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MINI REVIEW

Fatty acid composition, biological activity and authentication of marine fish oil

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Abstract

Marine fish oils (MFO) are known as sources of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), having beneficial effects on human health. MFO is considered an important raw material in the pharmaceutical, food, and cosmetics industries. It is classified as high price oil; therefore, MFO are subjected to adulteration with low price oils. This review aimed to provide a scientific view of the fatty acid composition, biological activity, and authentication of marine fish oil. Different species of fish have different nutritional content such as triacylglycerols composition, especially DHA, EPA, and lipid-soluble vitamins. Marine fish oil is one of the functional oils that have beneficial effects on human health. Marine fish oil has a high price in the market which is lucrative to be adulterated with lower-priced ones to gain economic profit, therefore reliable analytical methods for detecting the possible adulterated such as combined fourier transform infrared and chemometrics.

1. Introduction

Fish oil is a marine bioactive and has recently received much attention. Marine fish oil (MFO) contains a high level of omega-3-polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), vitamins (A, D and E) (Durmuş, 2019). The consumption of omega-3 fatty acid series (EPA and DHA) have beneficial human health effects, especially brain development for children (He *et al*., 2017), prevention of cognitive disorder and diabetes (Graciano *et al*., 2016), protective effect on type 2 diabetes factors via increased insulin-secretion and decrease pro-oxidant (Liu *et al*., 2022), cardioprotective effect by triglyceride-lowering effect, inhibiting platelet monocyte aggregation (Adkins and Kelley, 2010; Mone *et al*., 2022), remodeling skeletal muscle fiber via fat mass and obesity -associated gene/N6-methyladen-osine/DNA damage-induced transcript 4/peroxisome proliferator-activated receptor-γ coactivator 1α (FTO/m6A/DDIT4/PGC1α) signaling (Chen, Chen, Wu *et al*., 2022). In addition, omega-3 consumption from an animal source (>2 g/day) can improve inflammatory rheumatic disease activity (Sigaux *et al*., 2022), while the high dose (>6 g/day) of fish oil consumption as a triglyceride-lowering agent in hypertriglyceridemia patients (Durmuş, 2019).

Since the fatty acid composition of fish oils affects human health, therefore the nutritional information of oil is important. The fatty acid composition of fish varies between and within species; even dark and white muscles are affected by many factors such as diet,

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temperature, salinity, age, size, and season (Özogul *et al*., 2009). Several studies concerning the fat content and fatty acid profile of fish oil have been published, such as the fatty acid content of 20 species of marine fish from Straits Malacca (Abd Aziz *et al*., 2013), 34 marine fish waters from the Mediterranean Sea (Özogul *et al*., 2009) also a by-product from Chilean fishes (Rincón-Cervera *et al*., 2017). In addition, Srigley and Rader (2014) have reported the fatty acid profile of 46 commercially available marine oil omega-3 supplements.

The adulteration of high-priced edible oils with lower ones is typically motivated by economic gains. The practice of adulteration in oils has been a concern not only by consumers and producers but also by the government. Oil's quality and beneficial effects on human health decrease due to adulteration practices (Rohman *et al*., 2020). Our team has reviewed the authentication analysis of oil; such as the application of spectroscopy combined chemometrics for the analysis of fats and oils in food products (Rohman *et al*., 2020), molecular spectroscopy and chromatography combined chemometrics for authentication of fish oils (Rohman *et al*., 2021), application of Raman spectroscopy and chemometrics for fats and oils quality control (Windarsih *et al*., 2021). Specifically, this review aimed to discuss fatty acid compositions and biological activities of marine fish oil as well as its authentication analysis.

2. Methodology

During preparing this review, some articles appearing in several databases such as Web of Science, PubMed, and Google Scholar were explored. The keywords were carried using “Marine fish oil”, “biological activity”, “Fatty acid profiling”, and “authentication”. The inclusion criteria of selected articles were (a) studies regarding fatty acids composition of marine fish oil between 2009-2023, (b) studies on the biological activity of marine fish oil between 2009-2023, (c) studies on analysis and authentication of marine fish oil between 2009-2023; (d) all articles written in the English language.

3. Characterization

Determination of the physicochemical properties of marine fish oil (MFO) including yield, iodine value, acid value, anisidine value, peroxide value, saponification value, and oxidation products is important for fish oil characterization (Rohman, 2017). The extraction process affects the characterization of the oils because different extraction methods provide different physicochemical properties of MFO. A study on the extraction of Salmon oil using two types of extraction methods, namely the Soxhlet extraction technique and microwave-assisted extraction technique showed different yields. Salmon oil was extracted from three different parts, backbones, viscera, and heads. The oil obtained from the Soxhlet technique was 57±1% (backbones), 56±2% (heads), and 77±2% (viscera). Meanwhile, the yield obtained from microwave-assisted technique was 39.4±0.3%, 38±1%, and 71±2% for backbones, heads, and viscera respectively. Therefore, the use of the Soxhlet extraction method resulted in a higher extraction yield than using microwave (de la Fuente *et al*., 2022).

The parameters of acid value, conjugated diene, and peroxide value, are associated with the quality of MFO. A study on the characterization of shark liver oil has been carried out. Acid value become the most important parameter for assessing fish oil quality (Zhang *et al*., 2022). Acid value is correlated to the level of rancidity of the oils. In addition, peroxide value also can be used as an initial indicator of rancidity in unsaturated fats contained in oils. The peroxide value determines the primary oxidation in oils which is also associated with rancidification. It has been applied to measure the initial rancidity of different cooking oils subjected to light and heat (Kaleem *et al*., 2015). High peroxide value is associated with the rancid oils and fats although low peroxide value also may be observed from rancid oils and fats (Esfarjani *et al*., 2019). The determination of acid value from seven different oil extraction techniques of shark liver showed that the oil yielded from five extraction techniques complied with the requirement by the Codex Alimentarius Commission. The measurement of conjugated diene indicated the early stage of oil rancidity. Shark liver oil demonstrated a conjugated diene value of 2.04 μM. This result was still acceptable for fish oil samples indicating that the shark liver oil obtained has good stability from oxidation. In addition, the peroxide value indicated the degree of oxidation as well as the rancidity of the shark liver oil. All the shark liver oil samples demonstrated acceptable peroxide values that comply with the requirement (Quero-Jiménez *et al*., 2020)

Tengku-Rozaina and Birch (2013) have studied the physicochemical properties of MFO such as refined hoki oil, unrefined hoki oil, and unrefined tuna oil. Hoki oil and tuna oil were yellow but, hoki oil was darker yellow than tuna oil. The parameters such as unsaponifiable matter, peroxide value, and p-anisidine value were measured. Hoki oil presented a higher value of unsaponifiable matter accounted for (4.90–7.24%) compared to tuna oil (0.56%). Determination of the peroxide value showed that refined hoki oil and tuna oil had peroxide values that exceeded the maximum limit (5 meq O_2 per kg). Meanwhile, the peroxide value of unrefined hoki oil was 3.09 meq O_2 per kg, which is

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acceptable for consumption because it was lower than the maximum limit. The peroxide value is associated with the primary oxidation products in fish oils. On the other hand, the determination of p-anisidine value showed that unrefined tuna oil demonstrated high p-anisidine value and exceeded the maximum limit acceptable for human consumption. The p-anisidine value is correlated to the secondary oxidation products in fish oil. The high p-anisidine value indicated the presence of high PUFA in tuna oil. It is in accordance with the result of fatty acid measurement that the unrefined tuna oil contained 42.57% PUFA whereas PUFA in hoki oil was 28.79-30.13%. The results of the characterization showed that hoki oil had better stability than tuna oil.

The physicochemical of tuna oil obtained from different extraction methods including chemical solvent using Bligh and Dyer method, physical method, and enzymatic method has been determined. The parameters for evaluation include a refractive index, iodine value, acid value, saponification value, and peroxide value. The refractive index of tuna oil obtained from three extraction methods were similar; 1.4783±0.02, 1.4807±0.05, and 1.4803±0.01 for physical method, solvent method, and enzymatic method, respectively. The refractive index was measured at a temperature of 40^{o}C. The iodine value of tuna oil from chemical extraction methods had the highest value (62.11±0.8 g Iodine/100 g oil) compared to physical method (21.27±1.1 g Iodine/100 g oil) and enzymatic method (27.12±2.1 g Iodine/100 g oil). For other parameters such as saponification value, acid value, and peroxide value, tuna oil obtained from enzymatic extraction methods had the lowest value compared to the other two extraction techniques indicating better oil quality obtained from the enzymatic extraction method. The values were 164.21±0.08 mg KOH/g oil for saponification value, 1.96±0.7% oleic acid for acidity index, and 5.14±0.03 mEq O_2/kg oil) for peroxide value. These results indicated that the enzymatic extraction method could improve the quality of tuna oil (de Oliveira *et al*., 2017).

A study on the physicochemical properties of MFO over multiple years has also been conducted using third-party database. The parameters such as acid value, p-anisidine value, TOTOX limit, and peroxide value were observed. The acid value of marine fish oil showed that 2.1% of samples had an acid value exceeding 3 mg KOH/g oil, while the p-anisidine value of unflavored oil from 6.1% of samples exceeded 20. A number of 8.8% samples had TOTOX values more than the acceptable limit (more than 26). Moreover, the peroxide value from 13.9% of analyzed samples was high, and exceeded the maximum limit (more than 5 mEq O_2/kg). These results showed that most fish oil products in the market met the regulatory requirements (De Boer *et al*., 2018).

4. Fatty acid composition

Marine fish oils are widely accepted as healthy and highly nutritious food supplements. People usually think that different types of fish have the same nutritional content as fish oils. Therefore, it is important to be aware of the different nutrients of fish oils by providing nutritional content that is associated with various health-related effects, especially fatty acid content (Nurnadia *et al.,* 2011). The composition of fatty acid in 20 species of marine fish from the Straits of Malacca has been reported. Quantitative determination of fatty acid content regarding percentages of total fatty acids. This study revealed three samples as being outstanding based on the composition of fatty acid namely yellowstripe scad, moonfish and longtail shad. Overall marine fish samples contained fairly high amounts of PUFAs especially alpha -linolenic acid, eicosapentaenoic acid and docosahexaenoic acid as provide beneficial effects for human health (Abd Aziz *et al*., 2013).

Saturated fatty acids (SFAs) and palmitic acid (C16:0), contribute approximately 65% of the total SFAs in marine fish oils (Zhang *et al*., 2020). Based on Table 1, Rainbow sardine oil has the greatest SFA content (38.3%). The percentages of Monounsaturated fatty acids (MUFAs) present in the total fatty acids of the fish species varied over a wide range. The MUFAs in Mediterranean Hake oils constituted half of the total fatty acids up to 50.2%. The proportion of Polyunsaturated fatty acids (PUFAs) ranged from 16.1-51.4%. Shortfin mako oils contain the highest PUFAs. The greatest percentage of DHA is in European anchovy oil (20.7%), while Antarctic krill contains the highest EPA (17.8%).

Durmuş (2019) studied the fatty acid compositions of 13 different seafood species. This study was observed the most important fatty acid in most species were myristic acid, palmitic acid, palmitoleic acid heptadecanoic acid, stearic acid, vaccenic acid, oleic acid, linoleic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. The range of fatty acid contents was 27.67% to 36.59% for SFAs, 8.99% to 35.8% for MUFAs and 10.69% to 39.57% for PUFAs. The highest of total PUFAs was found in European squid, bogue, twaite shad, blue-spotted cornetfish and spiny gurnard. Overall seafood species in the Northeastern Mediterranean had high levels of EPA and DHA with PUFA/SFA ratio at least 0.45 except shi drun species.

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Table 1. Fatty acid content of marine fish oils.

Fish oil	Fatty acid content					References
	ΣSFAs	ΣMUFAs	ΣPUFAs	DHA (n-3)	EPA (n-3)	
Cod (*Gadus morua*) liver	16.7	46.6	26.1	12.5	9.6	Loftsson *et al*. (2016)
Fourspot megrim (*Lepidorhombus boscii*)	28.3	49.3	19.1	8.9	2.9	Guil-Guerrero *et al*. (2011)
Shortfin mako (*Isurus oxirinchus*)	23.6	21.9	51.4	25.2	11.3	
Great weeve (*Trachinus draco*)	28.5	18.2	38.6	20.6	10.8	
Axillary seabream (*Pagellus acarne*)	35.5	42.8	16.1	5.9	3.0	
Atlantic Mackerel (*Scomber scombrus*)	23.3	43.6	28.7	11.6	3.4	
European anchovy (*Eugraulis encrasicholus*)	24.1	14.2	47.9	20.7	10.0	
European pilchard (*Sardina pilchardus*)	35.8	24.5	31.7	11.8	9.1	
Forkbeard (*Phycis phycis*)	23.8	41.6	21.8	9.5	6.0	
Blue whiting (*Micromesistius poutasou*)	25.7	34.8	34.4	15.9	9.6	
Mediterranean Hake (*Merlucius merlucius Mediterraneus*)	24.9	50.2	21.0	8.0	5.4	
European Hake (*Merlucius merlucius*)	23.9	32.2	37.6	19.1	9.8	
Rainbow sardine fish (*Dussumieria acuta*)	38.3	14.9	43.7	17.4	15.4	Homayooni *et al*. (2014)
Shark (*Musteleus musteleus*)	32.6	33.3	34.1	17.2	1.9	Zarai *et al*. (2020)
White Shark (*Carcharodon carcharias*) liver	31.3	35.6	29.8	13.4	2.3	Pethybridge *et al*. (2014)
Tuna (*Thunnus albacares*)	27.9	27.7	44.3	8.7	6.1	Nazir *et al*. (2017)
Atlantic Salmon (*Salmon salar*)	19.6	43.2	30.8	5.5	5.3	Cascant *et al*. (2018); Pando *et al*. (2014)
Antarctic krill (*Euphausia superba*)	35.1	34.7	30.1	12.3	17.8	Xie *et al*. (2017)

*FA calculate as area % on TFA area

5. Biological activity of marine fish oil

Several researchers have reported the beneficial effects of marine fish oil such as antitumor, antidiabetic, and medication for cardiovascular disease. The biological activities of marine fish oil are related to the high contents of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

5.1 Antitumor

The inhibition of mammary carcinogenesis of marine polyunsaturated fatty acids (PUFA) such as EPA and DHA have been reported. Liu *et al*. (2018) examined the antitumor effect of marine or plant PUFA on pubertal mammary gland and tumor development in MMTV -*neu* (ndl)-YD5 mice. The males of MMTV-FVB were bred with wild-type females and fed either: 10% safflower (1), 10% flaxseed (2), 7% safflower plus 3% flaxseed (3), or 7% safflower plus 3% fish oil (4) diet. Female offspring were maintained on parental diets. This study revealed that the antitumor effects of EPA and DHA have greater potency than plant polyunsaturated fatty acids. Fish oil (3%) significantly down-regulated the expression of genes both in eicosanoid synthesis and inflammation. Anti-tumorigenic effects were associated with altered HER2, pHER-2, pAkt and Ki-67 protein expression.

The effect of lifelong exposure of n-3 PUFA, SFA and MUFA toward mitigation of mammary tumor (Her-2 Breast Cancer) on MMTV-neu (ndl) YD5 mouse models were examined. The fed model of maternal containing 10% safflower oil (n-6 PUFA), 3% menhaden oil + 7% safflower oil (marine n-3 PUFA), 3% flaxseed + 7% safflower oil (plant-based n-3 PUFA), 10% olive oil (MUFA or 10% lard (SFA). This study showed that the marine-derived n-3 PUFA best mitigates BC outcomes compared to other dietary fatty acids (Hillyer *et al*., 2020). In addition, fish oil supplementation delayed tumor onset, reduced mammary tumor multiplicity, improved mammary tumor apoptosis, decreased tumor protein expression of activated Akt, NFkB p65 and STAT3, reduced tumor mRNA expression of inflammatory mediators TNFα, IL-6, and leptin in the mouse modeled (obesity-associated mammary tumorigenesis in the MMTV-neu(ndl)-YD5) (Monk *et al*., 2021).

The effect of the maternal n-3 PUFA diet on breast cancer risk in female have been reported by Li, Li, Gao *et al*. (2018). In this study, the pregnant C57BL/6J mice were divided into several groups, namely the control group (fed a normal diet), a high-fat diet including Safflower oil, fish oil or flaxseed oil throughout gestation and lactation. Supplementation of fish oil in both pregnant dams and offspring could decrease the plasma concentration of 17β-estradiol, delay puberty onset, decrease the number of epithelial terminal end buds, reduce cell proliferation and increase apoptosis. In addition, maternal supplementation with fish oil-induced apoptosis signaling pathway, long noncoding RNA in

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p53 and inhibited signaling pathway of NF-kB and Jak-STAT. Thus, maternal supplementation of fish oil has a protective effect on the mammary tumor risk of female offspring.

5.2 Antidiabetic

The beneficial effects of fish oil have been reported on in vitro, in vivo and clinical studies. Graciano *et al*. (2016) have investigated the effect of EPA and DHA in combination with palmitic acid on pancreatic beta cell redox state and function. This study revealed that EPA and DHA could decrease peroxide production after 1 hour of incubation, while palmitic acid treatment was shown 48 hours after incubation. Therefore, all types of omega-3 could be used to increase insulin levels, enhance defense capacity of antioxidant enzymatic, and decrease pro-oxidant generating activities.

The glycolipid metabolism disorder was correlated with dietary fatty acid intake. Liu *et al*. (2022) reported the different effects of marine-derived and plant-derived omega-3 on the composition of erythrocyte fatty acids and glycolipid metabolism in type 2 diabetes mellitus patients. This study was conducted on 180 type 2 diabetes mellitus patients who were randomly assigned to three groups, 52 of them in the fish oil group, 50 in the perilla oil group, and 28 in the mixed linseed and fish oil at a dose of 3 g/day each for six-month intervention. This study revealed that the supplementation of fish oil could decrease the triglyceride level, while the perilla oil could regulate the glycolipid metabolism via decreased fast blood glucose. The supplementation of both sources of omega-3 PUFA significantly decreased C-peptide and insulin concentrations, apolipoprotein A1, IL-6 and serum total cholesterol levels.

In addition, Mone *et al*. (2022) stated that diabetes mellitus and dyslipidemia have an association with increasing the risk of coronary artery diseases. The potential molecular mechanism of plant-derived and marine-derived omega-3 PUFAs such as (a) expression of G-protein coupled receptor 120 (GPR120) on endocrine L cells lining the gut directly increased glucagon-like peptide-1 (GLP-1), (b) the activation of GPR40 by long chain fatty acids, GPR80 by medium chain fatty acids, and GPR41 and GPR43 by short chain fatty acids, and represent other via direct action or mediate by GLP-1 on pancreatic islets, or on adipose and hepatic tissue. The marine-derived omega-3 PUFA could decrease plasma levels of proprotein convertase subtilisin kexin type 9 (PCSK9). The inhibitor of PCKSK was used to reduce hypercholesterolemia, therefore marine omega-3 can be used for cardiovascular disease treatment.

5.3 Cardiovascular diseases

Cardiovascular disease is one of the leading causes of death worldwide. The elevated cardiovascular risk associated with air pollution exposure. It has been reported that the supplementation of omega-3 PUFA has attenuated adverse of cardiovascular disease after exposure to fine particulate (PM_{25}), however, the effect of omega-3 PUFA against short-term exposure to low-level air pollution in healthy participants is unclear. Therefore, this study aimed to show the protective effect of omega-3 PUFA against short-term exposure to low-level ambient air pollution. Sixty-two healthy participants with low and high dietary omega-3 PUFA were repeatedly assessed for at least 7 days with indicators used are blood lipids, markers of vascular inflammation, coagulation and fibrinolysis, heart rate variability (HRV), and repolarization. This study revealed that the participants with low dietary omega-3 intake and short-term exposure to both PM_{25} and 8-h ozone (O_3) were associated with changing all parameters such as total cholesterol, von Willebrand factor (vWF), tissue plasminogen activator, D-dimer, and very low-frequency HRV for PM_{25} exposure and total cholesterol, high-density lipoprotein, serum amyloid A, soluble intracellular adhesion molecule 1 and vWF for O_3 exposure (Chen, Zhang, Shen *et al*., 2022).

6. Authentication

Chemometrics can be described as the combination of mathematical and statistical techniques to overcome several problems in chemistry including processes or systems. In the field of analytical chemistry, chemometrics has been widely applied to assist in the improvement and development of instrumental methods (Brereton *et al*., 2017). Several chemometrics algorithms such as Principal Component Analysis (PCA), Discriminant Analysis (DA), Artificial Neural Networks (ANN), Principal Component Regression (PCR), Partial Least Squares Regression (PLSR), Soft Independent Modelling of Class Analogies (SIMCA), and Partial Least Squares – Discriminant Analysis (PLS-DA) can be employed to improve the quality of the analytical methods (Miller *et al*., 2018; Yehia and Mohamed, 2016). Chemometrics can be applied for spectroscopy-based foodstuff analysis and authentication due to the complex nature of the response obtained from spectroscopy instrumentation for further multivariate chemometric strategies (Biancolillo *et al*., 2020). These strategies become more popular since there were several cases of food adulteration and fraud motivated by economic pursuit (Li, Zhang, Li *et al*., 2018). Cheap oils such as palm oil, argemone oil, cottonseed oil, and rapeseed oil have been reported as adulterants for fat and

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oil products (Abhirami and Radha, 2015; Navya *et al*., 2017; Yadav, 2018).

Authentication of oils can be executed by combining outputs from analytical methods with chemometrics techniques. Previous studies from Irnawati *et al*. (2021) have successfully applied PCA for authenticating several oils aided by open-source chemometrics software (Irnawati *et al*., 2021). Other studies from Rohman *et al*. (2021) reported that a combination of Fourier Transform Infrared (FTIR) spectroscopy and other molecular spectroscopies with chemometrics offered promising methods for performing rapid screening to evaluate the adulteration of fish oil (Rohman *et al*., 2021). Figure 1 presents the illustration of chemometrics employment for marine fish oil authentication. A prediction model can be generated using a training data set. These data were labeled using an appropriate label as well as their categories or classes. The prediction model for authentication purposes has been generated using chemometrics modeling. The quality of the prediction models was evaluated using independent test data with the evaluation of performance by applying mathematics and statistical techniques. Studies on marine fish oil authentication aided by chemometrics techniques are reported in Table 2.

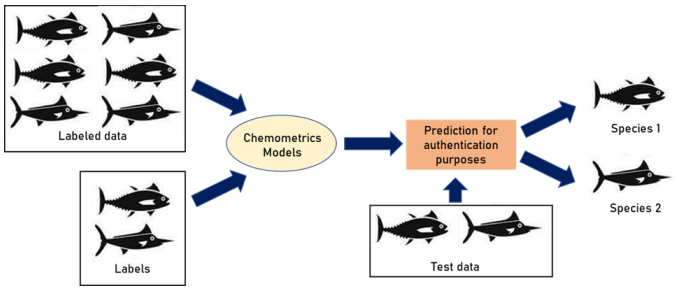

Figure 1. Authentication scheme for marine fish oil analysis using chemometrics techniques.

PCA, one of the most popular chemometrics techniques, has been widely applied in marine fish oil analysis. PCA was used not only for data visualization but also for exploratory data analysis. Several studies related to marine fish oil exploit PCA before applying other chemometrics techniques. Karunathilaka *et al*. (2019) used PCA to visually examine the distribution of triacylglycerol (TAG), concentrated ethyl ester (EE), and TAG+EE test samples. Further chemometrics techniques namely PLS-DA and PLSR were applied to differentiate and quantify several marine oils (Karunathilaka *et al*., 2019). PCA and PLSR were also generated in two studies by Mustafidah *et al*. (2021) and Killeen *et al*.

Table 2. Studies on marine fish oil authentication aided by chemometrics techniques.

No	Sources	Chemometrics Techniques	Aim of the studies	References
1	Ninety-five dietary supplements containing marine oil omega-3	PCA, PLS-DA, PLSR	Differentiating and quantifying several marine oils	Karunathilaka *et al*. (2019)
2	Eight fish oil samples obtained from fish	PLSR	Developing prediction models to evaluate percentages of fatty acids content	Nieto-Ortega *et al*. (2022)
3	Milkfish from a local market in Juwana Pati, Central Java, Indonesia	PCA, PCR, PLSR	Authenticating milkfish fish oil from palm oil as an adulterant	Mustafidah *et al*. (2021)
4	Twenty-eight cod liver oils	ANN	Determining cod liver oil adulteration with sunflower and canola oils	Giese *et al*. (2019)
5	Commercial fish oil supplements in capsules from Brazil, Canada, Spain, and the US	PCA, PLS-DA	Evaluating the lipid form of fish oil supplements	Amorim *et al*. (2021)
6	Grass carp (*Ctenopharyngodon idella*), Pacu (*Piaractus mesopotamicus*), and Catfish (*Ictalurus punctatus*)	DA	Evaluating the lipid profile of three fish species	Tonial *et al*. (2022)
7	White croaker surimi (*Argyrosomus argentatus*), hairtail surimi (*Trichiurus haumela*), and red coat surimi (*Nemipterus virgatus*)	PCA, LDA	Classifying marine fish surimi by according to the species	Zhang *et al*. (2017)
8	Industrially prepared fish fillets	PCA, LDA, SIMCA	Investigating the potential substitution of valuable species with cheaper ones	Alamprese and Casiraghi (2015)
9	Eleven commercial ω-3 polyunsaturated fatty acids oil supplements in soft gel (gelatin) capsules	PCA, PLSR	Investigating the suitability of FT-Raman spectroscopy for evaluating fish oil parameters	Killeen *et al*. (2017)
10	Cod liver oil (CLO)	PLSR	Quantification of CLO in binary mixture with corn oil	Man (2011)

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(2017). Both studies used PCA to characterize the oil samples whereas the quantification have been performed using PLSR. Milkfish from a local market in Juwana Pati, Central Java, Indonesia were successfully authenticated from palm oil as an adulterant by FTIR spectroscopy combined with PCA, PCR, and PLSR (Mustafidah *et al*., 2021). FT-Raman spectroscopy has been studied to evaluate fish oil parameters from samples of commercial ω-3 polyunsaturated fatty acids oil supplements in soft gel (gelatin) capsules. It was also found that oils with peroxide values of as low as 10 meq kg^{-1}, could be readily differentiated from oils that were within specification (7 meq kg^{-1}) (Killeen *et al*., 2017).

The dual combination of PCA and discriminant analysis such as LDA or PLS-DA was widely applied in marine fish oil authentication studies. PCA and LDA were successfully used to classify marine fish surimi according to the species and investigate the potential substitution of valuable species with cheaper ones in the case of industrially prepared fish fillets (Alamprese and Casiraghi, 2015; Zhang *et al*., 2017). These two studies employed infrared spectroscopy for the data acquisition process since the infrared spectra properties can be linked to the characteristics of each type of oil. PCA and PLS-DA were applied in evaluating the lipid form of fish oil supplements in capsules from Brazil, Canada, Spain, and the US (Amorim *et al*., 2021). TAGs and EEs were clearly distinguished. It was found that a better prediction model for TAG and EE fish oil samples can be generated using ATR-FTIR spectroscopy (R^2 = 0.99) compared to Raman spectroscopy (R^2 = 0.95).

7. Conclusion

Marine fish oil is rich in unsaturated fatty acids which have many beneficial effects on human health. MFO commanded a high price in the market which can be a target of adulteration practice, therefore reliable analytical technique such as Fourier transform infrared has been successfully applied for the authentication of MFO.

Conflict of interest

The authors declare no conflict of interest.

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