Impact of Spray Drying Encapsulation on Metabolite Profile and Quality of Fish Oil Products

Hilda Novianty[1*], Ayu Septi Anggraeni[1**], Indrawati Dian Utami[2], Retno Ayu Kurniasih[3], Lucky Prabowo Miftachul Alam[2], Selvia Oktaviyani[4], Diah Radini Noerdjito[5] and Suratno[1]

ABSTRACT

Encapsulation is one of the techniques to preserve fish oil compounds from deterioration and degeneration caused by oxidation. In the encapsulation process, the matrix wall protects fish oil substances, particularly when using the spray-drying method. This mechanism can induce changes in metabolite profiles while maintaining the quality of fish oil. In this study, metabolite changes were analyzed using high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS), alongside measurements of free fatty acids, acid values, and scanning electron microscope (SEM). The results showed distinct clusters between encapsulated and raw fish oil, with 75 metabolites identified, of which 35 were considered variables important in projection (VIP) metabolites. Statistical analysis revealed significant changes in 63 metabolites. Principal component analysis (PCA) clearly distinguished raw from encapsulated fish oil across a broad range, while the heatmap showed that most metabolites significantly differed among the treatment groups. PLS-DA identified 35 VIP metabolites, of which (1S,4S)-4-hydroxy-3-oxocyclohexanecarboxylic acid showed the highest VIP score. The VIP metabolites included 15 types of lipid derivatives, two amino acid derivatives, two carboxylic acid derivatives, two carbohydrate derivatives, two phenolic compounds, two aromatic compounds, and several minor substances. Additional findings showed a significant ($p<0.05$) decline in both free fatty acid and acid value levels for encapsulated fish oil, along with the formation of spherical encapsulated powder particles. The combination of HPLC-HRMS-based metabolomics with encapsulation analysis remains underexplored, and this study provides valuable insights into the encapsulation process for fish oil products.

Keywords: Encapsulation, Fish oil, HPLC-HRMS, Metabolite changes, Spray drying

INTRODUCTION

Fish oil consumption has increased significantly due to its high nutrient content of omega-3 (ω-3) fatty acids, such as eicosapentaenoic acid (EPA, $C_{20}H_{30}O_2$) and docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$), which are found in fish and seafood products and are widely used as functional foods, supplements, and medicines. This trend has arisen from the growing public awareness of the benefits of a healthy lifestyle. As a result, the global fish oil trading market was valued at USD 12.3 billion in 2022 and is expected to increase at a compound annual growth rate (CAGR) of 6.8% from 2022 to 2032, reaching USD 23.8 billion (FMI, 2023).

[1]Research Center for Food Technology and Processing-National Research and Innovation Agency, Yogyakarta, Indonesia
[2]Directorate for Laboratory Management, Research Facilities, and Science and Technology Park-National Research and Innovation Agency, Jakarta, Indonesia
[3]Diponegoro University, Semarang, Indonesia
[4]Research Center for Oceanography-National Research and Innovation Agency, Jakarta, Indonesia
[5]Research Center for Biosystematics and Evolution-National Research and Innovation Agency, Cibinong, Indonesia
[*]Corresponding author. E-mail address: hildanovianty2012@gmail.com
[**]Co-corresponding author. Email address: ayu.anggraeni07@gmail.com
Received 23 January 2025 / Accepted 5 August 2025

Fish oil is a derivative product of marine resources, extracted from fish processing waste and by-products (Zhang *et al*., 2021; Lee *et al*., 2022; Aitta *et al*., 2023). It is rich in polyunsaturated fatty acids (PUFAs), particularly omega-3 (ω-3) fatty acids such as EPA ($C_{20}H_{30}O_2$) and DHA ($C_{22}H_{32}O_2$), lipids soluble vitamins, lipophilic bioactives, and other bioactive compounds (Bakry *et al*., 2015; Ramos *et al*., 2021; Lee *et al*., 2022; Sultana *et al*., 2023). Several health benefits associated with the consumption of this functional lipid compound, including reducing the risk of cancer, cardioprotective effects, strengthening the immune system, lowering cholesterol, protection against free radicals, improved brain function, anti-aging effects, and potential treatment for various diseases. Previous studies have reported benefits conditions such as hypertension, obesity, inflammation, diabetes, autoimmune diseases, neurological disorder, and mental illnesses (Fernández-Cuesta *et al*., 2013; Kumar *et al*., 2017; Ramos *et al*., 2021). However, despite these beneficial properties, the high degree of unsaturated in PUFAs makes them highly susceptible to oxidative degradation, which can negatively affect the quality of fish oil. The quality of fish oil deteriorated due to oxidative degradation of PUFA compounds, which are prone to radical scavenging. This leads to the formation of volatile compounds and the development of a rancid odor (Bakry *et al*., 2015; Venugopalan *et al*., 2021). Encapsulation is an effective strategy to overcome this issue. The encapsulation mechanism involves surrounding the active substance with a secondary material that acts as an against environmental factors such as light and oxygen. This protective barrier prevents chemical or physical reactions with the environment that could destabilize the compound (Bakry *et al*., 2015; Kumar *et al*., 2021).

One common encapsulation technique is spray drying, which rapidly convert liquid dispersion into dry particles at high temperatures. Maltodextrin (MD) is often used as the wall material in this process (Díaz-Montes, 2023), while Tween serves as a surfactant to stabilize the liquid dispersion.

Although it has been widely stated that encapsulation enhances the stability of fish oil (Bakry *et al*., 2015; Kumar *et al*., 2017; Ayuni *et al*., 2021; Ramos *et al*., 2021; Venugopalan *et al*., 2021; Rahmani-Manglano *et al*., 2022; Sultana *et al*., 2023), there is a need to assess the metabolite changes in encapsulated fish oil compared to its raw form. Most previous studies have focused only on the chemical and physical characteristics of encapsulated fish oil (Kumar *et al*., 2017; Ayuni *et al*., 2021; Ramos *et al*., 2021; Rahmani-Manglano *et al*., 2022; Sultana *et al*., 2023), while compound-level changes between raw and encapsulated products have rarely been reported.

These compound changes due to the encapsulation process can be more precisely described through metabolomic analysis using High-Performance Liquid Chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS). Today, the metabolomic approach become increasingly popular in food research as it enables both qualitative and quantitative assessment of metabolites (Emwas *et al*., 2021). It is an effective technique for detecting chemical changes in metabolites influenced by external factors such as season, maturity, or physical/chemical/bioprocess treatments (Yang *et al*., 2020).

In this study, HPLC-HRMS was used to identify metabolite variability in fish oil matrices. This method enables the detection of numerous molecules in complex matrices and supports interpretation through statistical tools such as principal component analysis (PLS-DA) dendrograms, and heatmaps (Utpott *et al*., 2022). The comprehensive identification of fish oil compounds using HPLC-HRMS further supports the originality of this study in exploring the effects of encapsulation, a combination not widely investigated.

This study aimed to evaluate the impact of spray drying encapsulation on the metabolite profile using HPLC-HRMS-based metabolomics, in combination with quality parameter analyses (free fatty acid and acid value) and scanning electron microscopy (SEM), to provide new insights into the compound differences between raw and encapsulated fish oil products.

MATERIALS AND METHODS

The fish oil used in this study was purchased from a commercial online marketplace and labelled as 100% pure fish oil rich in squalene (manufacturer information not provided by the seller; country of origin: Indonesia). The product was stored at 4 °C in a dark bottle until further use to minimize oxidation. Maltodextrin [MD, dextrose equivalent (DE) 10–12] was also purchased from a commercial online marketplace and labelled as food grade (manufacturer information not provided by the seller). Other materials included Tween 80 (Merck), distilled water (OneMed, Indonesia), methanol (MS grade, Fisher Scientific Korea Ltd), sodium hydroxide (NaOH, Emsure, Germany), and Ethanol (95%, Emsure, Germany).

Production of encapsulated fish oil powder

Encapsulation was prepared by combining the active compound (fish oil) with a secondary material/encapsulating agent (MD) to form a homogenous solution. The mass ratio of raw fish oil (2%, 4%, and 6%)) to MD was 1:4, based on modifications of Bakry *et al*. (2016) and El-Messery *et al*. (2020). The MD solution was prepared by dissolving MD in distilled water using a magnetic stirrer (Heidolph, Germany) at 600 revolutions per minute (rpm) at room temperature for 10 min. Fish oil was then dispersed into the MD solution and homogenized using a high-speed homogenizer (T25 Digital Ultra-Turrax, Germany) at 10,000 rpm for 10 min. Tween 80 (2%, as a surfactant) was added to the mixture of fish oil and MD solution, and the emulsion was further homogenized at 10,000 rpm for 20 min using the same homogenizer. The resulting emulsion was stored at 4 °C until further analyses.

Encapsulated fish oil powder was produced by drying the emulsion using a spray dryer (B-290 Buchi Mini Spray Dryer, Switzerland) equipped with a standard 0.7 mm nozzle. The emulsion was fed into the spray nozzle through an automated pump system. The spray drying parameters were as follows: air inlet temperature of 130 °C, air outlet temperature of 65–70 °C , pump rate of 24%, aspirator rate of 9%, and chamber pressure of 60 mbar. The inlet temperature was selected based on El-Messery *et al*. (2020), with slight modifications. The resulting encapsulated fish oil powder was collected from the cyclone chamber and sample vessel, then stored at 4 °C with a 1 g silica gel pad in Ziplock plastic packaging for further analyses.

Metabolite extraction for HPLC-HRMS analyses

Samples preparation followed a modified method from Windarsih *et al*. (2022). A 100 mg portion of each sample (raw and encapsulated fish oil powder) was extracted with 4 mL of MS-grade methanol in a microcentrifuge tube. The mixture was homogenized using a sonicator (Elmasonic, Elma S100H, Germany, 37 kHz) for 30 min. The supernatant was collected using a syringe and filtered through a 0.22-µm nylon filter membrane into a clean microcentrifuge tube. One mL of the filtered supernatant was transferred into an HPLC vial for HPLC-HRMS analysis to evaluate metabolite profiles. A blank sample containing only MS-grade methanol was used as a control. All samples were prepared in triplicate.

HPLC-HRMS analyses

The HPLC-HRMS parameters were adapted from Windarsih *et al*. (2022). Metabolites from the extracted samples were characterized and analyzed using a HPLC-HRMS. Chromatographic separation was performed using a Thermo Scientific™ Vanquish™ UHPLC Binary Pump and a Thermo Scientific™ Accucore™ Phenyl-Hexyl analytical column (100 mm × 2.1 mm ID × 2.6 µm particle size). Mass spectrometric detection was carried out using a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ High-Resolution Mass Spectrometer.

The mobile phase consisted of: mobile phase A: MS-grade water containing 0.1% formic acid and 50 mM ammonium format; mobile phase B: MS-grade methanol containing 0.1% formic acid and 50 mM ammonium format. A gradient elution was employed at a flow rate of 300 µL·min^{-1}. The initial condition was 95% A, gradually reduced to 10% over 16 min, held at 10% for 4 min, and then returned to 95%. A until the 25-min mark. The column temperature was maintained at 40 °C, and the injection volume was 7 µL.

Untargeted screening was conducted in both positive and negative ionization modes using full MS/dd-MS^2 acquisition. Nitrogen was employed as the sheath, auxiliary, and sweep gases at 32, 8, and 4 arbitrary units (AU), respectively. The spray voltage was set at 3.30 kV, with a capillary temperature of 320 °C and an auxiliary gas heater temperature of 30 °C. The scan range was set from 66.7–1,000 $m \cdot z^{-1}$, with a resolutions of 70,000 for full MS and 17,500 for dd-MS^2.

Chemometrics analysis

In this metabolomics study, samples were differentiated and classified using principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) to distinguish between raw and encapsulated fish oil. The chemometric study was conducted using the free software MetabAnalyst 6.0. The chemical and metabolite regions derived from the untargeted study were incorporated as variables in the PCA and PLS-DA analyses. The chemometric analysis included four sample groups: raw fish oil (without encapsulation) and encapsulated fish oil at concentrations of 2%, 4%, and 6%. A total of 75 compounds were selected for analysis based on metabolomics assessment. Compound filtering was performed using the Thermo Scientific's Compound Discoverer software to reduce variable numbers while maintaining data diversity. Compounds were selected based on available names, DDA segments for specific ions, and best-match results using mzCloud database.

Prior to chemometrics analysis, data were normalized by sum, log-transformed (base 10), and standardized using relative standard deviation (RSD = SD/mean) and auto-scaling technique. PLS-DA interpretation involved a PLS-DA score plot, loading score, and variable importance in projection (VIP) value. PCA interpretation was based on the PCA score plot. Sample clustering was illustrated using dendrogram and heatmap techniques. Statistical analysis was performed using analysis of variance (ANOVA), and significant differences among clusters were assessed using Tukey's HSD post hoc test, with $p<0.05$ considered statistically significant.

Total free fatty acids and acid value analysis

Total free fatty acids (FFA) were determined using the procedure outlined in AOAC 969.33. (AOAC, 2005). A 14 g sample of either raw or encapsulated fish oil powder was mixed with 25% ethanol (95%). Phenolphthalein was used as an indicator. The mixture was titrated with 0.05 N of NaOH under vigorous shaking until a persistence pink color appeared and lasted for at least 30 s. The percentage of FFA was calculated using the following formula (Latip *et al*., 2014; Ayeloja *et al*., 2024a) :

$$\% \text{ FFA} = (V \times N \times 28.2) / W,$$

V = volume of 0.05 N NaOH (mL)
N = Normality of NaOH (N)
W = weight sample (g)

$$\text{Acid value} = \% \text{ FFA} \times 1.99$$

Results were expressed as means and standard deviations. Data were collected in triplicate. Statistical significance was determined using analysis of variance (ANOVA), and LSD tests were performed using CoSTAT statistical software.

Scanning electron microscopy (SEM)

The morphology of the encapsulated fish oil powder was observed using scanning electron microscopy (SEM, Hitachi SU3500, Japan). Approximately 0.5 g of the powder sample was mounted onto a specimen stub and placed in the specimen holder. Observation was conducted at 3,000× magnification (acceleration voltage: 3 kV; vacuum: 6 Pa) using an Ion Sputter Coater (Hitachi MC1000) with 100% gold coating. Image measurements were analyzed using the SEM SU3500 software package.

RESULTS

Metabolite analysis

Differential metabolites identified in raw and encapsulated fish oil

HPLC-HRMS detected a total of 75 metabolites from the extracted samples in both positive and negative ion modes. Among these 63 metabolites showed statistically significant differences (Supplementary Table 1). These metabolites showed clear distinctions between raw and encapsulated fish oil. Further interpretation of these metabolite profiles was performed using principal component analysis (PCA), a dendrogram, heatmaps, and partial least squares discriminant analysis (PLS-DA).

Principal component analysis (PCA) and clustering of metabolites

Metabolite clustering is shown in Figure 1a. The samples were clearly separated into distinct clusters, indicating that each formulation possesses unique characteristics contributing to its specific location in the principal component space. The total variance explained by PC_1 and PC_2 was 78.2%.

As shown in Figure 1a, the 4% and 6% of encapsulated fish oil samples were located on the positive side of PC_2, while the 2% encapsulated fish oil were positioned on the negative side of PC_1. In contrast, the raw fish oil group was located closer to the center, exhibiting a more homogenous profile. The PCA score plot showed statistically significant ($p<0.001$), indicating high variability among groups. Further evaluation of similar and differential clustering relationships is shown in Figure 1b. The dendrogram illustrated four distinct groups: 2% encapsulated fish oil, 4% encapsulated fish oil, 6% encapsulated fish oil, and raw fish oil. While the encapsulated groups shared some clustering patterns, each showed varying levels of internal variance. In contrast, the raw fish oil group formed a separate and distinct cluster from the encapsulated group.

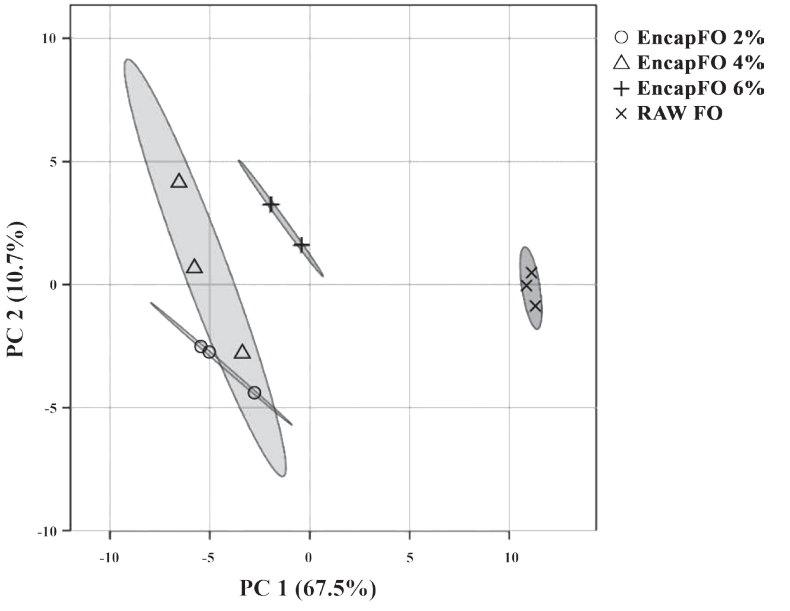
Heatmap diagram of fish oil (raw and encapsulated) metabolites

The heatmap provided a hierarchical clustering visualization of the metabolite profiles detected in the samples (raw and encapsulated fish oil in different concentrations). Samples were grouped based on similarities in metabolite abundance, with the intensity of each metabolite represented by a color gradient from blue (low abundance) to red (high abundance). Figure 2 displayed the 63 significantly different metabolites, visualized and clustered accordingly. Each cluster revealed similar metabolite compositions but differed in abundance intensity. While the encapsulated groups showed comparable variance patterns among themselves, they were clearly distinct from the raw fish oil cluster.

Partial least squares discriminant analysis (PLS-DA) of fish oil metabolites

PLS-DA confirmed the presence of differential metabolites within each cluster, with a high variable importance in projection (VIP) score range (Song *et al*., 2022). A total of 35 metabolites were identified as VIP metabolites (Supplementary Table 2), representing 15 types of lipids and their derivatives, two types of amino acid derivatives, two types of carboxylic acid derivatives, two types of carbohydrates and their derivatives, two types of phenolic compounds, two types of aromatic compounds, and other small molecule categories. As shown in Figure 3, clear separation among clusters was observed. Metabolites extracted from encapsulated fish oil showed a greater variety in intensity compared to those from raw fish oil, indicating changes in metabolite profiles induced by the encapsulation process.

(a)



(b)

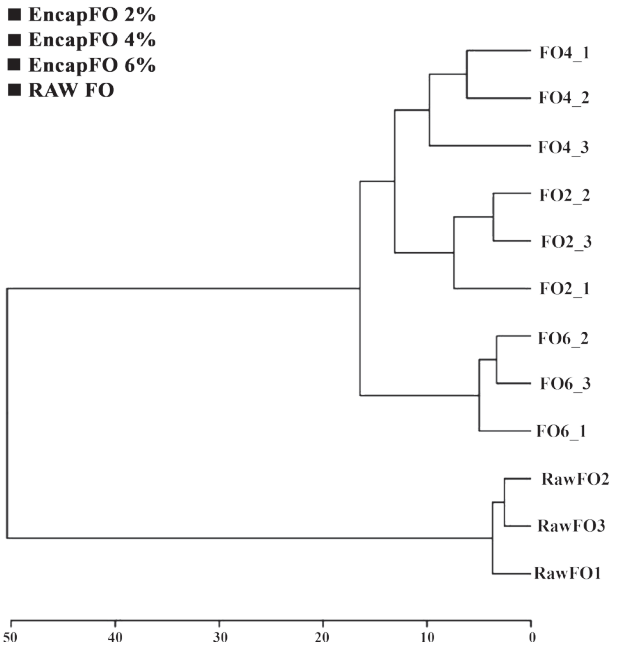


Figure 1. (a) Principal Component Analysis (PCA) score plot showing the clustering of raw and encapsulated fish oil samples based on metabolite profiles; (b) Hierarchical clustering dendrogram showing the similarity among raw fish oil (Raw FO) and encapsulated fish oil at 2% (EncapFO 2%), 4% (EncapFO 4%), and 6% (EncapFO 6%) concentrations.

Total free fatty acids (FFA) and acid value (AV) analyses of fish oil

The FFA and AV values of raw and encapsulated fish oil are shown in Figure 4. The FFA and AV of the samples showed statistically significant differences among treatments ($p<0.05$), as indicated by different superscript letters. Raw FO exhibited the highest FFA (1.71%) and AV (3.40 mg KOH·g^{-1}), significantly higher than all encapsulated fish oil samples. Among the encapsulated samples, F06 had the lowest FFA (1.21%) and AV (2.40 mg KOH·g^{-1}), both significantly different from F02 and F04. The Different among treatments, confirming that each treatment had a distinct impact.

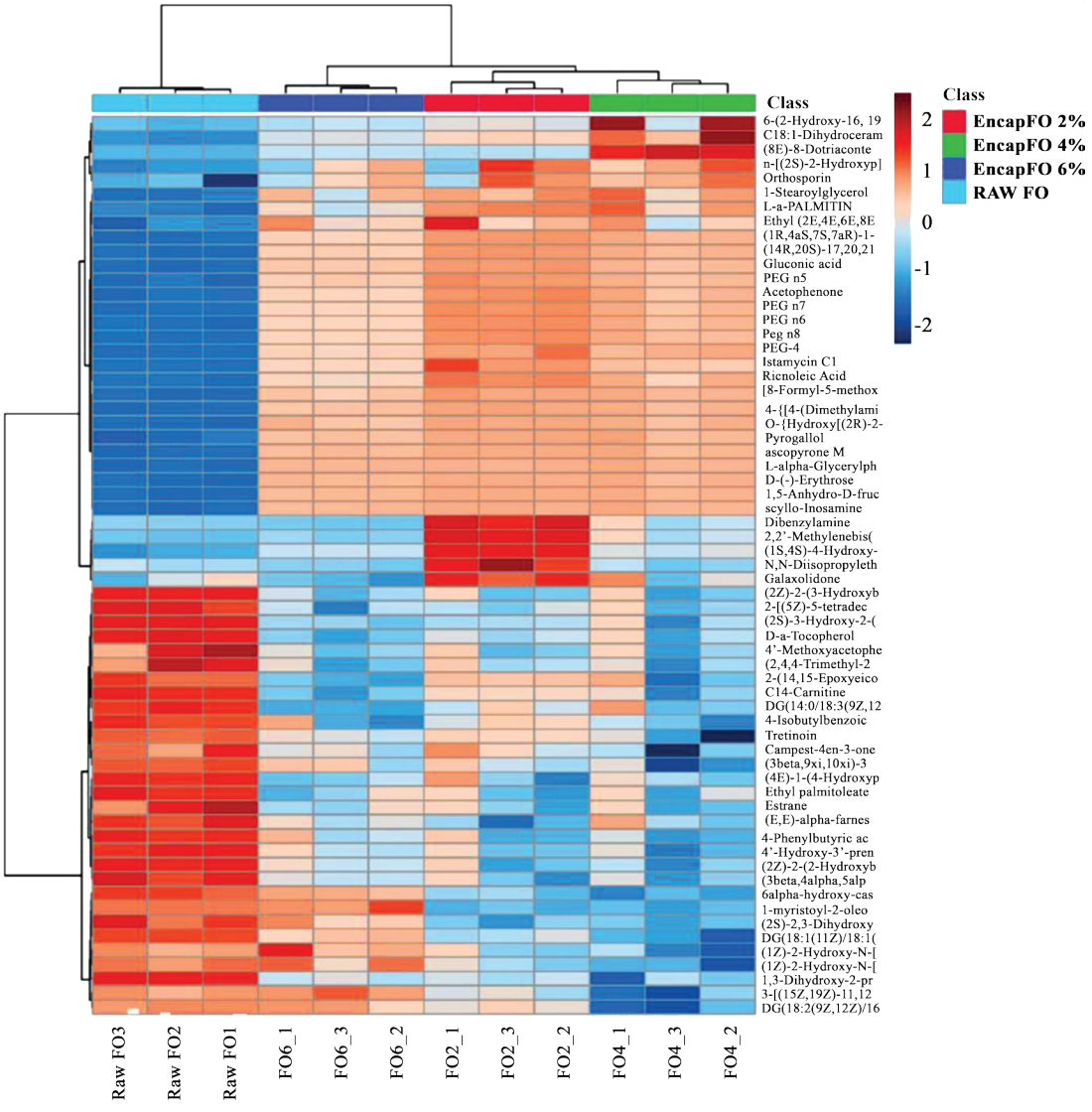


Figure 2. Heatmap showing the relative abundance of 63 metabolites in raw and encapsulated fish oil samples. Groups include raw fish oil (Raw FO), and encapsulated fish oil at 2% (EncapFO 2%), 4% (EncapFO 4%), and 6% (EncapFO 6%) concentrations. The color gradient represents metabolite abundance, with red indicating higher and blue indicating lower levels. Hierarchical clustering was applied to both samples and metabolites to visualize patterns of variation among treatments.

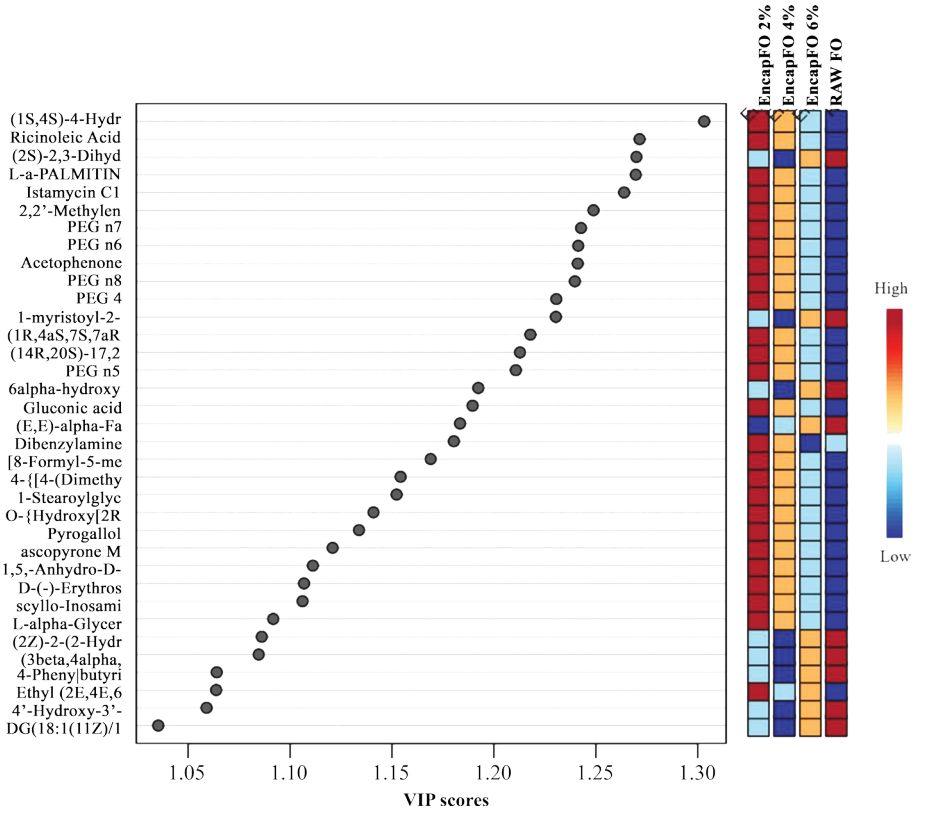


Figure 3. PLS-DA of the top 35 metabolites with the highest Variable Importance in Projection (VIP) scores, showing variance among raw and encapsulated fish oil samples. Groups include raw fish oil (Raw FO), and encapsulated fish oil at 2% (EncapFO 2%), 4% (EncapFO 4%), and 6 % (EncapFO 6%) levels. The heatmap indicates relative metabolite abundance, with red representing high and blue representing low concentrations.

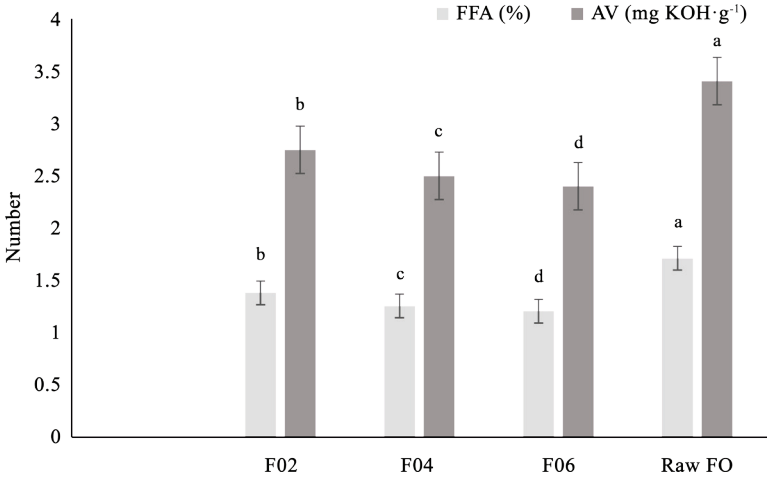


Figure 4. Percentage of total free fatty acid (FFA) and acid value (AV) of raw and encapsulated fish oil. Raw FO = raw fish oil; F02 = 2% encapsulated fish oil; F04 = 4% encapsulated fish oil; F06 = 6% encapsulated fish oil. Bars represent mean values (n = 3), and error bars represent ±SD. Different lowercase letters above bars for the same trait denote significant differences ($p<0.05$).

Scanning electron microscopy (SEM) of encapsulated fish oil powder

The surface morphology of control and encapsulated fish oil powder particles is presented in Figure 5. The control samples (Figure 5a) showed irregular, agglomerated, crushed, and wrinkled particles. In contrast, the encapsulated samples (Figure 5b, 5c, 5d) demonstrated that as the amount of fish oil increased in the formula, more spherical-shaped particles were formed through the spray drying process.

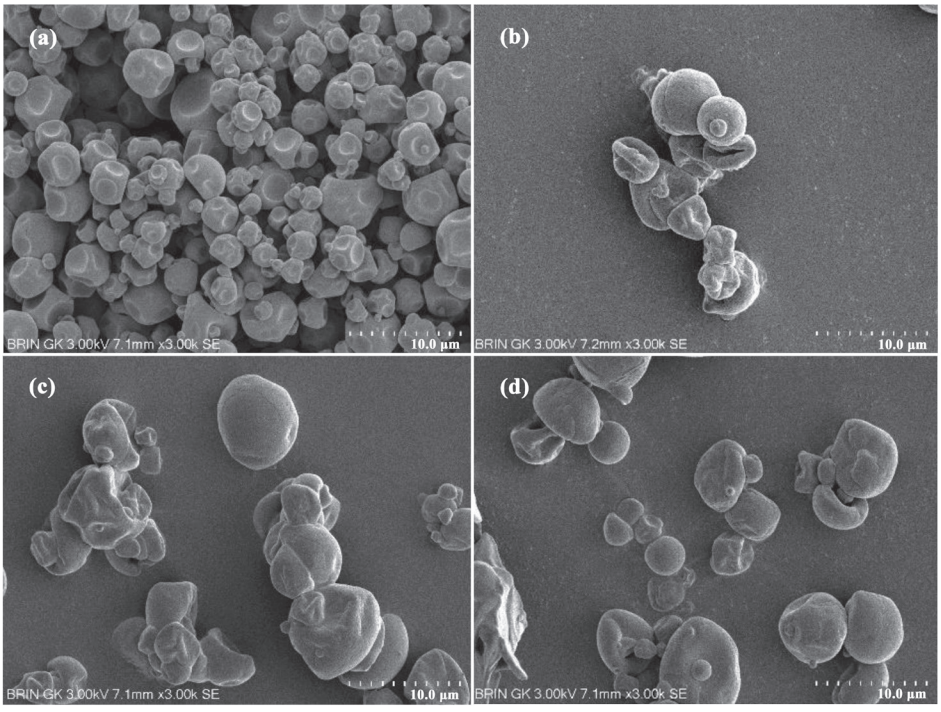


Figure 5. Morphology of encapsulated fish oil: (a) control/no fish oil loaded; (b) 2% of encapsulated fish oil powder; (c) 4% of encapsulated fish oil powder; (d) 6% of encapsulated fish oil powder.

DISCUSSION

In this study, when the emulsion of fish oil encapsulation goes through the feed at a high temperature during the spray dryer, it helps evaporate the water from the emulsion and turn it into powder (Sultana *et al*., 2023). This mechanism probably made metabolite changes in the fish oil compounds. Thermal processing could lead to an effect observed in the compositional and changing nutrients in the food matrix (Utpott *et al*., 2022). This study allowed metabolomics analysis to differentiate the metabolite changes between raw fish oil and encapsulated fish oil using a spray dryer to evaluate the quality of fish oil products. The significant metabolites were expressed in Supplementary Table 1. The differences in the variance of peak intensity range between metabolites among the clustering groups stimulate this significance.

The analysis of metabolite changes was interpreted using chemometrics analysis to differentiate the clustering. PCA is a multivariate statistical analysis method that often summarizes large-scale data into grouping descriptions (Isha *et al*., 2020; Utpott *et al*., 2022). The PCA was conducted to examine the metabolite profile, as illustrated in Figure 1a and 1b. PC_1 and PC_2 explain 78.2% of the total variability in the data. This indicates that the total differences among samples

can be effectively summarized and visualized in a two-dimensional plot. This cumulative variance suggests that PC_1 and PC_2 are sufficient to distinguish the major patterns and groupings in the data (as the separation between raw and encapsulated fish oil in different encapsulation concentrations). The encapsulated groups for 4% and 6% of encapsulated fish oil are in the exact location (the positive side of PC_2), and encapsulated groups for 2% is on the negative side of PC_1 (nearly to the positive side of PC_2). This was likely because these groups had more common metabolite profiles than the raw FO group.

As shown in Figure 2, the variance intensity of seventy metabolites was illustrated in each cluster group from -2 (lowest) to 2 (highest) intensity range. Most of the metabolites in the encapsulated group showed a color of intensity similar to that of the raw fish oil group. The primary metabolite in Figure 2 is shown in Figure 3, with each metabolite more clearly appearing, showing the different color intensity in each cluster. The highest score detected by the VIP (Supplementary Table 2) was reached by (1S,4S)-4-Hydroxy-3-oxocyclohexanecarboxylic. Statistics showed that (1S,4S)-4-Hydroxy-3-oxocy clohexanecarboxylic was significantly different in each cluster (Supplementary Table 1), and this metabolite belonged to the carboxylic acid group. Ricinoleic Acid, a fatty acid group, was the second highest and was significantly different in each cluster. Ricinoleic acid is an unsaturated omega-9 fatty acid and a hydroxy acid commonly found in various foods and in vegetable and seed oils (Nisbett *et al*., 2024). It constitutes approximately 90% of the fatty acid content in castor oil, where it exists primarily as triglyceride. The process of ricinoleate formation involves channelling the substrate through the hydroxyl rebound pathway (Buist, 2010).

Additionally, ricinoleic acid has been shown to reduce anxiety-like behaviour in male mice, demonstrating effects like those of antidepressants (Nisbett *et al*., 2024). The lowest intensity described in the diglyceride metabolite, DG-(18:1 (11Z)/18:1(9Z)/0:0), was also significantly different in each cluster (Supplementary Table 1). Overall, the VIP metabolites of encapsulated fish oil have high intensity compared to raw fish oil (Supplementary Table 2). Most of them were lipids and their derivative compounds, which belong to fatty acids, such as ricinoleic acid, docosadienoate (PUFA derivied), palmitin, glycerols, myristoyl, heptadecanoate, and octadecadienoate.

The high intensity of metabolites in encapsulated fish oil groups indicates that the encapsulation process by spray dryer could maintain the consistency of the fish oil substances. Figure 3 shows that 2% of encapsulated fish oil has more metabolites in the high-intensity range. The metabolites were discovered in this study, also found in other fish product studies, such as L-α-PALMITIN (syn. 1-Monopalmitoylglycerol), 4-Phenylbutyric acid (phenylbutyric acid), 1-Stearoylglycerol (-Stearoyl-glycero) were found in Li *et al*. (2021), Acetophenone (ketone group), 4'-Hydroxy-3'-prenylacetophenone (ketone group), D-(-)-Erythrose (aldehyde group) were found in Song *et al*. (2017), L-alpha-Glyceryl phosphorylcholine (syn. Cholineglycerophosphate) was found in Wen *et al*. (2019), 1-myristoyl-2-oleoyl-sn-glycerol (Myristoleicacid) was found in Kuvendziev *et al*. (2018), D.G.- (18:1(11Z)/18:1 (9Z)/0:0) (DG-derived) was found in Cao *et al*. (2022), (2S)-2,3-Dihydroxypropyl (13Z,16Z)-13, 16-docosadienoate (Docosanoate derived), (14R,20S) -17,20,21-Trihydroxy-2-methyl-17-oxido-11-oxo -12,16,18-trioxa-17lambda~5~-phosphahenicosan -14-yl, 16-methylheptadecanoate (Heptadecanoate derived) and Ethyl (2E,4E,6E,8E,10E,12E,14Z,16E)-2, 6,11,15-tetramethyl-17-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10,12,14,16-heptadecaoctaenoate (Heptadecanoate derived) were found in Irnawati *et al*. (2024). In addition to illustrating the various ranges between raw and encapsulated fish oil using Heatmap and PLS-DA, nutrition quality from FFA and AV was assessed to estimate the difference.

PUFA compounds in oil products could linked with radical scavenging and easily to hydrolysis or oxidative, which leads to the degradation of product quality. Degradation in oil products produced FFA which was presented because of the decomposition reaction of triglycerides (Tsado *et al*., 2018; Hidayah *et al*., 2022; Djamaludin *et al*., 2023; Ayeloja *et al*., 2024a) (Figure 4). If FFA is a product of hydrolysis reaction, then AV is a secondary product of the oxidative response which indicates rancidity (Özyurt *et al*., 2013;

Latip *et al*., 2014; Hidayah *et al*., 2022; Lee *et al*., 2022; Ayeloja *et al*., 2024b). Both become indicators of fish oil quality. This result has not reached the IFOS (International Fish Oil Standard) standard for FFA requirements (<1.13) (Djamaludin *et al*., 2023), but has reached requirements from other literature which stated that the acceptable limit for FFA value is about<8 (Ayeloja *et al*., 2024b) and for the AV has reached the requirements standard for fish oil from FAO (≤3 mg $KOH \cdot g^{-1}$) (FAO, 2017), both for the encapsulated oil samples. The result (Figure 4) showed a decline in FFA and AV from raw into encapsulated fish oil products. Statistical showed a significant effect ($p<0.05$) on the FFA and AV value between raw and encapsulated products. It showed that the encapsulation process could decline the FFA and AV number levels, showing that protecting fish oil's active substances from external factors could stimulate the degradation process.

Furthermore, SEM in Figure 5 described the morphology of encapsulated fish oil in more detail. The encapsulation of fish oil starts with the emulsion stages, which combine oil-active compounds with the second material wall through a series of homogenizations until the oil-active compounds are present within the second material wall (Sultana *et al*., 2023). This study found that fish oil encapsulation particles (Figure 5b, 5c, and 5d) appeared more spherical than the control (Figure 5a.) but had similar morphology particles (around 10 µm) among all fish oil concentration treatments. This finding is similar to the study of Rehman *et al*. (2021), and Rahmani-Manglano *et al*., 2022 showed a spherical particle formed while fatty acid was loaded in the encapsulation formulas. We predicted that the active compounds in fish oil had been entrapped within the second material wall.

This study found that the encapsulation mechanism was effective in protecting various metabolites detected during the analysis, including several PUFA-derived compounds. Chemometric showed that encapsulated fish oil exhibited higher metabolite intensity compared to raw fish oil (without encapsulation treatment). This protective effect was also reflected in lower levels of degradation indicators (FFA and AV value) on encapsulated fish oil than the raw fish oil sample. The encapsulation process involves enclosing fish oil compounds within a protective matrix (maltodextrin), using the spray drying method. This matrix acts as a physical barrier, shielding the compounds from environmental factors that contribute to oxidative degradation. Encapsulation is particularly important for fish oil, which is rich in PUFAs that are highly susceptible to oxidation when exposed to oxygen, heat, light, and moisture. Oxidation leads to the formation of undesirable compounds such as FFAs, peroxides, and aldehydes, which result in rancidity, off-flavors, and a substantial loss of nutritional value. Therefore, encapsulation provides an effective means of enhancing the stability and shelf life of fish oil by minimizing oxidative damage. By protecting the oil from environmental factors, encapsulation not only preserves the quality of fish oil but also ensures that its beneficial properties, such as omega-3 fatty acids, remain intact until consumption. This method ultimately supports the delivery of health benefits associated with fish oil while maintaining its sensory attributes.

CONCLUSIONS

This study showed that the encapsulation process using a spray dryer has stimulated fish oil metabolite changes. The result showed a distinguish cluster between encapsulated and raw fish oil. 35 VIP metabolites were discovered, which represented 15 types of lipids and their derivatives, two types of amino acids derivatives, two types of carboxylic acid derivatives, two types of carbohydrates and derived, two types of phenolic compounds, two types of aromatic compounds, and other small substances (formamides, iridoid, organic compound, sesquiterpenoids, sulfonic acid, glycogen, tetrose saccharide, hydroxy benzylidene, sterols, methyl ketone). According to Heatmap and PLS-DA visualized, most metabolites (VIP compounds) have a high range intensity in the encapsulated fish oil compared to raw fish oil. This study also showed a decline in FFA and AV levels for encapsulated fish oil and a Spherical-shaped particle formed by the spray dryer. The highest level of FFA and AV will lead to deterioration in the quality of fish oil. This indicated that encapsulation by spray drying

could protect the substance active of fish oil from quality degradation. SEM figure also showed an entrapping active compound in fish oil within the second matrix wall. This study result gave new insight into the positive side of the impact of the fish oil encapsulation process by spray drying method, which could protect the substance of fish oil compounds.

ACKNOWLEDGEMENTS

The Research Organization for Agriculture and Food of the National Research and Innovation Agency-Indonesia funded this study in 2024 with Grant-Project No. 6/III.11/H.K./2024. The authors acknowledge the facility's scientific and technical support from Advanced Characterization Laboratories Yogyakarta of the National Research and Innovation Agency through the https://elsa.brin.go.id/ channel.

LITERATURE CITED

Association of Official Analytical Chemists (AOAC) 2005. **Official Methods of Analysis of AOAC International.** https://www.researchgate.net/publication/292783651. Cited 2 Aug 2024.

Aitta, E., A. Damerau, A. Marsol-Vall, M. Fabritius, L. Pajunen, M. Kortesniemi and B. Yang. 2023. Enzyme-assisted aqueous extraction of fish oil from Baltic herring (*Clupea harengus membras*) with special reference to emulsion-formation, extraction efficiency, and composition of crude oil. **Food Chemistry** 424: 1–13. DOI: 10.1016/j.foodchem.2023.136381.

Ayeloja, A.A., W.A. Jimoh and A.O. Garuba. 2024a. Nutritional quality of fish oil extracted from selected freshwater fish species. **Food Chemistry Advances** 4: 2–7. DOI: 10.1016/j.focha.2024.100720.

Ayeloja, A.A., W.A. Jimoh and T.V. Oyewole. 2024b. Nutritional quality of fish oil extracted from selected marine fish species. **Food and Humanity** 2: 1–6. DOI: 10.1016/j.foohum.2023.100212.

Ayuni, D., J.N.W. Karyadi, A.D. Saputro and H. Yoshii. 2021. Effect of antioxidant and pro-oxidant on the stability of microencapsulated squalene by spray drying. **agriTECH** 41(1): 49–57. DOI: 10.22146/agritech.52741.

Bakry, A.M., S. Abbas, B. Ali, H. Majeed, M.Y. Abouelwafa, A. Mousa and L. Liang. 2015. Microencapsulation of oils: a comprehensive review of benefits, techniques, and applications. **Comprehensive Reviews in Food Science and Food Safety** 15(1): 143–182. DOI: 10.1111/1541-4337.12179.

Bakry, A.M., Z. Fang, Y. Ni, H. Cheng, Y.Q. Chen and L. Liang. 2016. Stability of tuna oil and tuna oil/peppermint oil blend microencapsulated using whey protein isolate in combination with carboxymethyl cellulose or pullulan. **Food Hydrocolloids** 60: 559–571. DOI: 10.1016/j.foodhyd.2016.04.026.

Buist, P.H. 2010. **Natural products structural diversity-i secondary metabolites: Organization and biosynthesis.** In: Comprehensive Natural Products II (eds. H. Liu and L. Mander), pp. 5–33. Elsevier Ltd., Canada.

Cao, Y., Q. Gao, X. Li, Y. Zhou, S. Dong, Y. Wang and Z. Dai. 2022. Integrated analysis of metabolomics and transcriptomics for assessing effects of fish meal and fish oil replacement on the metabolism of rainbow trout (*Oncorhynchus mykiss*). **Frontiers in Marine Science** 9: 1–17. DOI: 10.3389/fmars.2022.843637.

Díaz-Montes, E. 2023. Wall materials for encapsulating bioactive compounds via spray-drying: A review. **Polymers** 15: 1–26. DOI: 10.3390/polym15122659.

Djamaludin, H., T.D. Sulistiyati, A. Chamidah, P. Nurashikin, M. Roifah, H. Notonegoro and P.R. Ferdian. 2023. Quality and fatty acid profiles of fish oil from tuna by-products extracted using a dry-rendering method. **Biodiversitas** 24(11): 6100–6106. DOI: 10.13057/biodiv/d241131.

El-Messery, T.M., U. Altuntas, G. Altin and B. Özçelik. 2020. The effect of spray-drying and freeze-drying on encapsulation efficiency, *in vitro* bioaccessibility and oxidative stability of krill oil nanoemulsion system. **Food Hydrocolloids** 106: 1–9. DOI: 10.1016/j.foodhyd.2020.105890.

Emwas, A.H.M., N. Al-Rifai, K. Szczepski, S. Alsuhaymi, S. Rayyan, H. Almahasheer, M. Jaremko, L. Brennan and J.I. Lachowicz. 2021. You are what you eat: Application of metabolomics approaches to advance nutrition research. **Foods** 10: 1–20. DOI: 10.3390/foods10061249.

Fernández-Cuesta, A., L. León, L.Velasco and R. De la Rosa. 2013. Changes in squalene and sterols associated with olive maturation. **Food Research International** 54: 1885–1889. DOI: 10.1016/j.foodres.2013.07.049.

Food and Agriculture Organization of the United Nations (FAO). 2017. **Standard for fish oil.** https://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B329-2017%252FCXS_329e.pdf. Cited 4 Aug 2024.

Future Market Insight (FMI). 2023. **Fish oil market.** https://www.futuremarketinsights.com/. Cited 12 Oct 2023.

Hidayah, N., A. Rohman, M. Mustafidah and Irnawati. 2022. Physicochemical characterization and fatty acid profiles of fish oil from milkfish (*Chanos chanos* F.). **Food Research** 6(2): 265–270. DOI: 10.26656/fr.2017.6(2).222.

Irnawati, I., A. Windarsih, L.O.M.H. Nadia, A. Rohman and W.O. Fasrida. 2024. Quantification and classification of fatty acids in marine fish oil from Southeast Sulawesi using gas chromatography and chemometrics. **Journal of Applied Pharmaceutical Science** 14(3): 162–169. DOI: 10.7324/JAPS.2024.159140.

Isha, A., N.A. Yusof, R. Osman, M.Y. Wong and S.N.A. Abdullah. 2020. NMR-based metabolomics reveals effect of *Ganoderma boninense* infection on oil palm leaf at 30 days post-infection. **Plant OMICS** 13(1): 15–20. DOI: 10.21475/POJ.13.01.20.P2071.

Kumar, L.R.G., N.S. Chatterjee, C.S. Tejpal, K.V. Vishnu, K.K. Anas, K.K. Asha, R. Anandan and S. Mathew. 2017. Evaluation of chitosan as a wall material for microencapsulation of squalene by spray drying: Characterization and oxidative stability studies. **International Journal of Biological Macromolecules** 104: 1986–1995. DOI: 10.1016/j.ijbiomac.2017.03.114.

Kumar, L.R.G., H.S. Kumar, C.S. Tejpal, K.K. Anas, B.B. Nayak, K. Sarika, S.S. Greeshma, N.S. Chatterjee, S. Mathew and C.N. Ravishankar. 2021. Exploring the physical and quality attributes of muffins incorporated with microencapsulated squalene as a functional food additive. **Journal of Food Science and Technology** 58(12): 4674–4684. DOI: 10.1007/s13197-020-04955-9.

Kuvendziev, S., K. Lisichkov, Z. Zekovi, M. Marinkovski and Z.H. Musliu. 2018. Supercritical fluid extraction of fish oil from common carp (*Cyprinus carpio* L.) tissues. **The Journal of Supercritical Fluids** 133: 528–534. DOI: 10.1016/j.supflu.2017.11.02.

Latip, L.D., W. Zzaman and T.A.Yang. 2014. Application of response surface methodology to optimize the extraction of asian catfish (*Clarias batrachus*) oil using superheated steam treatment. **Food Science and Technology** 2(6): 69–76. DOI: 10.13189/fst.2014.020601.

Lee, S.C., D. Surendhiran and B.S. Chun. 2022. Extraction and encapsulation of squalene-rich cod liver oil using a supercritical process for enhanced oxidative stability. **Journal of CO_2 Utilization** 62: 1–12. DOI: 10.1016/j.jcou.2022.102104.

Li, R., Z. Sun, Y. Zhao, L. Li, X. Yang, J. Cen, S. Chen, C. Li and Y. Wang. 2021. Application of UHPLC-Q-TOF-MS/MS metabolomics approach to investigate the taste and nutrition changes in tilapia fillets treated with different thermal processing methods. **Food Chemistry** 356: 1–11. DOI: 10.1016/j.foodchem.2021.129737.

Nisbett, K.E., L.F. Vendruscolo and G.F. Koob. 2024. Indulging curiosity: preliminary evidence of an anxiolytic-like effect of castor oil and ricinoleic acid. **Nutrients** 16: 1–9. DOI: 10.3390/nu16101527.

Özyurt, G., A. Şimşek, M. Etyemez and A. Polat. 2013. Fatty acid composition and oxidative stability of fish oil products in Turkish retail market. **Journal of Aquatic Food Product Technology** 22(3): 322–329. DOI: 10.1080/10498850.2011.644882.

Rahmani-Manglano, N.E., M. Tirado-Delgado, P.J. García-Moreno, A. Guadix and E.M. Guadix. 2022. Influence of emulsifier type and encapsulating agent on the *in vitro* digestion of fish oil-loaded microcapsules produced by spray-drying. **Food Chemistry** 392: 1–9. DOI: 10.1016/j.foodchem.2022.133257.

Ramos, F. de M., V.S. Júnior and A.S. Prata. 2021. Impact of vacuum spray drying on encapsulation of fish oil: oxidative stability and encapsulation efficiency. **Food Research International** 143: 1–10. DOI: 10.1016/j.foodres.2021.110283.

Rehman, A., Q. Tong, S.M. Jafari, *et al*. 2021. Spray dried nanoemulsions loaded with curcumin, resveratrol, and borage seed oil: The role of two different modified starches as encapsulating materials. **International Journal of Biological Macromolecules** 186: 820–828. DOI: 10.1016/j.ijbiomac.2021.07.076.

Song, G., Z. Dai, Q. Shen, X. Peng and M. Zhang. 2017. Analysis of the changes in volatile compound and fatty acid profiles of fish oil in chemical refining process. **European Journal of Lipid Science and Technology** 120(2): 1–8. DOI: 10.1002/ejlt.201700219.

Song, R., X. Wang, S. Deng and N. Tao. 2022. Lipidomic analysis and triglyceride profiles of fish oil: Preparation through silica gel column and enzymatic treatment. **Food Research International** 162: 26–33. DOI: 10.1016/j.foodres.2022.112100.

Sultana, A., S. Adachi and H. Yoshii. 2023. Encapsulation of fish oil and essential fatty acids by spray drying. **Sustainable Food Technology** 1(6): 1–10. DOI: 10.1039/d3fb00099k.

Tsado, D.B., M.M. Ndamitso and A.I. Ajai. 2018. Determination of physicochemical properties and fatty acid profile of oil extract of blighia sapida fruit from selected areas in Niger State, Nigeria. **Nigerian Journal of Chemical Research** 23(1): 21–34.

Utpott, M., E. Rodrigues, A. de O. Rios, G.D. Mercali and S.H. Flôres. 2022. Metabolomics: an analytical technique for food processing evaluation. **Food Chemistry** 366: 1–10. DOI: 10.1016/j.foodchem.2021.130685.

Venugopalan, V.K., L.R. Gopakumar, A.K. Kumaran, N.S. Chatterjee, V. Soman, S. Peeralil, S. Mathew, D.J. McClements and R.C. Nagarajarao. 2021. Encapsulation and protection of omega-3-rich fish oils using food-grade delivery systems. **Foods** 10: 1–21. DOI: 10.3390/foods10071566.

Wen, X., Y. Hu, X. Zhang, X. Wei, T. Wang and S. Yin. 2019. Integrated application of multi-omics provides insights into cold stress responses in pufferfish Takifugu fasciatus. **BMC Genomics** 20(1): 1–15. DOI: 10.1186/s12864-019-5915-7.

Windarsih, A., Suratno, H.D. Warmiko, A.W. Indrianingsih, A. Rohman and Y.I. Ulumuddin. 2022. Untargeted metabolomics and proteomics approach using liquid chromatography-orbitrap high-resolution mass spectrometry to detect pork adulteration in *Pangasius hypopthalmus* meat. **Food Chemistry** 386: 1–9. DOI: 10.1016/j.foodchem.2022.132856.

Yang, C., J. Zhang, R. Zhong, Z. Guo, Q. Wang and Z. Zheng. 2020. Characterization of metabolomic differences in peanut worm *Sipunculus nudus* between breeding and nonbreeding seasons. **Aquaculture Reports** 16: 1–8. DOI: 10.1016/j.aqrep.2019.100271.

Zhang, Y., Q. Sun, S. Liu, S. Wei, Q. Xia, H. Ji, C. Deng and J. Hao. 2021. Extraction of fish oil from fish heads using ultra-high pressure pre-treatment prior to enzymatic hydrolysis. **Innovative Food Science and Emerging Technologies** 70: 1–11. DOI: 10.1016/j.ifset.2021.102670.