A review on column CO₂ fractionation methods from fish oil to omega-3, 6 and 9

Arsat Koto¹, Erliza Hambali¹², Ani Suryani¹²

¹Department of Agroindustrial Engineering, Faculty of Agricultural Technology, IPB University (Bogor Agricultural University), Bogor, West Java, Indonesia
²Surfactant and Bioenergy Research Center, IPB University, Baranangsiang Campus, Bogor, Indonesia

E-mail: arsatkotoipb@gmail.com

Abstract. Commercial interest in getting polyunsaturated fatty acid concentrates, especially omega-3 fatty acids such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) was increasing. Unsaturated fatty acids could prevent cardiovascular disease and improved cognitive function of the brain. Fish oil was the main source of fatty acids, especially omega-3 fatty acids. The content of omega-3 fatty acids (24%), omega-6 (5.37%) and omega-9 (12.6%) fish oil were quite high. Fish oil concentrate was needed in the food and pharmaceutical industries. Degradation of thermolabile compounds cannot be avoided in conventional separation methods. Some conventional oil separation methods had weaknesses and strengths. Column chromatography: the concentrate was very difficult to separate so it required a lot of solvents; distillation: low oil yield due to a lot of yawning; centrifugation: the results were heterogeneous; membrane: easy to plug; using solvents: low cost, technology exists, could be at room temperature and atmospheric pressure, the results were homogeneous. The choice of solvent determined the level of yield and safety. CO₂ as a solvent was non-polar, non-toxic, volatile, high diffusivity, low surface tension, low viscosity, good extract degradation, low critical temperature, (31 °C), could prevent thermal degradation, fast, cheap and efficient.

1. Introduction

Fish oil is one of the nutrients that contains fatty acids rich in benefits obtained from fish body tissues. Fish oil contains triglycerides from three fatty acids with phospholipids, glycerol ether and ester wax [1]. Fish oil contains about 25% of saturated fatty acids and 75% of unsaturated fatty acids. This is confirmed by [2] that omega-3 fatty acids from fish oil contains a little saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), but they contain a lot of polyunsaturated fatty acids (PUFA). The characteristics of fish oils are that they contain a variety of long chain fatty acids with a number of carbon atoms ranging from 14 to 22, and unsaturation levels ranging from one to six double bonds per molecule. In addition, the oil content and composition of fatty acids differ in each type of fish. Even differences are also found in similar fish that are influenced by diet, habitat, temperature, seasonal influences, age and sex [3, 4, 5].

The results of test conducted by [6] show that omega-3 (24%), omega-6 (5.37%) and omega-9 (12.6%) fatty acids are quite high. [7] confirms that omega-3 and omega-6 fatty acids are essential fatty acids. Essential fatty acids are fatty acids that cannot be produced by the body, but are needed so
that they are taken from food sources [8], because they are able to prevent cardiovascular disease, Alzheimer's, increase fetal development and better baby's immune response [9]. Although the human body can synthesize several types of fatty acids, several other types of fatty acids such as omega-3 (ω3) and omega-6 (ω-6) fatty acids are obtained through food or supplements [10]. Fish oil supplements are increasingly popular in the global market. Fish oil is widely used as a functional food and nutritional supplement [11]. Fish oil is easily oxidized by the air, easily hydrolyzed (acidic), can be soaped and polymerized. While the physical properties of fish oil has a smaller specific gravity than the specific gravity of water, refracting light at specific angles, has a certain degree of thickness and golden yellow [12].

Production of PUFA n-3 concentrate involves the trans-esterification of triacylglyceride (TAG) fish oil with alcohol (such as methanol and ethanol), obtaining methyl/ethyl ester (FAME/FAEE) fatty acids and glycerol [13]. Omega-3 needs to be separated from other fatty acid mixtures. Several conventional separation and purification methods can be carried out, such as distillation, centrifugation, column chromatography, membrane separation and solvent use [14].

2. Fish Oil Composition

The content of omega-3, omega-6 and omega-9 fatty acids is quite high in fish oil (Table 1) [6].

Table 1. Fish oil content

| Content       | Unit          | Result |
|---------------|---------------|--------|
| Anisidine     | Meq O₂/kg     | 7.97   |
| Peroxide value| Meq O₂/kg     | 2.49   |
| Acid value    | Mg KOH/g      | 3.37   |
| Iodine value  | Gi² 100 g     | 156    |
| Moisture content | %       | 0.340  |
| Free fatty acid | %          | 1.90   |
| Omega-3       | %            | 24.8   |
| EPA           | %            | 5.61   |
| DHA           | %            | 18.7   |
| Omega-6       | %            | 5.37   |
| Omega-9       | %            | 12.6   |

2.1 Omega-3 Fatty Acids

Omega-3 fatty acids are a group of unsaturated fatty acids that have multiple double bonds. The first double bond is lied in the third carbon atom of the methyl group and the next double bond is lied in the third carbon atom number of the previous double bond. Several types of omega-3 acids, including alpha-linolenic acid (ALA, 18: 3n-3), eicosapentaenoic acid (EPA, 20: 5n-3) and docosahexaenoic acid (DHA, 22: 6n-3) [15]. The molecular formula of omega-3 fatty acids that are found in fish oil is shown in Figure 1.
Fatty algae are used to extract EPA and DHA, which are important in maintaining health. Omega-3 fatty acids, especially EPA and DHA, are widely used in foods and supplements. The importance of omega-3 fatty acids is often ascribed to PUFAs long chain, especially EPA and DHA. The total content of EPA and DHA in various fishes varies and depends on the type of fish and its habitat. Fatty fish such as herring, mackerel and salmon have higher levels of omega-3 fatty acids than lean fish, such as cod. Meanwhile, shellfish such as crab (0.3%-0.4%), shrimp (0.2%-0.5%), and lobster (0.3%-0.4%) have omega-3 low. In addition, types of mollusks such as squid, oysters and shellfish contain low fatty acids (0.2%-0.6%) [18].

Omega-3 is able to prevent cardiovascular disease, Alzheimer's, increase fetal development and better baby's immune response [9], maintain mental health [19], as an anti-inflammatory and antiarrhythmic agent that is beneficial for heart function [20]. Omega-3 fatty acids, especially EPA and DHA are already widely used in foods and supplements, such as cereal-based products, milk and juice drinks. The total daily recommended EPA and DHA intake is depended on age. Children aged 1-8 years can consume up to 1.5 g; 2.0 g for ages 9-13 years and 2.5 g for 14-18 years. The amount of consumption can reach up to 3.0 g/ ay for adults [17].

2.2 Omega-6 Fatty Acids

Omega-6 (PUFA) which is consumed in excess without being offset by consumption of omega 3 can reduce LDL (Low Density Lipoprotein) cholesterol. However, High Density Lipoprotein (HDL) cholesterol also decreased. In addition, the disturbed balance between omega 3 and omega 6 causes blood to clot easily. Both of these are unfavorable because the decreased LDL/HDL ratio and easy blood clotting cannot prevent the occurrence of coronary heart disease, it can even trigger coronary heart disease [21].

2.3 Omega-9 Fatty Acids

Omega-9 (MUFA) has a protective power that can reduce blood LDL cholesterol, increase HDL cholesterol which is greater than omega 3 and omega 6, more stable than PUFA. Omega 9 fatty acids can prevent coronary heart disease (laboratory-tested and epidemiology), where research is always done using oils with low saturated fatty acid levels (about 5%). The results showed that omega-6 in the single form has negative properties because it is associated with increased production of eicosanoids (stimulants for tumor growth in animal experiments). But the presence of omega-3 and omega-9 in suitable proportions will have the potential to block the product of the eicosanoids compound [22].

3. Fish oil refining

Fish oils are extracted require a purification process to achieve the well quality characteristics that acceptable for human and animal consumption [23], while they contain insoluble impurities, phospholipids, free fatty acids, moisture, primary oxidation products, minerals, pigments and even
Persistent organic pollutants (POP) so that must be eliminated to get highest quality [24]. The traditional refining process includes several stages, such as degumming, neutralization, bleaching, deodorization and winterization. Each of the stages is especially important to remove the different classes of compounds (Table 2) and is the most studied and industrially applied process for the refining of fish oils. Furthermore, each of refining technology has its principle work (Table 3) [25]. [23] states that the use high temperature such as at neutralization, drying and deodorization process can make increasing peroxide and acidity value so that oil is susceptibility to oxidation and the formation of peroxides.

**Table 2. Minor oil components, effect on the quality and refining stages for its removal**

| Type of component               | Effect on oil quality                                          | Refining stage     |
|---------------------------------|-----------------------------------------------------------------|--------------------|
| Phospholipids                   | Sedimentation in the product, less oxidation stability         | Degumming          |
| Free fatty acids                | Acylglycerol pro-oxidants, lower stability to oxidation        | Neutralization     |
| Pigments                        | Decrease in sensory quality                                    | Bleaching          |
| Ions and metal complexes        | Harmful, pro-oxidants                                          | Neutralization     |
| Oxidation products              | They cause bad taste and rancidity, as well as being harmful   | Neutralization, bleaching, deodorization |
| Persistent organic pollutants   | Toxicity                                                        | Bleaching          |
| Humidity                        | Pro-oxidants                                                   | Drying             |

**Table 3. Technology with application potential in fish oil refining**

| Technology                      | Principle                                                                 | Limitation                      |
|---------------------------------|--------------------------------------------------------------------------|---------------------------------|
| Enzymatic degumming             | Modification of phospholipids with phospholipases to facilitate hydration | Possible oil instability after processing |
| Degumming by membranes Retention| Retention of phospholipids through the passage of crude oil through semipermeable membranes | Process conditions must be adapted for each kind of oil |
| Neutralization by molecular distillation | Purification by distillation at low pressures | High implementation costs at the industrial level |
| Enzymatic de-acidification Esterification | Esterification of fatty acids through a reaction catalyzed by lipases | Higher energy consumption compared to neutralization with alkali |
(Continuation of table 3)

| Technology         | Principle                                                                 | Limitation                                             |
|--------------------|---------------------------------------------------------------------------|--------------------------------------------------------|
| Nano-neutralization| Reaction of high pressure oil in a hydrodynamic cavitation reactor (nano-reactor), where high turbulence and cutting forces are created, which mixes the caustic solution and the oil very well, and eliminates phospholipids and other impurities | Possible secondary reactions with alteration of physical and organoleptic characteristics of the oil |

4. Fractionation methods

Some conventional and advanced oil separation and purification methods, including chromatography, distillation, winterization, membranes, formation of complexes with urea, enzymatic methods and using solvents (fractionation by supercritical fluids) [14, 25].

4.1 Using Chromatography

The separation process is based on the ability to absorb different compounds in the stationary phase. However, it is very difficult to separate, due to various polarities and most oxygenated compounds. As a result, it requires a lot of solvents [26]. Chromatographic methods can be used to purify PUFAs concentrates with a high degree of purity (> 95%) [27]. Supercritical liquids have the density and capacity of certain liquid solvents. However, it has lower viscosity and better diffusion, so it can be used as a carrier of substances such as the mobile phase in gas chromatography or as a solvent as a solvent in HPLC. This technique is known as supercritical fluid chromatography (SCFC) [28]. SCFC is very suitable for separating high purity omega-3 PUFAs, because it combines high selectivity from supercritical fluids and stationary phases [29]. The results of a study conducted by [30] showed that the production rate of DHA ethyl esters with a purity of 90% of 0.85% g/(kg ODS * h) and EPA ethyl esters with 53% purity of 0.23 g/(kg ODS * h) where supercritical CO₂ as the mobile phase and octadecylsilane (ODS) as the stationary phase.

4.2 Using Distillation

The process of splitting oil into different fractions based on its boiling point. However, some chemical compounds in oil are unstable and sensitive to high temperatures. This results in low oil yield, polymerization reaction and easy coke from the remaining fraction [31]. In addition, molecular distillation can be used for the separation and purification of substances that are sensitive to temperature, because they use high vacuum pressure (lower than absolute pressure of 1.000-500 kPa) [32, 33], and are also capable of removing organic pollutants [34]. This technology can also be applied to fish oil to get its concentrate (PUFA or MUFA) and several studies have been successfully carried out. For example, by [35, 36] EPA and DHA as ethyl esters. In addition, the results of a study conducted [37] that the total concentration of EPA and DHA from tuna oil has increased (from 32.11% to 82.23%). The same thing was reported by [36] that the concentration of acylglycerol from omega-3 increased in sardine oil (from 63% to 91%).

4.3 Using Winterization

Winterization is a process that involves the crystallization of a portion of the oil by controlling the cooling, followed by filtration. The aim is to separate saturated fatty acids from unsaturated fatty acids. The principle of separation is based on the melting point of fatty acids, which tends to the number of long chains and unsaturation. Unsaturated and monounsaturated fatty acids have higher melting points
and winterization so that they can be separated by filtration, whereas polyunsaturated fatty acids (PUFA) have lower melting points so that they remain liquid in the oil [38]. Organic solvents can be used in the winterization process to increase the rate of mass transfer and crystallization fraction of saturated fatty acids [39]. As the results of the study [40] showed that the best conditions when 40% hexane without freezing agitation were able to increase the concentration of unsaturated fatty acids by 9.2% and reduce saturated fatty acids by 13.3%. In addition, several other studies report that using hexane solvents can increase omega-3 concentrates compared without hexane solvents [41], using ethanol as a solvent and at -5 °C gives the best results in sardine oil [42], DHA and EPA experience an increase of 69% and 51.6%, respectively.

4.4 Using Membranes

The separation process is based on the ability to absorb different compounds in the stationary phase. This technology has been used in degumming, recovery of solvents in the extraction process, pigment removal, acidity reduction, concentrations of minor components, removal of waxes and emulsion separation [43]. This technology has been used in PUFA [44, 45]. As a result of studies conducted by [46] on salmon oil that saturated fatty acids have decreased (from 27.2% to 20.2%), while unsaturated fatty acids have increased (from 41.6% to 46.5%, with increasing DHA from 9.9% to 11.6%, and EPA from 3.6% to 5.6%). However, the method is easy to plug. As a result, it is necessary to clean, maintain and dispose of concentrates regularly [47].

4.5 Formation of Complexes with Urea

This technique is the simplest and most efficient technique for obtaining omega-3 PUFA concentrates as free fatty acids or triacylglycerol ethyl esters. That is, saturated and monounsaturated fatty acids are separated from polyunsaturated fatty acids found in urea solution. Saturated and monounsaturated fatty acids separate during the crystallization process, whereas PUFAs do not form complexes so they remain in the liquid fraction [48]. Furthermore, [49, 50] examines the relationship of urea and fatty acids, temperature and crystallization time. The results of the study [49] showed that the content of EPA and DHA was higher than 85.02% in tuna oil with a ratio of urea and fatty acids is 15, a temperature of -5 °C and a time period of 20 h. Also, [48] reported that EPA and DHA had increased, namely EPA from 15.39% to 19.76% and DHA from 17.45% to 29.61%.

4.6 Using Enzymatic Methods

Concentration by enzymatic method is based on the selectivity of several lipases for certain fatty acids or positions in the triacylglycerol molecule, catalyzing the hydrolysis reaction, alcoholysis or trans-esterification of triacylglycerol [51]. Some studies propose that this method is carried out before the application of other stages, such as distillation molecules or filtering with membranes, to obtain omega-3 fatty acid concentrates [25]. According to [52] that the use of supercritical CO₂ as a solvent is very good in the process of alcoholysis, because CO₂ is environmentally friendly, non-toxic and easily separated from the product so that it is suitable for use in enzymatic methods. Moreover it can be easily combined with other processes such as SCFE, FSCF, SCFC and encapsulation with supercritical CO₂ [29]. Furthermore, a study conducted by [52] on Sardinella aurita oil showed that 40% increased than usual when using supercritical CO₂.

4.7 Using Solvents

The separation process is based on differences in the solubility of different compounds to solvents, relatively low cost, available technology, can be at room temperature and at atmospheric pressure and is able to separate oil in different fractions, efficient, high yield [53].
4.8 Fractionation by Supercritical Fluids (FSCF).

Several studies have been successfully carried out using this method. [54] evaluated the fractionation of freshwater fish oil with quite low omega-3 PUFA content (10%). The best fractionation is obtained when isotherm 33 and 40 °C at 20.000 kPa. [55] also studied this method in tuna oil by using ethanol as a joint solvent. The results show that it is very effective and can restore PUFA. Because the complexity of fish oil is hampered so that the formation of methyl esters and triacylglycerol ethyl esters from fish oil increases and is more stable than fatty acids. This is obtained by hydrolysis of triacylglycerol (TAG) and fatty acid alkylation before FSFC [54, 29].

[56] studied the omega-3 concentrate from a mixture of commercial fatty acid esters obtained from fish oil using SFCF with supercritical fluid CO₂. They studied different pressures (10.000, 14.000, 15.000 and 30.000 kPa) and different column temperature (40, 50 and 60 °C). The results show that with increasing pressure and flow rate giving the best results, namely increasing DHA (from 24.54% to 49.57%), the EPA:DHA ratio decreases (from 1.61% to 0.65%), while fatty acids monounsaturated decreases (from 3.33% to 0.6%). [57] also reported that the best concentration of EPA (24.74%) and DHA (26.02%) at 60 °C compared to 40 °C (EPA= 4.28%, DHA= 7.53%) sardine oil. Likewise, the CO₂ density showed better DHA and EPA concentrations, increasing from 700 to 800 kg/m³, reaching compositions approaching 40% and 60% EPA and DHA, respectively.

5. Types of Solvents

Ethanol is a common solvent point and tends to be safe to use. Ethanol has a boiling point of 70 °C so that the extraction temperature used can attract all of components in the raw material [58]. According to [59], solvents which commonly used to extract fats are alcohol (methanol, ethanol, isopropanol, n-butanol), acetone, ether (diethyl ether, isopropyl ether, dioxane), halocarbons (chloroform, dichloromethane), hydrocarbons (hexane), benzene, cyclohexane, iso-octane), or mixtures of these solvents. [60] revealed that the greater the amount of organic solvents used in the extraction process, the higher the amount of dissolved components. The main factor in fractionation at low temperatures is the difference in melting point and solubility of the fat components to be separated. CO₂ is one of the organic solvents, non-polar, non-toxic, volatile, high diffusivity, low surface tension, low viscosity, good extract degradation, low critical temperature, (31 °C), can prevent thermal degradation, fast, cheap and efficient. In addition, supercritical CO₂ is more beneficial compared to other organic solvents, because it is considered a safe and non-flammable solvent [61].

Supercritical Fluid

Supercritical fluid conditions are formed when the fluid conditions are above its critical temperature and pressure. Unlike gases, supercritical fluid cannot be condensed into a liquid-gas state by pressure regulation. Supercritical fluids provide a large density range. This can be used to control the solubility of the solvent. Supercritical fluids are characterized by high density, low viscosity, and medium diffusivity between gases and liquids. This unusual property, instead makes supercritical fluid as an ideal and potential solvent.

Viscosity and Diffusivity of Supercritical Fluid.

In supercritical conditions, the interaction force between molecules is relatively low. It causes high mobility of molecules and causes supercritical fluid to have a lower viscosity than liquid, so supercritical fluid has the ability to penetrate into the solute so that it extracts a better solute. The viscosity and diffusivity of the supercritical fluid approaches the gas as the pressure is raised. Diffusivity will increase with increasing temperature. Although not as large as gas, supercritical fluid diffusivity is greater than liquid diffusivity, resulting in a greater mass transfer rate. Low viscosity and high diffusivity will make it easier for the solvent to penetrate the extracted material. The main advantage of supercritical fluid compared to liquid is greater diffusivity.
Density of Supercritical Fluid.

The ability of a solvent to dissolve a solute is expressed in terms of the number of solvents per unit volume. Supercritical fluid has a density that almost comparable to a liquid. As high density, many molecules can dissolve solutes. So that the ability of dissolving becomes greater.

Solubility of Supercritical Fluid.

Gas solubility in a solvent usually decreases with increasing temperature. But at high temperatures, close to the critical temperature of the solvent, the solubility of the gas generally rises in proportion to temperature. Most of solubility is expressed in units of mole fraction or Henry's constant. Many liquids are developed as solvents of supercritical states by heating and increasing pressure. The solvent that is often used for the supercritical fluid extraction process is CO₂ solvent. This is caused the low critical temperature of CO₂ allowing the experimental process to approach the ambient temperature. This condition is relatively easy to be achieved, because not too much energy is needed. Because it does not use non-organic solvents, so there is no solvent residue in the extract, besides that CO₂ is non-toxic and non-flammable and odorless. However, although CO₂ has some of these advantages, CO₂ has a limited ability to dissolve polar substances. The supercritical CO₂ characteristics can be increased by the addition a second component which is a polar substance and dissolves in supercritical CO₂.

Guenther [62] states that the conditions for a good solvent are selective, low boiling point (so easily separated by extract), not easy to react (chemical inert), cheap and not dangerous. The use of chemicals as solvents, such as chloroform, methanol and hexane can have an impact on human health and the environment, because chemical solvents are toxic. Solvent selection is important to obtain optimum yield, good oil quality and good for health [61, 63].

6. Conclusions

In this paper, the fractionation method and the selection of solvent type to separate fish oil content, especially the omega-3, omega-6 and omega-9 fatty acids have been discussed, and conclude that 1) using the solvent fractionation method is more profitable, because the low costs relatively, efficient, high yield, technology exists, can be at room temperature and at atmospheric pressure and able to separate oil in different fractions; 2) using type of CO₂ as solvent is safer, healthier and cheaper.

References

[1] Zheng S and Chen TC 2016 Fish, Fish Oil and Liver Cancer pp 249–262.
[2] Tengku-Rozaina TM and Birch EJ 2018 Effects of Low Temperature Solvent Fractionation on the Thermal Oxidative Stability and Antioxidant Activity of Refined Hoki Oil and its Derived Fractions 12 pp 1–6.
[3] Usyudus Z, Polak-Juszcza L, Dobrzanski Z and Malesa-Ciecwierz M 2007 Study on the Nutritive Value of Raw Fish Oils 4 pp 593–596.
[4] Nascimento VLV, Bermúdez VMS, Oliveira ALL, Kleinberg MN, Ribeiro RTM, Abreu RFA and Caricota JOB 2015 Characterization of a Hydrolyzed Oil Obtained From Fish Waste for Nutraceutical Application 2 pp 321-325.
[5] Estiasih T 2009 Fish Oil, Technology and Its Application for Food and Health Yogyakarta, Graha Ilmu.
[6] Intan DP 2018 Anisidine Value, Peroxide Value, Acid Value, Iodine Value, Moisture Content, Free Fatty Acid, and Omega Analyses Report of Analysis PT. Starfood International Certificate No. 187761 December 11, 2018.
[7] Sybille M, Editha G, Sascha R, Horst K, Ines L, Andreas W, Jan F 2017 Impact of Fish Species and Processing Technology on Minor Fish Oil Components Food Control 73 pp 1379-1387.
[8] Sanders TAB 2016 1 - Introduction: The Role of Fats in Human Diet, Elsevier Ltd.
[9] Swanson D, Block R and Mousa SA 2012 Omega-3 Fatty Acids EPA and DHA, Health 3 pp 1–7.
[10] Zhang Y, Wang Xiaosan, Xie D, Zou S, Jin Q and Wang Xingguo 2018 Synthesis and Concentration of 2-monoacylglycerols Rich in Polyunsaturated Fatty Acids pp 60–66.
[11] Adeoti IA dan Hawboldt K 2014 A Review of Lipid Extraction from Fish Processing by Product for Use as a Biofuel 63 pp 330-340.
[12] Mozuraityte R, Kristinova V, Standal IB and Carvajal AK 2016 Oxidative Stability and Shelf Life of Fish Oil, Elsevier Inc.
[13] Melgosa R, Sanz MT, Benito-Román Ó, Illera and Mousa 2019 Supercritical CO2 Assisted Synthesis and Concentration of Monoacylglycerides Rich in Omega-3 Polysaturated Fatty Acids pp 65–74.
[14] Zhang X, Ma H, Wu S and Wei W 2019 Sequential Fractionation of Lignin-Derived Pyrolysis Oil Via Extraction with a Combination of Water and Organic Solvents pp 2144–2159.
[15] Shahidi F 2001 Omega-3 Fatty Acids in Health and Disease AOCS Press.
[16] Ackman, RG 1982 Fatty Acid Composition of Fish Oil Academic Press Ltd London.
[17] Shahidi F 2011 Omega-3 Fatty Acids in Health and Disease AOCS Press.
[18] Barrow and Shahidi F 2008 Marine nutraceuticals and functional foods CRC Press Boca Raton FL.
[19] Lange KW 2020 Omega-3 Fatty Acids and Mental Health pp 1–13.
[20] Endo J and Arita M 2016 Cardioprotective Mechanism of Omega-3 Polyunsaturated Fatty Acids 1 pp 22–27.
[21] Haryadi W and ST 2006 Fraksinasi Asam Lemak Omega 3,6 dan 9 dari Daging Bekicot (Achatina fulica) Menggunakan Kolom Kromatografi 3 pp 316–321.
[22] Muchtadi TR 2000 Omega-3, Media Indonesia, 20 November 2000.
[23] Crexi, VT, Legemann-Monte, M Almeida de Souza L and De Almeida-Pinto L 2010. Production and Refinement of Oil from Carp (Cyprinus carpio) Viscera. Food Chemistry 3 pp 945-950.
[24] Huang, J and Sathivel S 2010 Purifying Salmon Oil Using Adsorption, Neutralization and Combined Neutralization and Adsorption process 1 pp 51-58.
[25] Méndez and JLH Concha 2018 Methods of Extraction, Refining and Concentration of Fish Oil as a Source Of Omega-3 Fatty Acids 3 pp 645–668
[26] Borisova YY, Tazeeva EG, Mironov NA, Borisov DN, Yakubova SG, Abilova GR, Sinyashin KO and Yakubov MR 2017 Role of Vanadylporphyrins in the Flocculation and Sedimentation of Asphaltenes of Heavy Oils with High Vanadium Content 12 pp 13382-13391.
[27] Dillon JT, Aponte JC, Tarozo Rand Huang Y 2013 Purification of Omega-3 Polyunsaturated Fatty Acids from Fish Oil Using Silver-Thiolate Chromatographic Material and High Performance Liquid Chromatography pp 18-25.
[28] Taylor LT 2009 Supercritical Fluid Chromatography for The 21st Century 3 pp 566-573.
[29] Rubio N, Beltrán S, JaimeI, Diego SM, Sanz MT and Carballido JR 2010 Production of Omega-3 Polyunsaturated Fatty Acid Concentrates 1 pp 1-12.
[30] Alkio M, González C, Jánti M and Aaltonen O 2000 Purification of Polyunsaturated Fatty Acid Esters from Tuna Oil with Supercritical Fluid Chromatography 3 pp 315-321
[31] Ibrahim D, Jobson M and Gosalbez GG 2017 Optimization-Based Design of Crude Oil Distillation Units Using Rigorous Simulation Models 23 pp 6728-6740
[32] Cerón IX, Cardona CA and Toro LA 2012 Simulación Del Proceso de Concentración de Aceite Esencial de Cidrón (Lippia Citriodora) Por deStilación Molecular de Película Descendente Ingeniería 1 pp 107-120.
[33] Prampano M, Prizzon S and Martinello MA 2005 Estudio de la Purificación de Ácidos Grasos, Tocoferoles y Esteroles a Partir del Destilado de Desodorización 3 pp 228-234.
[34] Olli JJ, Breivik H and Thorstad O 2013 Removal of Persistent Organic Pollutants in Fish Oils Using Short-Path Distillation with a Working fluid 3 pp 273-278.
[35] Solaesa ÁG, Sanz MT, Falkeborg M, Beltrán S and Guo Z 2016 Production and Concentration of Monoacylglycerols Rich in Omega-3 Polyunsaturated Fatty Acids by Enzymatic Glycerolysis and Molecular Distillation 190 pp 960-967.

[36] Oliveira ACM and Miller MR 2014 Purification of Alaskan Walleye Pollock (Gadus Chalcogrammus) and New Zealand Hoki (Macruronus novaezelandiae) Liver Oil Using Short Path Distillation 5 pp 2059-2076.

[37] Wang W, Li T, Ning Z, Wang Y, Yang B, Ma Y and Yang X 2012 A Process for the Synthesis of PUFA Enriched Triglycerides from High-Acid Crude Fish Oil 109 3 pp 366-371.

[38] Vazquez L and Akoh CC 2012 Enrichment of Stearidonic Acid in Modified Soybean Oil by Low Temperature Crystallisation 1 pp 147-155.

[39] Morales R, Munio MM, Perez R, Guadix A, and Guadix EM 2013 Lipids from Marine Sources Perez-Galvez and JP Berge (Ed.). Utilization of fish waste. Boca Raton EE UU CRC Press.

[40] Cunha DC, Crexi VT and De Almeida-Pinto LA 2009 Winterization of Fish Oil with Solvent 1 207-213.

[41] Tengku-Rozaina M and Birch EJ 2013. Enrichment of Omega-3 Fatty Acids of Refined Hoki Oil 8 pp 1111-1119.

[42] Diaz MA, Bonilla R, Hoyos JL and Benitez R 2016 Evaluacion de Refinacion de Aceite Extraido de Ensalaje de Subproductos de Trucha Arcoiris (Oncorhynchus mykiss) pp 351-354.

[43] De Morais-Coutinho C, Chiu MC, Basso RC Ribeiro, APB, Goncalves LÁG and Viotto LA 2009 State of Art of the Application of Membrane Technology to Vegetable Oils 5-6 pp 536-550.

[44] Ghasemian S, Sahari MA, Barzegar M and Ahmadi H 2016 Omega-3 Polyunsaturated Fatty Acids Concentration Using Synthesized Poly-vinylidene Fluoride (PVDF) Asymmetric Membranes 9 pp 1201-1210.

[45] Ghasemian S, Sahari MA, Barzegar M and Gavlish HA 2015 Concentration of Omega-3 Polyunsaturated Fatty Acids by Polymeric Membrane 11 pp 2411-2418.

[46] Linder M, Fanni J and Parmentier M 2005. Proteolytic Extraction of Salmon Oil and PUFA Concentration by Lipases 1 pp 70-76.

[47] O’Loughlin TE, Ngamassi FE, McKay P and Banerjee S 2018 Separation of Viscous Oil Emulsions Using Three-Dimensional Nanotetrapodal ZnO Membranes 4 pp 4894-4902.

[48] Homayooni B, Sahari MA and Barzegar M 2014 Concentrations of Omega-3 Fatty Acids from Rainbow Sardine Fish Oil by Various Methods 2 pp 743-748.

[49] Liu S, Zhang C, Hong P and Ji H.2006 Concentration of Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) of Tuna Oil by Urea Complexation 3 pp 203-209.

[50] Suriani NW, Lawalata HJ and Komansilan A 2014 Urea Crystallization on the Concentrate Making of Omega-3 Fatty Acid from Oil of Tuna Fish (Thunnus sp.) Canning By-product 7 pp 1981-1990.

[51] Correa C, Tejeda A, Martin AR, Garcia HS and Noriega JA 2017 Cinetica de Esterificacion Enzimatica de Acidos Grasos Poliinsaturados N-3 1 pp 17-28.

[52] Lin TJ, Chen SW and Chang AC 2006 Enrichment of n-3 PUFA Contents on Triglycerides of Fish Oil by Lipase Catalyzed Trans-esterification under Supercritical conditions 1-2 pp 27-34.

[53] Pinheiro C, Quina MJ and Gandoferreira L 2018 New Methodology of Solvent Selection for the Regeneration of Waste Lubricant Oil Using Greenness Criteria 5 pp 6820-6828.

[54] Lopes BLF, Sanchez-Camargo AP, Ferreira ALK, Grimaldi R, Paviani LC and Cabral FA. 2012. Selectivity of Supercritical Carbon Dioxide in the Fractionation of Fish Oil with a Lower Content of EPA+DHA pp 78-85.

[55] Ferdosh S, Sarker Z, Norulaini N, Akanda J, Ghafoor K and Kadir O 2014. Simultaneous Extraction and Fractionation of Fish Oil from Tuna By-product Using Supercritical Carbon Dioxide (SC-CO2) 2 pp 230-239.
[56] Perretti G, Motori A, Bravi E, Favati F, Montanari L and Fantozzi P 2007. Supercritical Carbon Dioxide Fractionation of Fish Oil Fatty Acid Ethyl Esters 3 pp 349-353.

[57] Letisse M and Comeau L 2008 Enrichment of Eicosapentaenoic Acid and Docosahexaenoic Acid from Sardine By-products by Supercritical Fluid 8 pp 1374-1380.

[58] Kealey, KS, M Rodney, JF Leo, F John, Margaret and Giovani 2004 Cocoa Extract Prepared from Cocoa Solids Having High Cocoa Polyphenol Content United States Patent. pp 1-7.

[59] Shahidi, F and Wanasundara UN 2002 Methods for Measuring Oxidative Rancidity in Fats and Oils Inc New York.

[60] Sumardjo, D 2006 Pengantar Kimia: Buku Panduan Kuliah Mahasiswa Kedokteran dan Program Strata 1 Fakultas Bioesakta Jakarta.

[61] Patel A, Matsakas L, Sartaj K and Chandra R 2020. Extraction of Lipids from Algae Using Supercritical Carbon Dioxide, Elsevier Inc.

[62] Guenther, E 1948 Minyak Atsiri UI Press Jakarta.

[63] Okolie and Chigozie L 2019 Influence of Conventional and Recent Extraction Technologies on Physicochemical Properties of Bioactive Macromolecules from Natural Sources 116 pp 827–39.