Urea Crystallization on the Concentrate Making of Omega-3 Fatty Acid from Oil of Tuna Fish (Thunnus Sp) Canning Byproduct

by Ni Wayan Suriani

Submission date: 22-Jun-2023 05:02PM (UTC+0700)
Submission ID: 2120809625
File name: rea_Crystallization_on_the_Concentrate_Making_of_Omega-3....pdf (420.51K)
Word count: 6315
Character count: 30403

www.sphinxsai.com

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.7, pp 1981-1990, November 2014

Urea Crystallization on the Concentrate Making of Omega-3 Fatty Acid from Oil of Tuna Fish *(Thunnus Sp)* Canning Byproduct

Ni Wayan Suriani[1]*, H.J.Lawalata[2], A.Komansilan[3]

[1]*Department of Chemistry, Manado State of University, Manado, 95618, North Sulawesi, Indonesia
[2]Department of Biology, Manado State of University, Manado, 95618, North Sulawesi, Indonesia
[3]Department of Physics, Manado State of University, Manado, 95618, North Sulawesi, Indonesia

*Corres.author : suriani_wayan@yahoo.com

Abstract : Tuna canning process has liquid waste byproduct that contains fish oil. Tuna oil is a good source of polyunsaturated (PUFA), especially EPA long-chain omega-3 *(eicosapentaenoic acid,* C20: 5 Ω -3) and DHA *(docosahexaenoic acid,* C22: 6 Ω-3). Characteristics of tuna canning byproduct liquid waste oil is 1.22% of moisture content, free fatty acid (FFA) of 3.99%, the copper (Cu) levels of 0.14 ppm and iron (Fe) levels of 4.39 ppm and meet IFOMA quality standards. The analysis result of fatty acid profile on oil from tuna canning liquid waste byproduct is 33.71% of SFA, 27.64% of PUFA, 1.39% of omega-6 fatty acid, 25.91% of omega-3 Total, 18.79% of MUFA and 13.97% of omega-9 fatty acid. Urea Crystallization can increase the PUFA from 27.64% to 70.88%, enrichment level of 2.56 times, the total content of omega-3 fatty from 25.91% up to 70.05%, enrichment level of 2.70 times, the EPA content of 4.16% to 9.87%, enrichment level of 2.37 times, DHA from 21.38% up to 59.68%, enrichment level of 2.79 times and the amount of EPA + DHA from 25.54% up to 69.55 %, the enrichment level of 2.72 times.
Keywords: Urea crystallization, concentrate of 0mega-3 fatty acids, liquid waste, tuna fish oil.

I. Introduction

Oil quality depends also on the processing process, the fish oil which is derived from canning and flouring, has free fatty acids (FFA) ranged from 4-20%[1]. Guerard reported that the solid waste generated from tuna processing industry consists of muscle (after the meat is taken), viscera, gills, dark meat / muscle, head, bone, and skin, and this waste can be as much as 70 % of the fish body[2].

Fish oil is a good source of polyunsaturated (PUFA) omega-3, especially the EPA and DHA long chain omega-3 fatty acids[3]. Health benefits of omega-3 fatty acids have been reported for a number of diseases including cardiovascular disease, hypertension, atherosclerosis, brain development, diabetes, cancer, arthritis, inflammation, autoimmune and neurological disorders[4]. Because of the nature of the health benefits, the omega -3 fatty acids has great potential as a functional food ingredient.

Tuna oil is a good source of polyunsaturated omega-3 (Ω-3 PUFA), especially long-chain fatty acid omega-3 of EPA *(eicosapentaenoic acid,* C20: 5 Ω -3) and DHA *(docosahexaenoic acid,* C22: 6 Ω-3)[3]. Long-chain omega-3 fatty acid is considered beneficial to the growth and development throughout the life cycle and plays an important role in the prevention and treatment of coronary arteries (arteriosclerosis, hypertension,

arthritis, and impaired immune response[5-6]. Due to the nature of the health benefits, the omega-3 fatty acids have great potential as a functional food ingredient.

Some things that affect the properties of the oil is the fatty acid constituents, i.e. saturated fatty acids (SFA) and unsaturated fatty acids (UFA), which consists of *mono-unsaturated fatty acids* (MUFA) and *poly-unsaturated fatty acid* (PUFA) or *high unsaturated fatty acids*. The biochemist and nutritionist know it as unsaturated fatty acids omega-3, omega-6 and omega -9. PUFAs have influence that can decrease cholesterol level. The excessive consumption of PUFA (omega -6) without consumption of omega -3 can decrease LDL cholesterol, but HDL cholesterol is also reported decreased. In addition, the disrupted balance of omega -3 and omega -6 cause blood to clot easily. These two things are not beneficial because the ratio of LDL / HDL (Coronary Heart Disease Index / CHD) is decreased. Easily the blood to clot, cannot prevent the occurrence of CHD, even can lead to CHD. Therefore, it should also consume MUFA[7].

Grundy states that MUFA can lower cholesterol (LDL-cholesterol) so MUFA began to receive attention. One type is the omega -9 MUFA (oleic) which is based on research in 1992, 1998, 1999 and 2000, concluded that omega -9 has a power protection that can lower LDL blood cholesterol, increase HDL cholesterol greater than omega -3 and omega -6, and is more stable compared with PUFA. It can be seen from the people who live in the Mediterranean region who are rare getting coronary heart disease because of high consumption of omega -9 and omega -3. While in the western region (USA and Europe), the fat intake has a ratio of 10: 1 (omega-6, omega -3), which is considered unhealthy[8].

Oil obtained from fish meal processing is blackish brown and cause unpleasant odors that are very stingy. But actually, fish oil contains long-chain unsaturated fatty acids that are omega-3 configured, i.e. the fatty acids that have the first double bond at the third carbon atom counted from the methyl group. These fatty acids are especially *eicosapenta-enoat* (EPA) and *docosa-hexsaenoat* (DHA), are reported at doses of 3g / day reduces the risk of heart disease by a decrease in plasma triacylglycerols, blood pressure and platelet aggregation [9]. Reduction of serum triacylglycerols (TAG) with dietary omega-3 PUFA has been supported by studies in mice[10]. Consumption of EPA and DHA are found to reduce the risk of ischemic heart disease (Lemaitre)[11] and heart attacks in humans[12].

In the fish oil, there are omega-3, i.e. eicosapentaenoat acids (EPA) and docosahexsaenoat acid (DHA). In addition to EPA and DHA, fish oil also contains 18: 4 ω-3, 20: 4 ω-3 and even 18: 5 ω-3. While the linolenic fatty acid (LNA, 18: 3 ω-3) is relatively rare, but are abundant in the seeds of certain plants, such as rapeseed oil, soybean oil and black currant seed. Besides fish oil as a source of omega -3 fatty acids, it is also a good source of omega-6 fatty acid, linoleic acid and arachidonic acid[13].

Fish that contain much EPA and DHA are fish that mainly live in the cold and deep sea water. omega -3 fatty acids are derived from Plankton. Fish can convert linolenic acid into EPA and DHA, but not so efficient. This is because most fish cannot synthesize omega -3 fatty acids in itself but is synthesized from phytoplankton which is consumed by the fish, and concentrated on the food chain[14].

Omega-3 fatty acids can be considered as a basic component of daily nutrition for the beneficial effect. Omega-3 fatty acids dietary recommendation has been made by health authorities in different countries[4]. Dietary guidelines from the UK recommend an average daily intake of 0.2g / day of EPA and DHA[15]. Daily intake of 0.5 to 1.0 grams of EPA and DHA has been recommended by the American Heart Association (Lichtenstein)[16] and by The American Dietetic Association Canada[17]. International Society for the Study of Lipids and fatty acids (ISSFAL) recommends 2.2 g / day of ALA and 650 mg of EPA plus DHA per day with a minimum of 220 mg of EPA and DHA per day[18].

II. Material and Methods

2.1. Tools and Materials Research

The used materials in this stage are: Waste liquid, distilled water, Urea PA, NaOH, HCl, EDTA, hexane PA, EtanolPA, Methanol PA.

The used tools in this study includes the *Rotary Vacuum Evaporator, refrigerator,* digital scales, pH-meters, *magnetic stirer,* separating *funnel,* glassware, tools used for the analysis of fatty acid profile is GC *(Gas Chromatography)*

2.2. Research Methods

This study was designed in several phases of the study are: The Omega-3 fatty acid concentrate making from liquid waste oil[19].

a. Saponification

A total of 1000 g of liquid waste oil byproduct of tuna processing is mixed with 2000 ml of a solution of NaOH (5N) in water / ethanol. Reflux for 30 minutes at 60 ° C on a hot plate with stirer. After saponification, add 400 ml of distilled water. NaOH solution is prepared by dissolving 480 g of NaOH and 5 g of Na2EDTA in 400 ml of water, is added to a solution of 1600 ml of ethanol.

b. Extraction of fatty acids

4000 ml of hexane is added to the saponification result and stir for 1 hour, move to separating funnel, let stand for 1 hour to form 2 layers, the top layer containing unsoapped substance and discard. Furthermore, concentrated HCl is added to the bottom layer, while stirring with a *stirrer* until a pH of 4 to move to split funnel, let stand for 1 hour to form 2 layers, grab the top layer and evaporate with a vacuum rotary evaporator at 30 ° C until all the solvent is gone (for 2 hours).

c. Crystallization of urea

The fatty acid extraction results is added to the hot urea solution (temperature 60-65 ° C) in methanol and stirred at a constant speed for 5 minutes. The amount of urea is dissolved in accordance with the ratio of urea : fatty acids, i.e. 3 : 1 [19], and the solution is heated until clear. The volume of used methanol is 200 ml for fish oil from 25 g. The urea solution and oil are stirred for 5 min, and the urea is left to form crystals for 24 hours at a temperature of 10° C. Then do the filtration to separate the urea crystals with solvent containing Omega-3 fatty acids.

d. Extraction of omega-3 fatty acids

Each 3 liters of the filtrate, add 1 liter of n-hexane and 0.5 liters of concentrated HCl, and the compound is stirred for 1 hour by a stirrer and moved to a separating funnel, let stand 1 hour to form 2 layers, then the top layer (hexane) is separated (I). A total of 1,5 liters of water is added to the bottom layer, and this layer is then extracted again with 1 liter of hexane, stirred for 30 minutes, put on separating funnel for 1 hour to form 2 layers, grab the top layer (II). Both extract I and II are mixed, evaporate at 30 ° C with a rotary vacuum evaporator until the solvent n-hexane-out (2 hours).

III. Results and Discussion

This study aims to determine the physicochemical characteristics including moisture content, free fatty acid, peroxide number, Cu and Fe content and fatty acid profiles including the content of SFA, MUFA, PUFA, omega-3, omega-6, omega-9, EPA and DHA and the effectiveness index test of fish oil from tuna fish processing byproduct liquid waste in Bitung city, North Sulawesi.

The analysis is performed to determine that the produced fish oil meets the quality standards as edible oil of *the International Association of Fish Meal Manufacturers* (IFOMA). The obtained result is fish oil containing SFA, MUFA, PUFA, omega - 3, omega-6 and omega-9, which is supported by the characterization value of fish oil.

3.1. The Fish Oil Characteristics from Tuna Canning Byproduct Liquid Waste in Bitung, North Sulawesi

Descriptions of average and variance of chemical characteristics variable and fatty acid profile of fish oils from liquid waste variable are presented in Table 3.1 and Table 3.2.

Characteristics of fish oil from tuna canning byproduct liquid waste, solid waste, and culled fish including moisture content, free fatty acid, peroxide number, levels of iron (Fe) and the levels of copper (Cu) are presented in Table 3.1.

Table 3.1: Characteristics of Waste Liquid fish oil byproduct canning tuna.

Parameters	Liquid Waste	IFOMA Standard
Water Content (%)	1.22 ± 0.002	<1
FFA (%)	3.99 ± 0.17	1-7
Peroxide Number (meq / Kg)	15.81 ± 0.53	3-20
Cu (ppm)	0.14 ± 0.03	<0.3
Fe (ppm)	4.39 ± 0.16	From 0.5 to 0.7

3.1.1. Water Content

Oil from liquid waste has an average water content of 1.22%. The water remains in the byproduct oil of fish canning because at the time of oil extraction, there is addition of distilled water and at the evaporation at a temperature of 30°C there may still be a little bit of water that does not evaporate. When compared with the IFOMA standard (moisture content <1) then the moisture content in this study is slightly higher. In the hydrolysis reaction of fats or oils, it will produce free fatty acids and glycerol. Hydrolysis reaction that can result in damage to the oil or fat occurs because the presence of some water in the oil or fat. This reaction will lead to hydrolysis rancidity that produces the rancid flavor and smell on the oil. The higher the moisture content of fish oil, the faster the hydrolysis reaction and the lower quality fish oil[20] .

3.1.2. Levels of free fatty acids

Analysis of the acid number / free fatty acids is required to determine the degree of hydrolysis of lipids, i.e. how many fatty acids that is non-bonded with glycerol. The number of high free fatty acid will accelerate the damage to the oil. Oil from liquid waste contains average FFA levels of 3.99%, Toisuta reports that tuna fat contents in head of 1.1%, skin of 2%, liver of 1.99% and gonads of 3,83%. The presence of free fatty acids in the fat / oil is usually used as an initial indicator of damage to fats / oils because of the hydrolysis process[21]. The presence of free fatty acids is very closely related to the water content in the oil which can facilitate the hydrolysis of oils or fats.

3.1.3. Numbers Peroxide

The analysis of peroxide is needed to determine the primary oxidation phenomena, the most common damage is rancidity by oxidation that often called auto-oxidation. During the auto-oxidation reaction to form peroxide, the reaction will be accelerated due to the presence of light, the increase in temperature, the presence of oxygen, moisture and the presence of other catalysts[22].

Fish oil from liquid waste has an average of 15.81 peroxide (meq / kg). Fe can accelerate the oxidation because it acts as a catalyst for oxidation reactions. Peroxide value in this study still meets the standards IFOMA (peroxide value of 3-20 meq / kg). This number can be used to determine the level of oxidation of fat or oil. Fats or oils may contain unsaturated fatty acids that can be oxidized and produce a peroxide compound. The presence of peroxide in fish oil is presumed as a result of the precooking process. According Pak heating to high temperatures causes the primary oxidation reaction in the oil and will produce peroxide. When fats oxidize, the peroxide compound will increase[23].

3.1.4. Levels of Copper (Cu)

Metal is a catalyst in the auto-oxidation process of oil, although fatty foodstuffs generally contain very small metal[24]. Analysis of cooper (Cu) content is conducted because its existence is to catalyze the auto-oxidation. The liquid waste oil contains an average of Cu of 0.14 (ppm). The value of Cu concentration on liquid waste oil in this study is in accordance with the IFOMA standards.

3.1.5. Levels of Iron (Fe)

Just like the presence of Cu metal, metal iron (Fe) also acts as a catalyst in the auto-oxidation reaction on fish oil. Oil from liquid waste contains a Fe average of 4.39 (ppm). Fe content values in this study do not meet the IFOMA standards (0.5-0.7 ppm values). In fish, metals such as Fe and Cu generally form complexes with proteins. The metal content analysis of iron (Fe) and copper (Cu) is conducted because of the presence of both these metals in fish oil are thought to contribute to the oxidation reaction. Hamilton, states the metal is a catalyst in the auto-oxidation process on oil, although fatty foodstuffs generally contain very little metal[24].

3.2. Oil Fatty Acid Profile from Tuna Processing Byproducts Liquid in Bitung City, North Sulawesi

Average and variance description of variables in the three groups of oils obtained from liquid waste, solid waste, and culled fish are presented in Table 3 2.

Table 3.2: Fatty Acid Profile of Fish Oil from Tuna Processing Byproducts Liquid Waste.

Fatty Acid Profile (% Relative)	Oil from liquid waste
Capric acid C10:0	0.1 ± 0.01
Lauric acid C12:0	1.9 ± 0.43
Myristic acid C14:0	4.21 ± 0.36
Palmitic acid Asam C16:0	19.32 ± 0.46
Stearic acid C18:0	6.25 ± 0.9
Arakidic acid C20:0	0.51 ± 0.16
Behenic acid C22:0	2.24 ± 0.08
Total SFA	33.71 ± 0.56
Palmitoleic acid C16:1	4.82 ± 0.01
Oleic acid C18:1 (Omega-9)	13.97 ± 0.86
Total MUFA	18.79 ± 0,.5
Linoleic acid C18:2 (Omega-6)	1.39 ± 0.05
Linolenic acid C18:3 (Omega-3)	0.37 ± 0.9
EPA(Eicosapentaenoic) (Omega-3)	4.16 ± 0.08
DHA(Docosahexaenoic) (Omega-3)	21.38 ± 0.38
Total Omega-3	25.91 ± 0.17
Total PUFA	27.64 ± 0.75
Total Unsaturated Fatty Acid	46.43 ± 1.34

As a clearer picture to show the proportion of broiler meat fatty acids as shown in Figure 3.1. as follows:

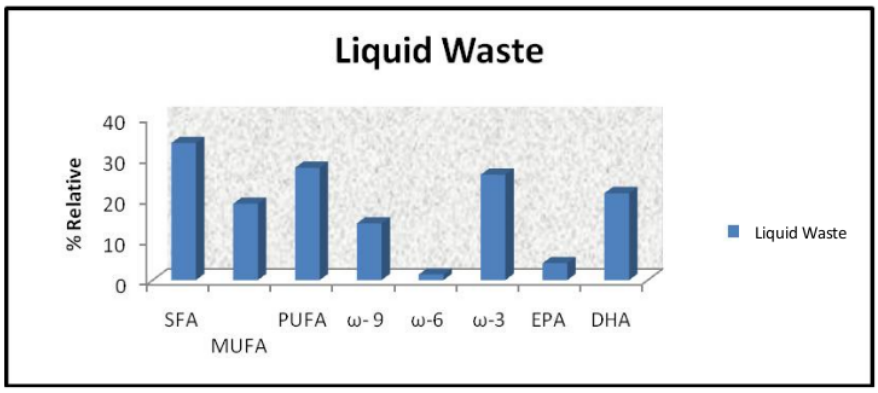

Figure 3.1. The proportion of fatty acids SFA, MUFA, PUFA, Omega-9, Omega-6 'Omega-3, EPA and DHA in liquid waste fish oil.

3.2.1. Content of *Total Saturated Fatty Acids* (SFA).

The analysis results of *Saturated fatty acids* (SFA) shows that the oil from liquid waste contains c*apric acid* (C10: 0), *lauric acid* (C12: 0), *myristic acid* (C14: 0), *palmitic acid* (C16: 0), *stearic acid* (C18: 0),

anachidic acid (C20: 0), *behenic acid* (C22: 0). Oil from liquid waste contains a SFA total average of 33.71%. Toisuta reports that the highest amount of saturated fatty acids (SFA) on the byproduct of the tuna is the head of 30.82%, skin of 26.85%, gut of 16.95%, liver of 26.56% and gonad (reproduction organs) of 21.31%, the highest value of total saturated fatty acids is at the head of 30.82%[21].

3.2.2. *The total* content of *Monounsaturated Fatty Acids* (MUFA).

The analysis results of *Monounsaturated fatty acid* (MUFA) indicates that oil from liquid waste, solid waste and culled fish contains p*almitoleic acid* (C16: 1n-7) and *oleic acid* (C18: 1n-9). Fish oil from liquid waste contains a MUFA total average of 18.79%. Fatimah reports that byproduct oil of tuna canning (liquid waste) contains MUFA of 15.51%[25].

3.2.3. The total content of PUFA.

The analysis results of *polyunsaturated fatty acid* (PUFA) shows that the oil from liquid waste contains *linoleic acid* (C18: 2n-6), *linolenic acid* (C18: 3n-3), EPA *(eicosapentaenoic)* and DHA *(Docosahexaenoat)*. The test results in the table above show that oil from liquid waste gives an PUFA total average of 27.64%. The analysis results of *Unsaturated Fatty Acid* (USFA) indicate that oil from liquid waste contains USFA total of 46.43%. Fatimah reports that byproduct oil from tuna canning (liquid waste) contains PUFA of 33.26%[25].

3.2.4. The content of Omega-9 Fatty Acids

The analysis results of o*leic acid* (C18: 1n-9) or omega-9 fatty acid shows that fish oil from liquid waste contains omega-9 fatty acid of 13.97%. This is consistent with the report by Fatimah that the byproduct oil of tuna canning (liquid waste) contains omega-9 acid of 12.69%[25].

3.2.5. The content of Omega-6 Fatty Acids

The analysis results of *linoleic acid* (C18: 2n-6) or omega-6 fatty acid shows that the oil from liquid waste contains the highest omega-6 fatty acid of 1.39%. Fatimah reports that byproduct oil of tuna canning (liquid waste) contains omega-6 fatty acidof 0.71%[25].

3.2.6. The total content of Omega-3 Fatty Acids

The analysis result shows that the oil from liquid waste contains a total of omega-3 fatty acid consisting of *linolenic acid* (C18: 3n-3), EPA and DHA. Oil from liquid waste contains omega-3 fatty acid for a total of 27.64%. The test results in the table above show that oil from liquid waste contains an omega-3 average of 25.91%. Fatimah reports that byproduct oil of tuna canning (liquid waste) contains an omega-3fatty acid total of 32.55%[25].

3.2.7. The content of EPA

Oil from liquid waste contains EPA of 4.16%. This is because the long-chain unsaturated fatty acids such as EPA are more susceptible to oxidation, it relates to the high free fatty acids contained in the liquid waste. reports that of tuna fish oil from liquid waste contain EPA of 4.8%[26]. Visentainer mentions that the tuna *(Thunnus thynnus)* oil has EPA and DHA content of 4.7% and 36.3%[27].

3.2.8. The content of DHA

Oil from liquid waste contains the highest DHA of 21.38%. The liquid waste is taken from tuna cannery that is derived from the results of precooking. Howe reports that tuna oil from flouring waste contains EPA of 4.8% and DHA of 22.4%[26]. The used raw materials for flouring process is tuna canning and filleting solid waste, i.e. *viscera,* red meat, fins, tail, and head. While the eye, eye pads, and the brain is part of tuna fish head which is rich with omega-3 fatty acids, especially DHA. Furthermore Yowono shows that the fish oil that is extracted by solvents from eye and eye pads waste of tuna, has EPA levels of 5.1% and DHA of 26.2% for *Yellowfin* tuna types and EPA levels of 5.9% and DHA level of 24.1% for the *Skipjack* tuna type[28].

According to Elisabeth that the content of tuna fish oil fatty acids EPA *(eicosapentanoat acid)* of about 3.64% (w / w) and DHA *(docosahexanoat acid)* of approximately 14.64% (w / w)[29]. Benefits and functions of omega-3 fatty acids are very important for the human body including, linolenic fatty acids (omega-3) is used to maintain the structural parts of cell membranes, and has an important function in the development of the brain,

then EPA *(eicosapentaenoic acid)* and DHA *(docosahexaenoic acid)* are useful to educate the brain, helping to lower triglyceride levels as well[30].

According to Imran and Sahgk, some benefits of omega -3 fatty acids are able to cures diabetes, atherosclerosis, prevents cancer, and strengthens the immune system[31]. In addition to fatty acids, omega-3 (EPA-DHA) is an essential fatty acid that cannot be synthesized by the body from the diet food by fish. So the food products that supply rich omega -3 fatty acids are very important. Fish such as tuna, anchovies, trout and salmon are a major source of EPA and DHA[32].

The research results that conducted by Toisuta also shows that the highest amount of single unsaturated fatty acid content of tuna byproducts oil is oleic fatty acid (C18: 1n9c) including: head of 17.76%, leather of 15.21%, intestine of 6.97%, liver 4.85% and *gonads* of 5.94%. While polyunsaturated fatty acids *docosahexaenoic acid* (DHA = C22: 6n3) including: head of 16.41%, skin of 25.10%, intestine of 25.44%, liver of 6.91% and gonads of 41.50%[21].

Many factors influence the composition of fatty acids in fish oil. Visentainer mentions that the fluctuations in the quality of fish food that phytoplankton affect the levels of omega-3 fatty acids in fish oil. Further explained that the factors affecting the composition of fatty acids is the species, sex, sexual maturity, body size, location of capture, water temperature, type of food, and season[27].

Therefore, based on these conclusions, the oil from liquid waste containing good fatty acid profiles and which still meet the IFOMA standards is used for the manufacture of omega-3 fatty acid concentrate by urea crystallization.

3.3. Omega-3 fatty acids concentrate making by urea crystallization of liquid waste oil.

The purpose of making omega-3 concentrate is to increase the levels of fatty acids in the oil and remove components other than omega-3 fatty acids that are not desired such as cholesterol and saturated fatty acids, also get the intake of high omega-3 fatty acids to maintain the fat intake low. The used method in this study is the method of urea crystallization. Urea crystallization method is based on the formation of urea complexes - saturated fatty acids more rapidly than urea complex formation of unsaturated fatty acids[33]. This method is more effective for the separation of fatty acids made by the presence of the double bond, not based on physical properties such as freezing point and solubility[34]. Urea crystallization method is influenced by the ratio of urea: fatty acids, long crystallization, and the crystallization temperature. In this research, the omega-3 concentrates manufacture on the ratio of urea : the optimum fatty acid is 3: 1 and the optimum crystallization time is 24 h at 10°C[25,19]. The analysis results of the fatty acid profile of the oil from liquid waste and omega-3 concentrate (3 replications) are presented in Table 3.3.

Table 3.3: Fatty acids profile of the fish oil from liquid waste and Omega-3 concentrate of fish oil urea crystallization results from liquid waste.

Fatty acid profile (% relative)	Oil from liquid waste	Omega-3 Concentrate
Capric acid C 10=0	0.10 ± 0.01	1.27 ± 0.11
Lauric acid C 12=0	1.90 ± 0.43	0
Myristic acid C 14=0	4.21 ± 0.36	0.38 ± 0.06
Palmitic Acid C 16=0	19.32 ± 0.46	2.48 ± 0.95
Stearic acid C 18=0	6.25 ± 0.9	0.31 ± 0.01
Arachidic Acid C 20=0	0.51 ± 0.16	0.59 ± 0.06
Behenic acid C 22=0	2.24 ± 0.08	2.66 ± 0.18
Total SFA	33.71 ± 0.56	7.68 ± 1.22
Palmitoleic acid C 16=1	4.82 ± 0.01	1.19 ± 0.12
Oleic acid C 18=1 (Omega-9)	13.97 ± 0.86	1.51 ± 0.02
Total MUFA	18.79 ± 0.65	2.71 ± 0.15
Linoleic acid C 18=2 (Omega-6)	1.39 ± 0.05	0.83 ± 0.05
Linolenic acid C 18=3 (Omega-3)	0.37 ± 0.9	0.50 ± 0.11
EPA	4.16 ± 0.08	9.87 ± 0.37
DHA	21.38 ± 0.38	59.68 ± 1.16
EPA + DHA	25.54 ± 0.33	69.55 + 0.80

Total Omega-3	25.91 ± 0.17	70.05 ± 0.91
Total PUFA	27.64 ± 0.75	70.88 ± 0.95
Total USFA	46.43 ± 1.34	73.59 ± 0.80

The data of omega-3 fatty acids concentrate profile test in Table 3.3 shows that the SFA content of 33.71% down to 7.68%, MUFA from 18.79% down to 2.71%, omega-9 fatty acid from 13.97% down to 1.51% and omega-6 fatty acid from 1.39 down to 0.83%. Urea crystallization method can eliminate the double bond saturated fatty acids and unsaturated fatty acids (MUFA). In the process of urea crystallization is an effective technique to get PUFA concentrates in the form of free fatty acids. This technique can eliminate saturated fatty acids and acid monoenoat[35].

Saturated and unsaturated fatty acids can be separated by urea crystallization due to differences in both the alkyl chain linearity. Saturated fatty acids have a straight alkyl chain, and unsaturated fatty acids have grooves on the double bond[36,33]. Yeo and Harris states that at the inclusion complex of urea, hydrogen bonded urea molecules form a channel *(tunnel)* which is parallel. The urea tunnel structure is stable if the tunnel is filled by the guest compound with dense-array. The urea tunnel has a diameter ties based on the van der Waals radius which varies between 5.5 and 5.8 A. Only molecules that are suitable may be guest compounds forming inclusion complexes. PUFA from 27.64% up to 70.88%, enrichment level of 2.56 times, the total content of omega-3 fatty acid from 25.91% up to 70.05%, enrichment level of 2.70 times, EPA content of 4.16% up to 9.87%, the enrichment level of 2.37 times, DHA from 21.38% up to 59.68%, enrichment level of 2.79 times, and the amount of EPA + DHA from 25.54% up to 69.55%, the level of enrichment 2.72 times[37]. Yuwono's research results show the levels of EPA + DHA that is obtained in oil concentrate of tuna eye and eye pad, is 83.9% with the initial EPA + DHA content of 31.3% so that the enrichment level is 2.62 times[28]. Estiasih also reports the analysis results of the response to omega-3 fatty acids concentrate from tuna byproducts flouring using Central Composite Design with EPA + DHA levels between 61.16 to 82.33%[19].

EPA + DHA enrichment level of fish oil, that is the levels of EPA + DHA in omega-3 fatty acid concentrate divided EPA + DHA levels in tuna flouring byproduct fish oil, is 2.90 to 3.93 times. The results of this study shows the lower EPA + DHA level at omega-3 fatty acid concentrate from liquid waste fish oil than the previous studies, but still higher than those reported by Fatimah, that the EPA + DHA level of tuna canning byproducts (liquid waste) omega-3 fatty acid concentrate of 50.66% on the ratio of urea: 3 fatty acids is 3 : 1, and at 24-hour long crystallization the obtained EPA + DHA level is 50.21%[25]. This may be due to different materials and the tuna fishing areas are different. The results of this study shows a higher enrichment level than the Yuwono's research which allegedly caused by the lower EPA + DHA level in tuna flouring byproduct oil[28].

The low EPA and DHA levels means the more saturated fatty acids that have a higher preference form complexes resulting in the more formation of urea inclusion complexes. Visentainer mentions that the fluctuations in the quality of fish food that phytoplankton affect the levels of omega-3 fatty acids in fish oil. Further explained that the factors affecting the composition of fatty acids is the species, sex, sexual maturity, body size, location of capture, water temperature, type of food, and season[27].

Therefore, based on the analysis result of omega-3 concentrate fatty acid profiles, EPA + DHA contents, and the highest omega -3fatty acid total of the oil from the tuna canning byproduct liquid waste in Bitung City, North Sulawesi, may be processed into microcapsules that will substitute into jerky minced chicken.

Conclusion

1. Characteristics of tuna canning byproducts liquid waste is moisture content of 1.22%, free fatty acid (FFA) of 3.99%, the copper (Cu) level of 0.14 ppm, the iron (Fe) level of 4.39 ppm, and still meet the IFOMA quality standards.

2. The analysis results of the fatty acid profile of oil from tuna canning byproduct liquid waste is to contain SFA of 33.71%, PUFA of 27.64%, omega-6 fatty acid of 1.39% and omega-3 fatty acid Total of 25.91%, MUFA of 18,79%, and omega -9 fatty acid of 13.97%.

3. Urea Crystallization can increase PUFAs levels of 27.64% up to 70.88%, enrichment level of 2.56 times, the total content of omega-3 fatty acid from 25.91% up to 70.05%, enrichment level of 2.70 times, EPA content of 4.16% up to 9.87%, the enrichment level of 2.37 times, DHA from 21.38% up to 59.68%, enrichment level of 2.79 times, and the EPA + DHA amount of 25.54% up to 69.55%, the enrichment level of 2.72 times.

References

1. Murtini JT. Jamal Basmal dan Nurul Hak. 1992. *Teknologi pengolahan bagi pengembangan industri produk perikanan bukan bahan makanan*. Dalam prosiding pusat penelitian dan pengembangan perikanan No. 23. Departemen Pertanian. Jakarta.
2. Guerard F, Guimas L, Binet A. 2002. *Production of tuna waste hydrolysates by a commercial neutral protease preparation*. J Mol Catal B-Enyme 19-20:489–98.
3. Klinkesorn, U.; H-Kittikun, A.; Chinachoti, P.; Sophanodora,P.2004. *Chemical transesterification of tuna oil to enriched omega-3 polyunsaturated fatty acids*. *Food Chem*., *87*: 415-421
4. Gogus, U. and Smith, C. 2010. *n-3 Omega fatty acids: a review of current knowledge*. Int. J. Food Sci. and Technol. 45: 417-436.
5. Harris, W. S. 2004. *Fish oil supplementation:* evidence for health benefits. *Cle*Veland *Clin*. *J*. *Med*., *71*:209-221.
6. Uauy, R.; Valenzuela, A.2000, *Marine oils:* the health benefits of n-3 fatty acids. *Nutrition*, *16*: 680-684.
7. Muchtadi, T.R., 2000, *Asam Lemak Omega 9 dan Manfaatnya bagi Kesehatan,* Med Indonesia, Edisi 29 November .
8. Grundy, S. M. 1986. *Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol*. N. Engl. J. Med.314:745-748.Mensink (1987
9. Breslow, J. L. 2006. *n-3 fatty acids and cardiovascular disease*. Am. J. Clin. Nutr. 83: S1477-S1482.
10. Dasgupta, S. and Bhattacharyya, D. K. 2007. *Dietary effect of eicosapentaenoic acid (EPA) containing soyphospholipid*. J. Oleo Sci. 56: 563-568.
11. Lemaitre, R. N., King, I. B., Mozaffarian, D., Kuller, L. H., Tracy, R. P. and Siscovick, D. S. 2003. *n-3 Polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults*: the Cardiovascular Health Study. Am. J. Clin. Nutr. 77: 319.
12. Bhatnagar D., Durrington P.N., 2013. *"Omega 3 fatty acids: their role in the prevention and treatment of atherosclerosis related risk factors and complications"*, Int J Clin Pract, 57 (4):305-314.
13. Nettleton, J.A. 1995. *Omega-3 Fatty Acids and Health*. New York: Chapmain and Hall.
14. Almatsier Sunita. 2003. *Prinsip Dasar Ilmu Gizi*. Jakarta: Gramedia Pustaka Utama.
15. Ruxton, C. and Derbyshire, E. 2009. *Latest evidence on omega-3 fatty acids and health*. Nutr. Food Sci.39: 423-438.
16. Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., Franch, H. A., Franklin, B., Kris-Etherton, P., Harris, W. S. and Howard, B. 2006. *Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee*. *Circulation 114: 82-96*
17. Kris- Etherton , P.M., W.S. Harris., and Appel, L.J. 2003. Fish consumption,fish oil, omega 3 fatty acid andcardiovascular disease, Arterioscler, Thromb.Vasc. Biol., 23:20-31.
18. ISSFAL (*International Socitey for the Study of Fatty acids and Lipids*). 2004. *Recommendations for intake of polyunsaturated fatty acids in healthy adults*. ISSFAL News 11: 12-18.
19. Estiasih T, Kgs. Ahmadi, Fithri Choirun Nisa, dan Fitriyah Kusumastuti, 2009. Optimasi Kondisi Pemurnian Asam Lemak Omega-3 Dari Minyak Hasil Samping Penepungan Tuna (Thunnus sp) Dengan Kristalisasi Urea. J. Teknol. dan Industri Pangan, Vol. XX No. 2 : 135-142
20. Winarno, F.G. 2002. Kimia Pangan dan Gizi. Gramedia Pustaka Utama. Jakarta.
21. Toisuta ,B.R., B. Ibrahim, Herisuseno, S. 2014. *Characterization of fatty acid from By Product of Skipjacktuna*(*Katsuwonus pelamis*).Global Journal of Biology,Agrikulture & Health Sciences vol.3(1) : 278 – 282
22. Suwetja, I.K. 2011. Biokimia Hasil Perikanan. Media Prima Aksara Jakarta.
23. Pak, Chol S. 2005. *Stability and Quality of Fish Oil During Typical Domestic Application*. Universitas Wonsan. Korea.
24. Hamilton, R. J. 2000. *Edible Oil Prosesing*. *Sheffield Academic Press*. *England*

25. Fatimah Ai Imas Faidoh. 2008.Optimasi Kristalisasi Urea pada Proses Pembuatan Konsentrat Asam Lemak Omega-3 dari Minyak Hasil Samping Pengalengan Ikan Tina (Thunnus sp), Skripsi. Jurusan Teknologi Hasil Pertanian Fakultas Teknologi Pertanian Universitas Brawijaya Malang
26. Howe, PRC. Downing JA. Grenyer BFS. Grigonis-Deane EM and Bryden WL. 2002. *Tuna fishmeal as source of DHA for n-3 PUFA enrichment of pork, chiken,end eggs*. Lipids, 37:1067-1076.
27. Visentainer JV, Noffs MA, Carvalho PO, Almeida VV, Oliveira CC,Souza NE (2007). *Lipid content and fatty acid composition of 15 marine fish species from the southeast coast of Brazil*. J AmerOil Chem Soc 84:543–547
28. Yuwono S. 1993. Pemanfaatan Limbah Mata Ikan Tuna sebagai Sumber Asam Lemak Omega-3. Skripsi. Fakultas Teknologi Pertanian, IPB, Bogor.
29. Elizabeth J. 1992. Isolasi Asam Lemak Omega-3 dari Hasil Limbah Industri Pengalengan Ikan Tuna. Thesis S2. Program Pascasarjana, Institut Pertanian Bogor, Bogor.
30. Leblanc J.C, Volatier J.L, Aouachria N.B, Oseredczuk M, Sirot V. 2008.*Lipid and fatty acid composition of fish and seafood consumed in France*.Journal of food composition and analysis 21: 8-16.
31. Imran S, Saghk S. 1997. Fatty acid composition and cholesterol content of mussel and shrimp consumed in Turkey.J.Marine sciences 3 (3): 179-189.
32. Surette, M. E. 2008. *The science behind dietary omega-3 fatty acids*. Can. Med. Assoc. J. 178: 177.
33. Hayes D.G. 2002. Urea inclusion compound formation. INFORM, 13: 781-783.
34. Wanasundara, U.N.&Shahidi, F. 1999. *Concentration of omega 3-polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions*. Food Chemistry65: 41-49.
35. Wu M, H Ding, S Wang S Xu. 2008. Optimizing conditions for the purification of linoleic acid from sunflower oil by urea complex fraction. J. Am. Oil Chem. Soc. 85(7):677-684.
36. Guil-Guerrero, JL, E-H. Belarbi. 2001. Purification process for cod liver oil polyunsaturated fatty acid. JAOCS 78: 472-484.
37. Yeo L, KDM Harris. 1999. *Temperature-dependent structural properties of a solid urea inclusion compound containing chiral guest molecules: 2-bromotetradecane/urea*. Can. J. Chem. 77: 2105-2118.

*****

Urea Crystallization on the Concentrate Making of Omega-3 Fatty Acid from Oil of Tuna Fish (Thunnus Sp) Canning Byproduct

ORIGINALITY REPORT

13%	12%	3%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

5%

★ www.semanticscholar.org

Internet Source

Exclude quotes	Off	Exclude matches	< 2%
Exclude bibliography	On		