Journal of Applied Pharmaceutical Science Vol. 14(03), pp 162-169, March, 2024
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2024.159140
ISSN 2231-3354

Quantification and classification of fatty acids in marine fish oil from Southeast Sulawesi using gas chromatography and chemometrics

Irnawati Irnawati[1]*, Anjar Windarsih[2], La Ode Muhamad Hazairin Nadia[3], Abdul Rohman[4], Wa Ode Fasrida[1]

[1]Study Program of Pharmacy, Faculty of Pharmacy, Halu Oleo University, Kendari, Indonesia.
[2]Research Center for Food Technology and Processing (PRTPP), National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia.
[3]Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari, Indonesia.
[4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia.

ARTICLE HISTORY

Received on: 28/10/2023
Accepted on: 06/02/2024
Available Online: 05/03/2024

Key words:
Fish oil, *Epinephelus* sp., *Lutjanus* sp., discrimination, gas chromatography.

ABSTRACT

Marine fish oils contain various fatty acids (FAs) which are important for human health. Due to the variation of species and origins, the FA compositions were truly affected. This research aimed to quantify FAs of fish oils from various *Epinephelus* sp. and *Lutjanus* sp. obtained from different locations around Southeast Sulawesi coast Indonesia using gas chromatography with flame ionization detector and to create classification models using chemometrics techniques. Different locations affected the amounts of FAs from each type of fish oil. The FA composition of fish oil consisted of 45%–61% saturated FAs, 19.16%–39.30% monosaturated FAs, and 11.51%–30.20% polyunsaturated FAs. Supervised pattern recognition chemometrics such as partial least square-discriminant analysis (PLS-DA), sparse PLS-DA, and orthogonal PLS-DA could be used for the classification of fish oils extracted from *Epinephelus* sp. and *Lutjanus* sp. FAs of tricosanoate (C23:0), trans-9-elaidate (C18:1t), linolelaidate (C18:2t), cis-11,14,17-eicosatrienoate (C20:3), palmitate (C16:0), stearate (C18:0), cis-9 oleate (C18:1), and myristate (C14:0) were considered for their significant contributions in discriminating *Epinephelus* fish oils and *Lutjanus* fish oils (variable importance in projection score > 1). Heatmap analysis showed that the distribution of each FA in all fish oil samples. This study could be very useful for the authentication and quality control of fish oils based on their FA compositions and classification analysis using chemometrics.

INTRODUCTION

Fish is the best source of fatty acids (FAs), especially polyunsaturated FAs (PUFAs) (eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA). These FAs are key nutrients whose beneficial role in human health such as the prevention of mammary tumors [1], cardioprotective effects [2], antidiabetic effect [3,4], and anti-inflammatory [5]. Fish oil is widely accepted as healthy food; therefore, providing nutritional value information especially PUFAs contents is important [6].

FA composition of fish oil has been published by several researchers such us 34 marine water fish from the Mediterranean sea [7], 20 species of marine fish, and 4 species of shellfish from Malacca straits [8], microalga oil and fish oil from Ocean Nutrition Canada Ltd. [9]. Each species of fish even in white and dark muscle has a different FA composition. This difference is affected by several factors such as type and abundance of food, habitat, salinity, temperature, size, life stage, and age [7].

Some methods have been reported for the identification and determination of FA in fish oils, including gas chromatography (GC)-mass spectrometry for the identification and determination of EPA and DHA in fish oil capsules [10]. Raman combined partial least squares regression (PLSR) for analyzing omega-3 in an intact fish oil capsule [11], Fourier transform infrared (FTIR), near-infrared (NIR), and Raman spectroscopic combined

*Corresponding Author
Irnawati Irnawati, Study Program of Pharmacy, Faculty of Pharmacy, Halu Oleo University, Kendari, Indonesia.
E-mail: irnawati.vhina @ gmail.com

chemometrics, namely PLSR for prediction of EPA, DHA, and total omega-3 FAs in fish oil supplement [12–14]. In addition, an esterified omega-3 supplement commercial was identified using FTIR-attenuated total reflectance (ATR) spectra, while ^{1}H NMR spectra were used to determine the relative concentration of DHA and EPA [15]. Viswanathan *et al.* [16] have reported using reverse phase high-performance liquid chromatography coupled with mass spectrometry for the quantification of ethyl ester of EPA and DHA. Meanwhile, GC with flame ionization detection (GC-FID) has often been used to quantify and profile the FAs in fish [17,18]. However, GC generating a massive amount of data and retrieving complex biochemical information demands powerful statistical tools, namely chemometrics [19]. In addition, Xiao *et al.* [18] have stated that chemometrics technique was proposed as s powerful tool for the determination of dynamic multiparametric of living systems. Thus, this study aimed to quantify and classify FAs in marine fish oil from Southeast Sulawesi using GC and chemometrics.

MATERIALS AND METHODS

Materials

Twenty species of marine fish were collected from different locations around the Southeast Sulawesi coast, Indonesia (Fig. 1). Each location consists of 5 kg/species with a weight range of 300–500 g to minimize variation possibilities and preserve the homogeneity of fish samples. All samples were collected fresh and caught within a period of 0–24 hours. The fish samples were eviscerated, filleted, packed flake ice in a cooling box, and sent to the Farmacy Laboratory of Halu Oleo University. The filleted fish samples were dried at a temperature of 80°C–90°C (2 × 24 hours) using an oven (Stuart Scientific) according to the methods described by Alinafiah *et al.* [17] with slight modification, powdered (Philips blender), and then stored at 4°C for future analysis. *n*-Hexane, heptane, ethanol, and all reagents used for analysis were of analytical grade and were supplied by E. Merk Darmstadt, Germany.

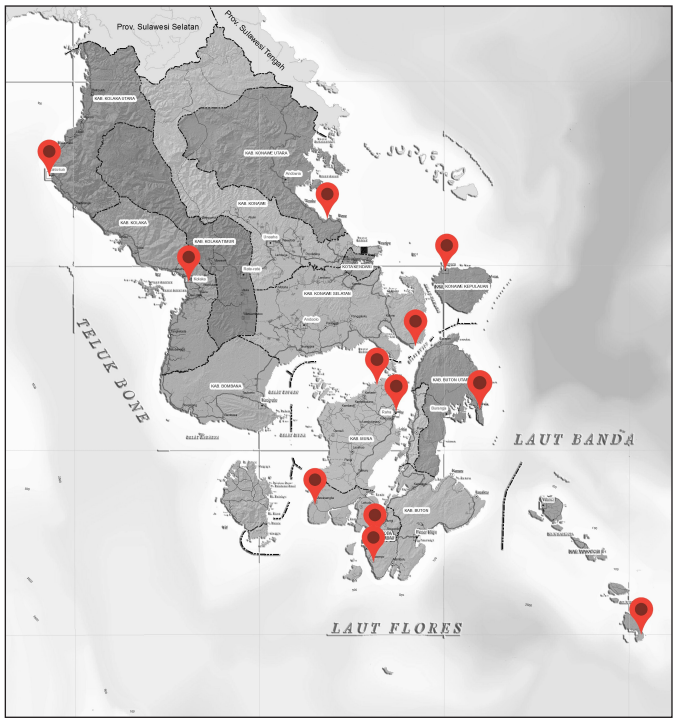


Figure 1. Location of sample collection.

Fish oils extraction

Marine fish oils were extracted using Soxhlet methods according to the methods described by Rincón-Cervera *et al.* [6] with slight modifications. Powdered fish (50 g) were placed in a Soxhlet apparatus and extracted with 750 ml *n*-hexane for 2 hours at a 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.

Oils purification

Crude fish oils were purified using bentonite at a ratio of 1% (w/w) of oils weight, centrifuged (Boeco Germany) for 5 minutes, filtered, and stored in a dark bottle in a freezer for subsequent analysis [20].

FAs analysis

The FA analysis of marine fish oils was carried out according to Irnawati *et al.* [21]. FAs were derived from FAs methyl esters (FAMEs). FAMEs of each oil sample (0.4 g) were prepared using an alkali catalyzed (1.5 ml of methanolic potassium hydroxide) and in a crew-capped glass test tube. The mixtures were heated at 60°C for 10 minutes, cooled at room temperature, and then added 2 ml of boron trifluoride-methanol. The sample mixtures were heated again under the same conditions; when cooled, 1 ml of heptane was added with 1 ml of sodium chloride and shacked. Finally, the upper heptane layer was collected for FA analysis using an Agilent GC (7890B) equipped flame ionization detector and an HP-88 capillary column (100 m × 0.3 mm × 0.2 µm). The temperature program was 5 minutes at 100°C, heating until 240°C at a rate of 4°C/minute, holt time at 15 minutes. The temperature of the injector was 260°C with a split ratio of 10:1, and injection volume was 1 µl. The detector temperature was 260°C. Helium was used as carrier gas (1.8 ml/minute). Peak identification was carried out by comparing the retention time from CRM47885 FAME Mix standards (Supelco) (Fig. 2).

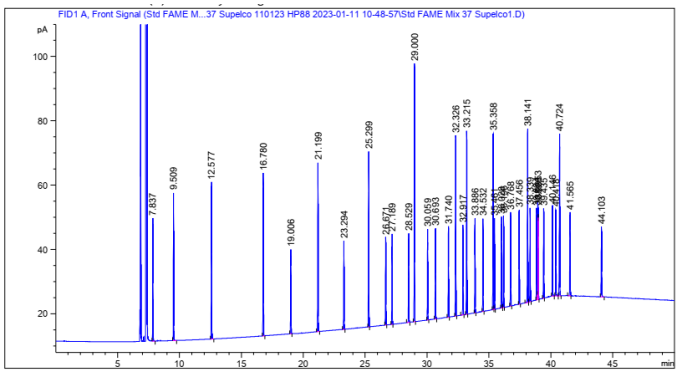


Figure 2. Chromatogram of FAMEs mix standards (CRM47885) using a GC (Agilent, 7890B) with flame ionization detector.

Classification of marine fish oil

Pattern recognition analysis unsupervised techniques, namely principal component analysis (PCA) and supervised techniques, namely partial least-square-discriminant analysis (PLS-DA), sparse variant PLS-DA (sPLS-DA), and orthogonal PLS-DA (OPLS-DA) were used in this study. The concentration of FA compositions was used as a variable during the classification of marine fish oil from different origins.

RESULT AND DISCUSSION

FAs analysis of marine fish oil

In this study, FAs from marine fish oils extracted from *Epinephelus* sp. and *Lutjanus* sp. were measured using GC-FID. The sampling of marine fish was done from several locations in South East Sulawesi province as illustrated in Figure 1. Different locations affected the compositions of FAs in each type of fish oil due to the difference in environment which is associated with the difference in nutrition sources. Table 1 shows the result of FAs analysis using the GC-FID technique from *Epinephelus* sp. and Table 2 from *Lutjanus* sp. fish oils. Total saturated FAs (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) percentages for both ranged from 45.59% to 61.42%, 20.32% to 39.30%, and 12.97% to 24.56%, respectively. The important FAs identified in most samples were myristic/C14:0 (1.89%–8.49%), palmitate/C16:0 (24.30%–33.68%), heptadecanoic/C17:0 (1.49%–2.87%),

Table 1. FA composition measured using a GC-FID from *Epinephelus* sp. fish oils.

FA (%)	*E. coioides* from Muna	*Epinephelus macrospilos* from Kolaka	*Epinephelus sexfasciatus* from West Muna	*Epinephelus sexfasciatus* from North Buton	*E. merra* from Buton	*E. merra* from Wakatobi	*E. merra* from South Konawe	*E. merra* from Bau-Bau
Laurate	1.11^{a}	0.19^{d}	0.18^{g}	0.13^{f}	0.23^{b}	0.10^{e}	0.20^{c}	0.1^{g}
Myristate	6.04^{a}	3.99^{f}	3.24^{g}	4.01^{e}	4.98^{b}	4.44^{d}	4.90^{c}	1.89^{h}
Pentadecanoate	1.02^{e}	1.32^{c}	1.35^{b}	1.52^{a}	1.07^{d}	0.78^{g}	1.01^{f}	0.10^{h}
Palmitate	27.15^{f}	28.90^{e}	29.37^{d}	33.07^{a}	30.84^{c}	26.44^{g}	31.51^{b}	25.52^{h}
Heptadecanoate	1.72^{g}	2.63^{b}	2.84^{a}	2.14^{c}	2.02^{f}	2.08^{e}	1.49^{h}	2.11^{d}
Stearate	11.01^{b}	13.06^{a}	13.06^{a}	10.38^{d}	10.75^{c}	9.92^{e}	10.29^{d}	10.77^{c}
Arachidate	0.29^{c}	0.12^{f}	0.18^{e}	0.96^{b}	0.99^{a}	0.11^{g}	0.26^{d}	0.10^{h}
Heneicosanoate	0.42^{d}	0.10^{g}	0.33^{e}	0.20^{f}	0.57^{c}	0.87^{b}	0.91^{a}	0.10^{g}
Docosanoate	0.10^{e}	0.10^{e}	0.17^{d}	0.10^{e}	0.36^{b}	0.31^{c}	0.45^{a}	0.10^{e}
Tricosanoate	6.89^{b}	5.34^{d}	4.67^{e}	3.13^{h}	3.89^{g}	3.90^{f}	5.53^{c}	8.66^{a}
Lignocerate	4.99^{a}	2.60^{f}	3.19^{c}	2.27^{h}	2.98^{e}	2.52^{g}	3.37^{b}	3.09^{d}
SFA	60.74	58.35	58.58	57.91	58.68	51.47	59.92	52.54
Myristoleic	0.12^{d}	0.11^{e}	0.10^{f}	0.10^{f}	0.21^{c}	0.95^{a}	0.25^{b}	0.1^{f}
cis-10-pentadecenoate	0.19^{c}	0.25^{b}	0.17^{d}	0.10^{e}	0.16^{d}	0.62^{a}	0.26^{b}	0.1^{e}
Palmitoleate	7.24^{c}	6.72^{d}	4.94^{g}	8.11^{a}	6.15^{e}	5.05^{f}	7.72^{b}	2.50^{h}
cis-10-heptadecenoate	0.98^{c}	0.49^{e}	1.22^{b}	1.49^{a}	0.76^{d}	0.30^{g}	0.44^{f}	0.1^{h}
trans-9-elaidate	0.1^{g}	0.81^{e}	3.15^{c}	3.83^{a}	3.20^{b}	1.01^{d}	0.78^{f}	0.1^{g}
cis-9-oleate	11.04^{f}	13.57^{c}	11.77^{d}	16.87^{a}	11.22^{e}	15.77^{b}	10.59^{g}	10.29^{h}
cis-11-eicosenoate	0.81^{b}	0.11^{g}	0.31^{e}	0.11^{f}	0.62^{c}	0.43^{d}	1.19^{a}	0.10^{h}
Erucate	0.10^{f}	0.35^{b}	0.21^{d}	0.10^{f}	0.27^{c}	0.12^{e}	0.38^{a}	0.10^{f}
Nervonate	2.22^{bc}	0.13^{f}	2.34^{b}	0.35^{f}	1.75^{e}	1.88^{de}	2.11^{cd}	5.77^{a}
MUFA	22.8	22.54	24.21	31.06	24.34	26.13	23.72	19.16
Linolelaidate	3.97^{c}	3.61^{d}	0.66^{f}	0.11^{g}	0.60^{f}	1.17^{e}	5.67^{a}	4.38^{b}
Linoleate	2.19^{a}	1.56^{c}	1.34^{d}	1.49^{c}	1.77^{b}	0.19^{e}	2.15^{a}	0.10^{e}
gamma-Linolenic	0.75^{ab}	0.55^{d}	0.67^{bc}	0.61^{cd}	0.81^{a}	0.19^{ef}	0.21^{e}	0.10^{f}
Linolenate	0.40^{e}	0.45^{d}	0.73^{b}	0.58^{c}	0.23^{h}	0.27^{f}	0.24^{g}	3.18^{a}
cis-11,14-eicosadienoate	0.73^{b}	0.97^{a}	0.54^{c}	0.10^{g}	0.47^{d}	0.11^{f}	0.39^{e}	0.10^{g}
cis-8,11,14-eicosatrienoate	0.86^{a}	0.41^{e}	0.65^{b}	0.57^{c}	0.42^{d}	0.24^{f}	0.10^{g}	0.1^{g}
cis-11,14,17-eicosatrienoate	0.31^{b}	0.10^{e}	0.30^{b}	0.16^{d}	0.10^{e}	0.42^{a}	0.25^{c}	0.10^{e}
cis-5,8,11,14-eicosatetraenoate	0.10^{e}	0.29^{b}	0.11^{d}	0.13^{c}	0.93^{a}	0.10^{e}	0.10^{e}	0.10^{e}
cis-13,16-docosadienoate	0.14^{c}	0.11^{e}	0.12^{d}	0.11^{e}	0.10^{f}	0.56^{a}	0.40^{b}	0.10^{f}
cis-5,8,11,14,17-eicosapentaenoate	0.10^{f}	0.13^{d}	0.11e	0.11^{e}	0.30^{c}	1.99^{a}	1.02^{b}	0.10^{f}
cis-4,7,10,13,16,19-docosahexaenoate	7.16^{g}	11.25^{d}	12.09^{c}	7.54^{f}	11.22^{e}	17.35^{b}	6.04^{h}	21.84^{a}
PUFA	16.71	19.43	17.32	11.51	16.95	22.59	16.57	30.2

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

Different lowercase letters within a row are significantly different in Tukey's test ($p < 0.05$).

Table 2. FA composition measured using a GC-FID from *Lutjanus* sp. fish oils.

FA (%)	*L. malabaricus* from South Konawe	*L. malabaricus* from Bau-Bau	*L. malabaricus* from Wakatobi	*L. malabaricus* from Konawe Kepulauan	*L. malabaricus* from North Konawe	*L. malabaricus* from North Kolaka
Laurate	0.44^{a}	0.14^{d}	0.14^{d}	0.18^{c}	0.10^{f}	0.13^{e}
Myristate	5.86^{e}	8.49^{a}	7.18^{c}	6.09^{d}	4.81^{f}	3.54^{i}
Pentadecanoate	0.92^{l}	0.90^{k}	2.03^{j}	1.27^{i}	1.12^{g}	1.61^{h}
Palmitate	29.56^{b}	28.66^{d}	25.27^{h}	26.18^{g}	29.11^{b}	26.91^{f}
Heptadecanoate	1.87^{d}	2.12^{b}	1.74^{g}	2.22^{e}	1.93^{c}	1.77^{f}
Stearate	7.89^{c}	13.44^{a}	9.98^{abc}	10.17^{abc}	9.22^{abc}	9.14^{abc}
Arachidate	0.11^{h}	0.17^{f}	0.28^{cd}	0.19^{e}	0.10^{h}	0.30^{c}
Heneicosanoate	0.36^{f}	0.40^{e}	1.08^{a}	0.24^{j}	0.60^{b}	0.25^{i}
Docosanoate	0.10^{e}	0.10^{e}	0.42^{ab}	0.10^{e}	0.10^{e}	0.21^{d}
Tricosanoate	0.13^{f}	3.58^{d}	3.78^{cd}	0.20^{f}	3.67^{d}	0.12^{f}
Lignocerate	2.89^{e}	2.15^{h}	2.39^{fg}	3.13^{bcd}	3.35^{b}	2.61^{ef}
SFA	50.73	60.75	54.89	50.57	55.38	47.19
Myristoleic	0.41^{d}	1.99^{a}	1.00^{b}	0.22^{e}	0.10^{j}	0.13^{j}
cis-10-pentadecenoate	0.16^{fg}	0.18^{g}	0.46^{cd}	0.15^{fg}	0.95^{a}	0.10^{g}
Palmitoleate	7.08^{ab}	4.05^{f}	5.61^{de}	6.64^{bc}	6.11^{cde}	7.68^{a}
cis-10-heptadecenoate	1.79^{a}	0.78^{e}	0.33^{e}	1.00^{cd}	0.59^{cde}	1.79^{a}
trans-9-elaidate	3.27^{b}	3.00^{c}	1.13^{cd}	3.32^{b}	0.10^{d}	4.29^{ab}
cis-9-oleate	13.70^{d}	9.76^{f}	16.25^{c}	11.90^{e}	9.92^{f}	22.60^{a}
cis-11-eicosenoate	0.33^{e}	0.32^{e}	1.25^{bcd}	0.70^{de}	0.41^{e}	0.17^{e}
Erucate	0.17^{e}	0.15^{e}	0.10^{e}	0.10^{e}	0.10^{e}	0.53^{a}
Nervonate	1.29^{f}	0.84^{g}	1.88^{cde}	1.53^{ef}	2.04^{bcd}	1.60^{def}
MUFA	28.20	21.07	28.01	25.56	20.32	38.89
Linolelaidate	0.13^{b}	0.3^{b}	0.21^{b}	0.11^{b}	2.51^{b}	0.11^{b}
Linoleate	1.73^{c}	1.27^{ef}	0.2^{h}	1.29^{ef}	1.21^{f}	1.42^{de}
gamma-Linolenic	0.56^{e}	0.94^{c}	0.13^{i}	0.7^{d}	0.1^{j}	0.15^{h}
Linolenate	0.59^{cd}	0.41^{e}	0.4^{e}	0.52^{de}	0.39^{e}	0.44^{de}
cis-11,14-eicosadienoate	0.41^{c}	0.54^{bc}	0.21^{e}	0.43^{c}	0.1^{e}	0.23^{de}
cis-8,11,14-eicosatrienoate	0.70^{b}	0.92^{a}	0.1^{d}	0.67^{b}	0.1^{d}	0.46^{c}
cis-11,14,17-eicosatrienoate	4.17^{a}	0.21^{b}	0.53^{b}	4.09^{a}	0.1^{b}	4.09^{a}
cis-5,8,11,14-eicosatetraenoate	0.12^{e}	0.23^{a}	0.1^{g}	0.17^{c}	0.1^{g}	0.17^{c}
cis-13,16-docosadienoate	0.18^{c}	0.99^{a}	0.62^{b}	0.14^{c}	0.1^{c}	0.16^{c}
cis-5,8,11,14,17-eicosapentaenoate	0.41^{c}	0.48^{c}	1.7^{b}	0.45^{c}	0.1^{c}	0.22^{c}
cis-4,7,10,13,16,19-docosahexaenoate	11.56^{h}	12.49^{g}	13.6^{e}	15.99^{b}	19.31^{a}	7.04^{i}
PUFA	20.56	18.78	17.8	24.56	24.12	14.49
Laurate	0.20^{b}	0.20^{b}	0.10^{f}	0.17^{c}	0.10^{h}	0.80^{e}
Myristate	3.23^{j}	7.87^{b}	4.67^{g}	2.91^{k}	4.35^{c}	3.50^{i}
Pentadecanoate	1.28^{f}	1.90^{a}	1.17^{d}	1.36^{e}	1.06^{b}	1.14^{b}
Palmitate	26.88^{f}	24.30^{i}	33.68^{a}	27.44^{e}	29.58^{i}	25.30^{h}
Heptadecanoate	1.49^{h}	1.12^{i}	1.75^{g}	1.82^{e}	1.10^{ab}	1.94^{c}
Stearate	5.90^{c}	10.11^{abc}	10.81^{abc}	9.49^{abc}	11.69^{d}	11.25^{ab}
Arachidate	0.37^{b}	0.19^{e}	0.14^{g}	0.40^{a}	0.27^{k}	0.20^{e}
Heneicosanoate	0.42^{d}	0.35^{g}	0.47^{c}	0.10^{l}	0.21^{abc}	0.30^{h}
Docosanoate	0.32^{c}	0.37^{bc}	0.47^{a}	0.10^{e}	0.39^{bd}	0.34^{c}
Tricosanoate	1.72^{e}	4.82^{bc}	5.76^{b}	0.30^{f}	4.84^{bc}	7.06^{a}
Lignocerate	1.35^{i}	2.70^{def}	1.80^{h}	2.08^{gh}	3.30^{bc}	3.96^{a}

Continued

FA (%)	*L. malabaricus* from South Konawe	*L. malabaricus* from Bau-Bau	*L. malabaricus* from Wakatobi	*L. malabaricus* from Konawe Kepulauan	*L. malabaricus* from North Konawe	*L. malabaricus* from North Kolaka
SFA	45.59	54.66	61.42	46.78	57.49	56.47
Myristoleic	0.16^{h}	0.99^{c}	0.10^{j}	0.18^{f}	0.10^{j}	0.17^{g}
cis-10-pentadecenoate	0.44^{de}	0.23^{f}	0.67^{b}	0.10^{g}	0.51^{c}	0.26^{ef}
Palmitoleate	7.04^{ab}	5.72^{de}	5.30^{e}	6.88^{abc}	6.34^{cd}	6.28^{bcd}
cis-10-heptadecenoate	1.06^{bcd}	0.43^{de}	0.61^{cde}	1.73^{ab}	0.48^{de}	0.50^{de}
trans-9-elaidate	5.84^{a}	5.52^{a}	3.17^{bc}	5.42^{a}	5.77^{a}	5.46^{a}
cis-9-oleate	22.58^{a}	9.67^{f}	12.11^{e}	20.74^{b}	7.94^{g}	7.47^{h}
cis-11-eicosenoate	1.08^{cd}	2.08^{a}	1.26^{bcd}	0.13^{e}	1.78^{ab}	1.53^{abc}
Erucate	0.26^{cd}	0.22^{d}	0.39^{b}	0.37^{bc}	0.19^{de}	0.17^{de}
Nervonate	0.84^{g}	1.97^{bcd}	2.81^{a}	2.20^{bc}	2.06^{bc}	2.34^{b}
MUFA	39.30	26.83	26.42	37.75	25.17	24.18
Linolelaidate	1.8^{ab}	0.41^{b}	0.74^{ab}	0.18^{b}	0.28^{b}	0.19^{b}
Linoleate	5.36^{a}	1.26^{ef}	1.47^{d}	3.47^{b}	0.23^{h}	0.81^{g}
gamma-Linolenic	0.22^{f}	0.20^{g}	1.74^{a}	0.56^{e}	0.99^{b}	0.15^{h}
Linolenate	1.13^{b}	0.46^{de}	1.03^{b}	1.40^{a}	0.76^{c}	0.21^{f}
cis-11,14-eicosadienoate	0.4^{cd}	0.19^{e}	0.73^{a}	0.69^{ab}	0.17^{e}	0.21^{e}
cis-8,11,14-eicosatrienoate	0.47^{c}	0.10^{d}	0.89^{a}	0.67^{b}	0.11^{d}	0.42^{c}
cis-11,14,17-eicosatrienoate	0.89^{b}	0.20^{b}	0.75^{b}	4.49^{a}	0.25^{b}	0.14^{b}
cis-5,8,11,14-eicosatetraenoate	0.11^{f}	0.10^{g}	0.22^{b}	0.13^{d}	0.1^{g}	0.1^{g}
cis-13,16-docosadienoate	0.56^{b}	0.23^{c}	0.18^{c}	0.12^{c}	0.11^{c}	0.1^{c}
cis-5,8,11,14,17-eicosapentaenoate	0.55^{c}	1.96^{ab}	0.29^{c}	0.11^{c}	1.85^{ab}	2.38^{a}
cis-4,7,10,13,16,19-docosahexaenoate	5.94^{j}	14.00^{d}	4.93^{k}	4.31^{l}	13.31^{f}	15.78^{c}
PUFA	17.43	19.11	12.97	16.13	18.16	20.49

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

Different lowercase letters within a row are significantly different in Tukey's test ($p < 0.05$).

stearate/C18:0 (5.90%–13.38%), tricosanoate/C23:0 (0.12%–8.66%), lignocerate/C24:0 (1.80%–4.99%), palmitoleic/C16:1 (4.05%–8.11%), oleate/C18:1c (9.76%–22.58%), nervonate/C24:1 (0.13%–5.77%), linolelaidate/C18:2t (0.11%–5.67%), linoleate/C18:2c (0.19%–5.36%), and cis-4,7,10,13,16,19-docosahexaenoate, DHA/C22:6 (4.31%–21.84%).

Palmitic acid (C16:0) and stearic acid (C18:0) were the dominant SFA, contributing 44.46%–54.60% and 12.94%–23.11% of the total SFA of lipids for all samples, respectively. Palmitoleic acid (C16:1) and oleic acid (C18:1c) were found to be dominant in MUFA, accounting for 19.22%–26.11% and 46.32%–57.41% of total MUFA of lipids, respectively. Similarly, several researchers reported that palmitic acid (C16:0) is a dominant SFA and palmitoleic acid (C16:1) and oleic acid (C18:1c) are major MUFA [7,22]. While the most represented PUFA were linolelaidate (C18:2t), linoleate (C18:2c), and 4,7,10,13,16,19-docosahexaenoate, DHA (C22:6), accounting for 0.96%–34.22%, 0.84%–30.75%, and 26.72%–72.32% of total PUFA of lipids, respectively.

Classification of marine fish oil

PCA using variables of FA composition was performed to differentiate fish oils from *Epinephelus* sp. and *Lutjanus* sp. Figure 3 indicates the score plot between two fish oils of *Epinephelus* sp. and *Lutjanus* sp. with principal component 1 (PC1) of 50.1% and PC2 of 19.8%. The samples were almost overlapped each other showing a high similarity of their variables (FA composition) between the two groups observed through PCA. The reduction of original variables into several PCs was done in PCA to reduce the variables to be more effective for sample differentiation [23]. However, because PCA is an unsupervised technique that is usually used for initial sample grouping, it is required to apply supervised pattern recognition techniques such as PLS-DA to obtain better discrimination between two groups of fish oil samples. Supervised pattern recognition techniques such as PLS-DA are often used to evaluate the PCA result due to their capability for better classification between samples. Figure 4A shows that *Epinephelus* sp. fish oil could be better discriminated from *Lutjanus* sp. fish oil using PLS-DA compared to the PCA result using FA composition as the variables with PC1 of 31.8% and PC2 of 30.5%. It indicated that the latent variables used in creating the PLS-DA model could maximize the variation to obtain better discrimination between two fish oils. *Epinephelus* sp. fish oil samples from different locations appeared in a tight cluster compared to *Lutjanus* sp. fish oil samples. It showed more variability of FA compositions within *Lutjanus* sp. fish oils. Using PLS-DA, only several samples of *Lutjanus* sp. fish oils overlapped with samples from *Epinephelus* sp. fish oils,

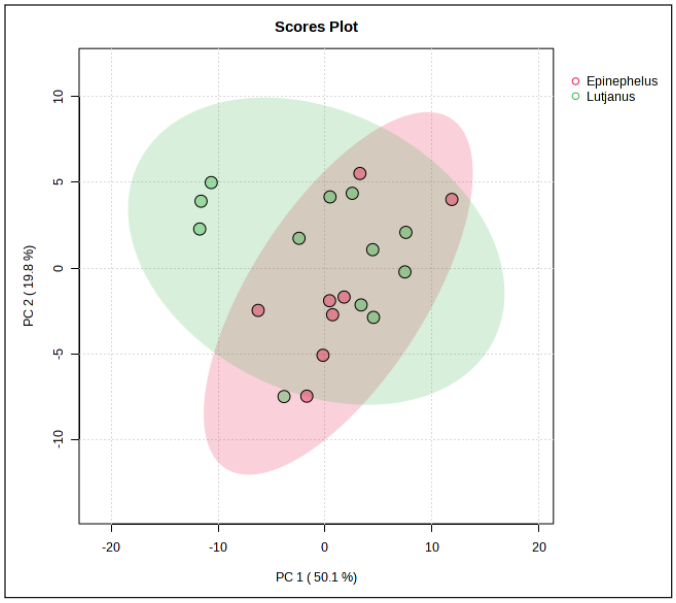
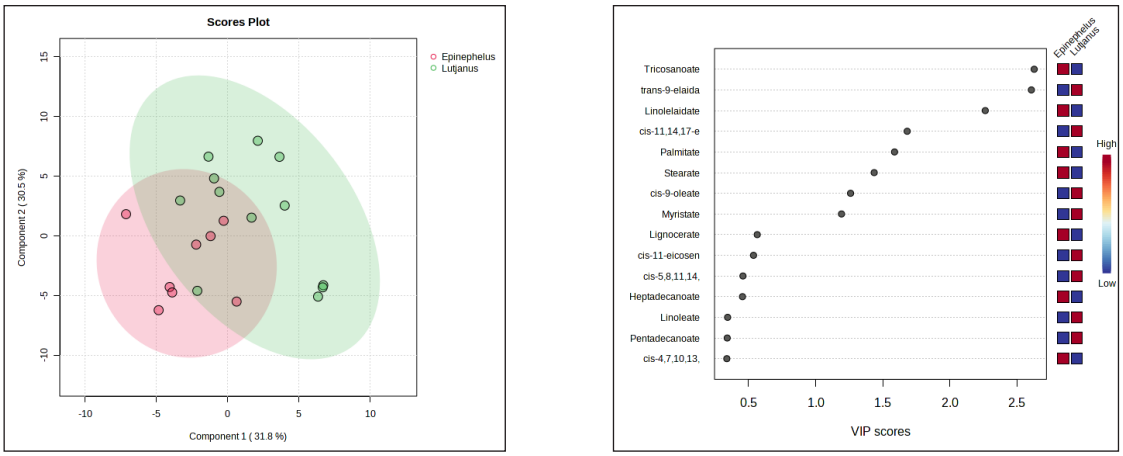


Figure 3. Score plot of PCA.

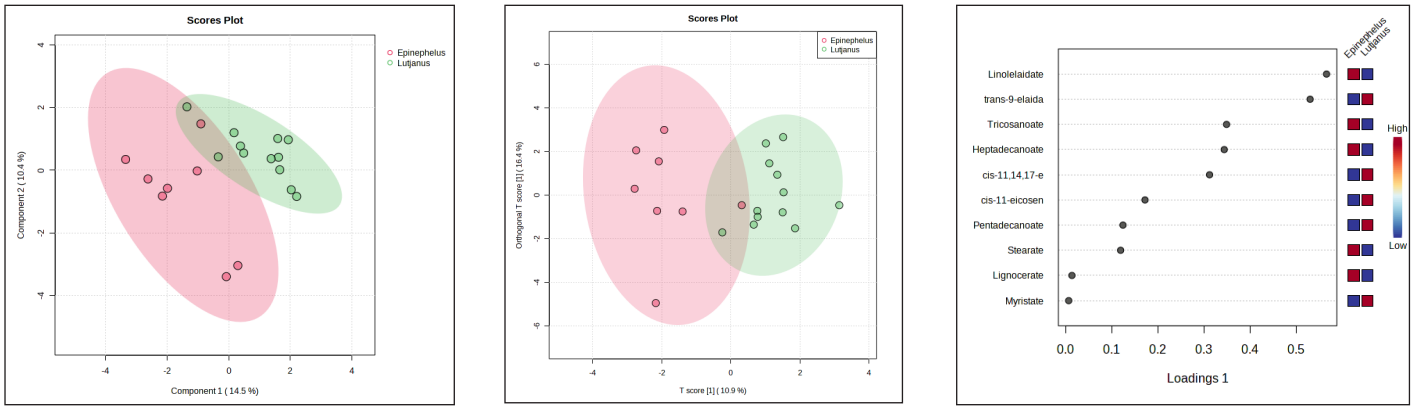
indicating the good performance of PLS-DA to discriminate between group samples. Through variable importance in projection (VIP) analysis in the PLS-DA model, variables of FAs that have a high contribution in discriminating fish oils from *Epinephelus* sp. and *Lutjanus* sp. could be identified as shown in Figure 4B. The FAs of tricosanoate (C23:0), trans-9-elaidate (C18:1t), linolelaidate (C18:2t), cis-11,14,17-eicosatrienoate (C20:3c), palmitate (C16:0), stearate (C18:0), cis-9-oleate (C18:1c), and myristate (C14:0) had important roles in discriminating *Epinephelus* sp. fish oils and *Lutjanus* sp. fish oils because they had VIP value larger than 1.0 [24,25]. FAs of tricosanoate (C23:0), linolelaidate (C18:2t), palmitate (C16:0), and stearate (C18:0), were significantly high in *Epinephelus* sp. fish oils whereas FAs of trans-9-elaidate (C18:1t), cis-11,14,17-eicosatrienoate (C20:3c), cis-9-oleate (C18:1c), and myristate (C14:0) were found to be in high levels in *Lujtanus* sp. fish oils.

The extension of PLS-DA analysis such as sPLS-DA and OPLS-DA was tried to classify *Epinephelus* sp. fish oils and *Lutjanus* sp. fish oils. Both sPLS-DA (Fig. 5A) and OPLS-DA (Fig. 5B) demonstrated better results for discriminating



A B

Figure 4. Score plot of (A) PLSDA and (B) analysis of VIPs value.



A B C

Figure 5. Score plot of (A) sPLSDA, (B) score plot of OPLS-DA, and (C) identification of important variables in sPLS-DA through loadings 1.

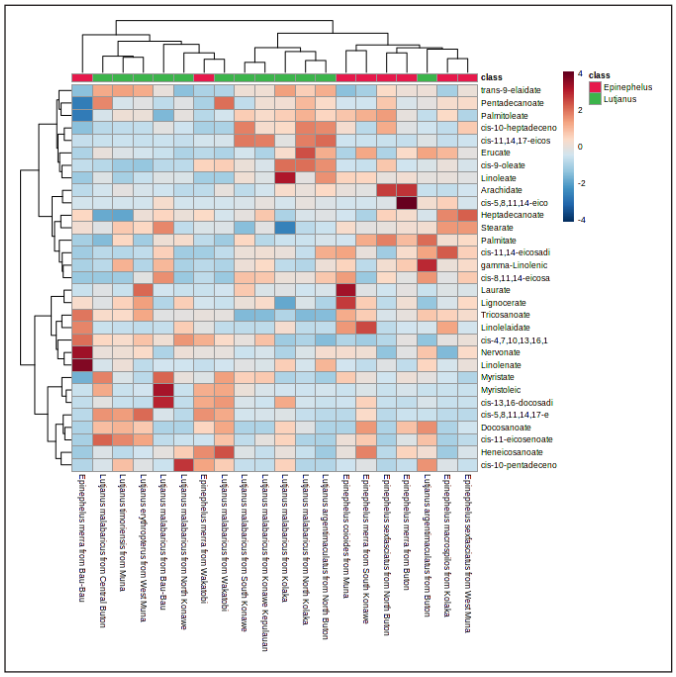


Figure 6. Heatmap analysis.

Epinephelus sp. fish oils and *Lutjanus* sp. fish oils. Better cluster separation were observed both in sPLS-DA and OPLS-DA between two groups of fish oils indicating better discrimination between the two groups. In sPLS-DA, the step of variable selection and classification was performed in one step simultaneously, meanwhile in OPLS-DA the variables were subjected to an orthogonal process. As a consequence, they often resulted in clearer discrimination and classification [26]. The variables which significantly contributed to sample discrimination in sPLS-DA were investigated through the first loading of sPLS-DA (Fig. 5C). The FAs of trans-9-elaidate (C18:1t), cis-11,14,17-eicosatrienoate (C20:3c), cis-11-eicosenoate (C20:1c), pentadecanoate (C15:0), and myristate (C14:0) were found to be high in *Lutjanus* sp. fish oils. Meanwhile, FAs of linolelaidate (C18:2t), tricosanoate (C23:0), heptadecanoate (C17:0), stearate (C18:0), and lignocerate (C24:0) were high in *Epinephelus* sp. fish oils.

Heatmap analysis (Fig. 6) for fish oil samples using all variables used could be used to observe the distribution of FAs in all fish oil samples extracted from various *Epinephelus* sp. and *Lutjanus* sp. A high amount of nervonate (C24:1c) and linolenate (C18:3c) was found only in *Epinephelus merra* from Bau-Bau. cis-5,8,11,14-eicosatetraenoate (C20:4c) was extremely high in *E. merra* from Buton. Meanwhile, laurate (C12:0), myristoleic (C14:1c), and linoleate (C18:2c) were significantly high only in *Epinephelus coioides* from Muna, *Lutjanus malabaricus* from Bau-Bau, and *L. malabaricus* from Kolaka, respectively. Heatmap analysis is very useful to identify the distribution pattern of compounds in certain samples which also could be used to monitor selected compounds that become targets for such authentication and quality control purposes.

CONCLUSION

GC-FID combined with supervised pattern recognition namely sPLS-DA and OPLS-DA offered a simple and convenient tool for quantification and discrimination of *Epinephelus* sp. and *Lutjanus* sp. The proportion of SFAs including tricosanoate, heptadecanoate, and stearate was greater in *Epinephelus* sp., meanwhile *Lutjanus* sp. showed the highest content of unsaturated FAs such as trans-9-elaidate, cis-11, 14, 17-eicosatrienoate, and cis-11-eicosanoate. Thus, it is used the main differences between *Epinephelus* sp. and *Lutjanus* sp.

ACKNOWLEDGMENT

The authors thank Kemendikbudristekdikti for financial support during this study through *Hibah Penelitian Dasar Unggulan Perguruan Tinggi* 2023 with contract number 41/UN29.20/PG/2023.

AUTHOR CONTRIBUTIONS

Irnawati concepted and designed research, prepared the manuscript, and made critical thinking on the manuscript, Anjar Windarsih prepared the manuscript and made critical thinking on the manuscript, Wa Ode Fasrida performed research activities, data acquisition, La Ode Muhamad Hazairin Nadia performed the statistical analysis and data interpretation, Abdul Rohman critically analyzed the manuscript. All authors read and agree on the published version of the manuscript.

CONFLICTS OF INTEREST

All authors declared that there are no conflicts of interest.

ETHICAL APPROVALS

In this study, the fish samples were obtained from local fishermen (caught within 0-24 hours), the fishes were not alive, thus, ethical approval is not required.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Liu J, Abdelmagid SA, Pinelli CJ, Monk JM, Liddle DM, Hillyer LM, *et al.* Marine fish oil is more potent than plant-based n-3 polyunsaturated fatty acids in the prevention of mammary tumors. J Nutr Biochem. 2018;55:41–52.
2. Adkins Y, Kelley DS. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J Nutr Biochem. 2010;21:781–92.
3. Mone P, Varzideh F, Kansakar U, Infante C, Lombardi A, de Donato A, *et al.* Omega-3 fatty acids coordinate glucose and lipid metabolism in diabetic patients. Lipids Health Dis. 2022;21:1–4.
4. Liu H, Wang F, Liu X, Xie Y, Xia H, Wang S, *et al.* Effects of marine-derived and plant-derived omega-3 polyunsaturated fatty acids on erythrocyte fatty acid composition in type 2 diabetic patients. Lipids Health Dis. 2022;21:1–14.
5. Sigaux J, Mathieu S, Nguyen Y, Sanchez P, Letarouilly JG, Soubrier M, *et al.* Impact of type and dose of oral polyunsaturated fatty acid supplementation on disease activity in inflammatory rheumatic

diseases: a systematic literature review and meta-analysis. Arthritis Res Ther. 2022;24:100.
6. 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. Euro J Lipid Sci Technol. 2017;119:1600516.
7. Özogul Y, Özogul F, Çiçek E, Polat A, Kuley E. Fat content and fatty acid compositions of 34 marine water fish species from the Mediterranean sea. Int J Food Sci Nutr. 2009;60:464–75.
8. Abd Aziz N, Azlan A, Ismail A, Mohd Alinafiah S, Razman MR. Quantitative determination of fatty acids in marine fish and shellfish from warm water of straits of Malacca for nutraceutical purposes. Biomed Res Int. 2013;2013:284329.
9. 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 Bioproc Tech. 2014;7:2410–22.
10. Yi T, Li SM, Fan JY, Fan LL, Zhang ZF, Luo P, *et al.* Comparative analysis of EPA and DHA in fish oil nutritional capsules by GC-MS. Lipids Health Dis. 2014;13:1–6.
11. Killeen DP, Card A, Gordon KC, Perry NB. First use of handheld raman spectroscopy to analyze omega-3 fatty acids in intact fish oil capsules. Appl Spectrosc. 2020;74:365–71.
12. Bekhit MY, Grung B, Mjøs SA. Determination of omega-3 fatty acids in fish oil supplements using vibrational spectroscopy and chemometric methods. Appl Spectrosc. 2014;68:1190–200.
13. Karunathilaka SR, Mossoba MM, Chung JK, Haile EA, Srigley CT. Rapid prediction of fatty acid content in marine oil omega-3 dietary supplements using a portable Fourier transform infrared (FTIR) device & partial least-squares regression (PLSR) analysis. J Agric Food Chem. 2017;65:224–233.
14. Killeen DP, Marshall SN, Burgess EJ, Gordon KC, Perry NB. Raman spectroscopy of fish oil capsules: polyunsaturated fatty acid quantitation plus detection of ethyl esters and oxidation. J Agric Food Chem. 2017;65:3551–8.
15. Lopes TIB, Pereira ES, Freitas DdS, Oliveira SL, Alcantara GB. Spectral profiles of commercial omega-3 supplements: an exploratory analysis by ATR-FTIR and ^{1}H NMR. J Food Sci Technol. 2020;57:1251–7.
16. Viswanathan S, Verma PRP, Ganesan M, Manivannan J. A novel liquid chromatography/tandem mass spectrometry (LC–MS/MS) based bioanalytical method for quantification of ethyl esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and its application in pharmacokinetic study. J Pharm Biomed Anal. 2017;141:250–61.
17. Alinafiah SM, Azlan A, Ismail A, Rashid NKMA. Method development and validation for omega-3 fatty acids (DHA and EPA) in fish using gas chromatography with flame ionization detection (GC-FID). Molecules. 2021;26:6592.
18. Xiao M, Qian K, Wang Y, Bao F. GC-MS metabolomics reveals metabolic differences of the farmed Mandarin fish *Siniperca chuatsi* in recirculating ponds aquaculture system and pond. Sci Rep. 2020;10:1–8.
19. Pollo BJ, Teixeira CA, Belinato JR, Furlan MF, de Matos Cunha IC, Vaz CR, *et al.* Chemometrics, comprehensive two-dimensional gas chromatography and "omics" sciences: basic tools and recent applications. TrAC Trends Anal Chem. 2021;134:116111.
20. Quero-Jiménez PC, Felipe LAA, Prieto García JO, Rodríguez MEJ, De La Torre López JB, Montenegro ON, *et al.* Local Cuban bentonite clay as potential low-cost adsorbent for shark liver oil pool purification. J Pharm Pharmacogn Res. 2021;9:525–36.
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. 2022;12:115–22.
22. Durmuş, M. Fish oil for human health: omega-3 fatty acid profiles of marine seafood species. Food Sci Technol. 2019;39:454–61.
23. Paul A, De P, Harrington B. Chemometric applications in metabolomic studies using chromatography-mass spectrometry. TrAC Trends Anal Chem. 2021;135:116165.
24. Mabuchi R, Ishimaru A, Tanaka M, Kawaguchi O, Tanimoto S. Metabolic profiling of fish meat by GC-MS analysis, and correlations with taste attributes obtained using an electronic tongue. Metabolites. 2019;9:1–11.
25. Jahani R, Yazdanpanah H, van Ruth SM, Kobarfard F, Alewijn M, Mahboubi A, *et al.* Novel application of near-infrared spectroscopy and chemometrics approach for detection of lime juice adulteration. Iran J Pharm Res. 2020;19:34.
26. Jiménez-Carvelo AM, Martín-Torres S, Ortega-Gavilán F, Camacho J. PLS-DA vs sparse PLS-DA in food traceability. A case study: authentication of avocado samples. Talanta. 2021;224:121904.

How to cite this article:
Irnawati I, Windarsih A, Nadia LOMH, Rohman A, Fasrida WO. Quantification and classification of fatty acids in marine fish oil from Southeast Sulawesi using gas chromatography and chemometrics. J Appl Pharm Sci. 2024;14(03):162–169.