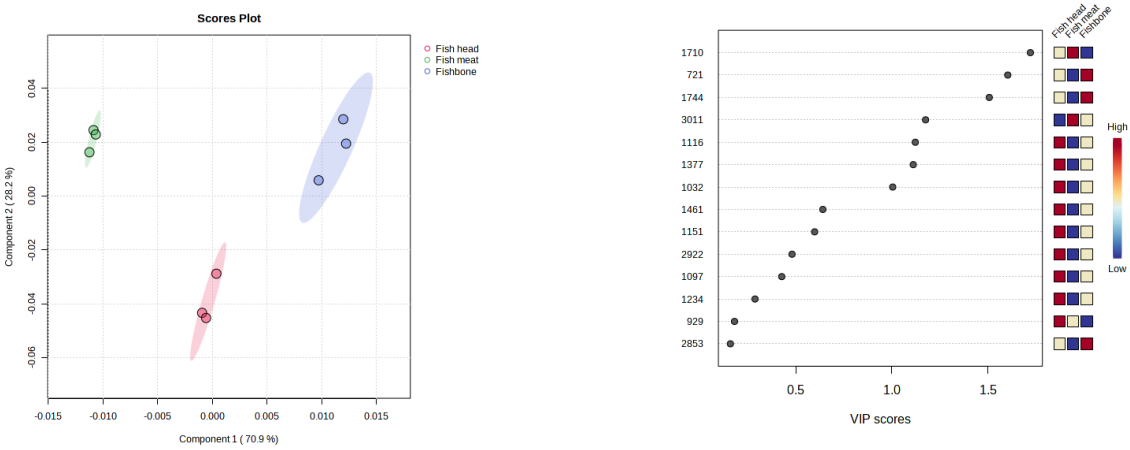


Figure 2. PCA Score plot of fish meat, fish head, and fish bone of goatfish



[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

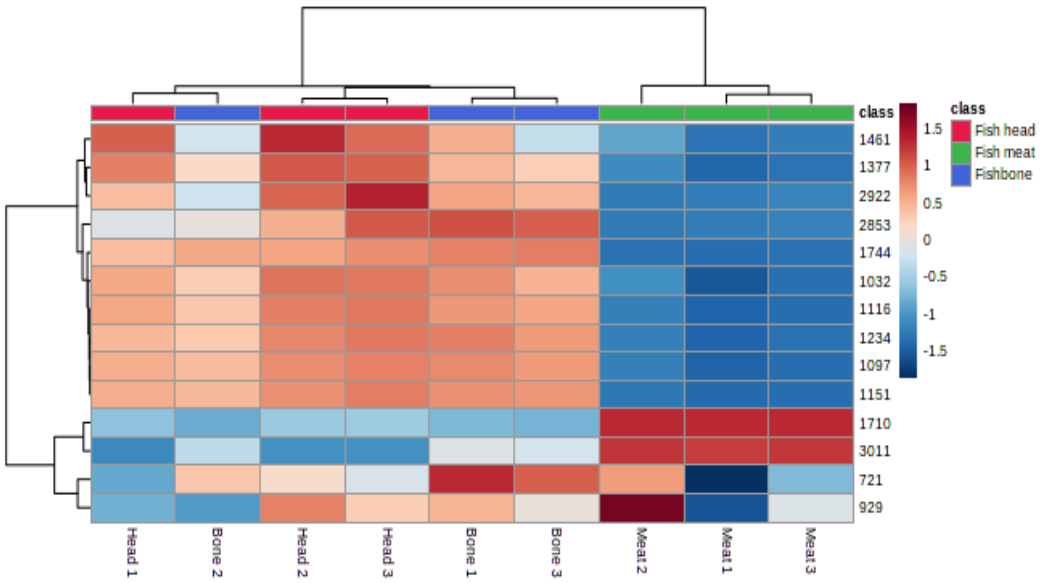


Figure 4. Heatmap analysis

http://dx.doi.org/10.29228/jrp.824
J Res Pharm 2024; 28(5): 1465-1471

Figure 4 demostrated the heatmap analysis to observe the distribution each variable. High intesity 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 higly 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 patter recognition PLS-DA could be used as a rapid analytical technique for authentication of fish oil. This technique also did not involved 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 minimized 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 70-80^{o}C (for 2 x 24 hours) using oven (Stuart Scientific), powdered (Philips blender), and then stored at 4^{o}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^{o}C. After extraction was complete, n-hexane was evaporated at 50^{o}C using a rotary evaporator (Stuart). The oils were collected, weighed, and stored at 4^{o}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^{o}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, shacked 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 x 250 µm x 0.25 µm). The temperature programmed from 150^{o}C to 200^{o}C at rate of 15^{o}C/min, heating until 250^{o}C at rate of 3^{o}C, hold time at 30 min. The temperature of injector was 250^{o}C with a split ratio of 10:1, injection volume was 1 µL. Detector temperature was 260^{o}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.

Acknowledgements: The authors would like to thank Directorate of Research Halu Oleo University for supporting this research through scheme *Penelitian Internal* 2023 with contract number 029h/UN29.18/KU/2023 awarded to Dr. Irnawati.

Author contributions: Concept – I.I., A.R.; Design – I.I., A.R.; Supervision – A.J., M.I.Y.; Resources – S.A., I.I.; Materials – S.A., A.R.; Data Collection and/or Processing – I.I., S.A.; Analysis and/or Interpretation – I.I., S.A., A.J., M.I.Y.; Literature Search – I.I., A.J., M.I.Y.; Writing – I.I.; Critical Reviews – A.R., A.J., M.I.Y.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] Longenecker K, Langston R, Bolick H, Crane M, Donaldson TJ, Franklin EC, Kelokelo M, Kondio U, Potuku T. Rapid reproductive analysis and length–weight relations for five species of coral-reef fishes (Actinopterygii) from papua new guinea: *Nemipterus isacanthus, parupeneus barberinus, kyphosus cinerascens, ctenochaetus striatus* (perciformes), and *balistapus undulatus* (Tetraodontiformes). Acta Ichthyol Piscat. 2017; 47: 107–124. https://doi:10.3750/AIEP/02146

[2] Lukoschek V, McCormick MI. Ontogeny of diet changes in a tropical benthic carnivorous fish, *Parupeneus barberinus* (Mullidae): Relationship between foraging behaviour, habitat use, jaw size, and prey selection. Mar Biol. 2001; 138: 1099–1113. https://doi:10.1007/S002270000530/METRICS

[3] Russ GR, Bergseth BJ, Rizzari JR, Alcala AC. Decadal-scale effects of benthic habitat and marine reserve protection on Philippine goatfish (F: Mullidae). Coral Reefs. 2015; 34: 773–787. https://doi:10.1007/S00338-015-1296-9/METRICS

[4] Durmuş M. Fish oil for human health: Omega-3 fatty acid profiles of marine seafood species. Food Sci Technol. 2019; 39: 454–461. https://doi:10.1590/fst.21318

[5] He Y, Li J, Kodali S, Chen B, Guo Z. Rationale behind the near-ideal catalysis of *Candida antarctica* lipase A (CAL-A) for highly concentrating ω-3 polyunsaturated fatty acids into monoacylglycerols. Food Chem. 2017; 219: 230–239. https://doi:10.1016/j.foodchem.2016.09.149

[6] Srigley CT, Rader JI. Content and composition of fatty acids in marine oil omega-3 supplements. J Agric Food Chem. 2014; 62: 7268–7278. https://doi:10.1021/jf5016973

[7] Vongsvivut J, Miller MR, McNaughton D, Heraud P, Barrow CJ. Rapid discrimination and determination of polyunsaturated fatty acid composition in marine oils by FTIR spectroscopy and multivariate data analysis. Food Bioprocess Technol. 2014; 7: 2410–2422. https://doi:10.1007/s11947-013-1251-0

[8] Alamprese C, Casiraghi E. Application of FT-NIR and FT-IR spectroscopy to fish fillet authentication. LWT. 2015; 63: 720–725. https://doi:10.1016/j.lwt.2015.03.021

[9] Cheng JH, Dai Q, Sun DW, Zeng XA, Liu D, Pu HB. Applications of non-destructive spectroscopic techniques for fish quality and safety evaluation and inspection. Trends Food Sci Technol. 2013; 34: 18–31. https://doi:10.1016/J.TIFS.2013.08.005

[10] Dalle Zotte A, Ottavian M, Concollato A, Serva L, Martelli R, Parisi G. Authentication of raw and cooked freeze-dried rainbow trout (*Oncorhynchus mykiss*) by means of near infrared spectroscopy and data fusion. Food Res Int. 2014; 60: 180–188. https://doi:10.1016/J.FOODRES.2013.10.033

[11] Ottavian M, Fasolato L, Facco P, Barolo M. Foodstuff authentication from spectral data: Toward a species-independent discrimination between fresh and frozen–thawed fish samples. J Food Eng. 2013; 119: 765–775. https://doi:10.1016/J.JFOODENG.2013.07.005

[12] Karunathilaka SR, Choi SH, Mossoba MM, Yakes BJ, Brückner L, Ellsworth Z, Srigley CT. Rapid classification and quantification of marine oil omega-3 supplements using ATR-FTIR, FT-NIR and chemometrics. J Food Compos Anal. 2019; 77: 9–19. https://doi:10.1016/j.jfca.2018.12.009

[13] Mustafidah M, Irnawati I, Lukitaningsih E, Rohman A. Authentication analysis of milkfish fish oil using the combination of FTIR spectroscopy and chemometrics. Food Res. 2021; 5: 272–278. https://doi:10.26656/fr.2017.5(2).607

[14] Zhang XY, Hu W, Teng J, Peng HH, Gan JH, Wang XC, Sun SQ, Xu CH, Liu Y. Rapid recognition of marine fish

surimi by one-step discriminant analysis based on near-infrared diffuse reflectance spectroscopy. Int J Food Prop. 2017; 20: 2932–2943. https://doi:10.1080/10942912.2016.1261153

[15] Yi T, Li SM, Fan JY, Fan LL, Zhang ZF, Luo P, Zhang XJ, Wang JG, Zhu L, Zhao ZZ, Chen HB. Comparative analysis of EPA and DHA in fish oil nutritional capsules by GC-MS. Lipids Health Dis. 2014; 13: 190. https://doi:10.1186/1476-511X-13-190

[16] Rincón-Cervera MÁ, Villarreal-Rubio MB, Valenzuela R, Valenzuela A. Comparison of fatty acid profiles of dried and raw by-products from cultured and wild fishes. Eur J Lipid Sci Technol. 2017; 119: 1600516. https://doi:10.1002/ejlt.201600516

[17] Irnawati I, Windarsih A, Indrianingsih AW, Apriyana W, Ratnawati YA, Nadia LOMH, Rohman A. Rapid detection of tuna fish oil adulteration using FTIR-ATR spectroscopy and chemometrics for halal authentication. J. Appl. Pharm. Sci. 2023; 13: 231–239. https://doi:10.7324/japs.2023.120270

[18] Ali NAM, Tukiran NA. Detection of pork in used cooking oil using Fourier Transform Infrared Spectroscopy. Int J Allied Heal Sci. 2021; 5: 2110–2110.

[19] de la Fuente B, Pinela J, Mandim F, Heleno SA, Ferreira ICFR, Barba FJ, Berrada H, Caleja C, Barros L. Nutritional and bioactive oils from salmon (*Salmo salar*) side streams obtained by Soxhlet and optimized microwave-assisted extraction. Food Chem. 2022; 386: 132778. https://doi.org/10.1016/j.foodchem.2022.132778

[20] Earlia N, Muslem, Suhendra R, Amin M, Prakoeswa CRS, Khairan, Idroes R. GC/MS Analysis of fatty acids on Pliek U Oil and its pharmacological study by molecular docking to filaggrin as a drug candidate in atopic dermatitis treatment. Sci World J. 2019; 2019: 8605743. https://doi:10.1155/2019/8605743

[21] Irnawati I, Riyanto S, Martono S, Windarsih A, Rohman A. Physicochemical properties and antioxidant activities of pumpkin seed oil as affected by different origins and extraction methods. J Appl Pharm Sci. 2021; 12: 115-122. https://doi.org/10.7324/JAPS.2022.120312.