Recovery of tuna virgin fish oil and formulation as a product model of emulsion food supplement

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Abstract. The aims of this study were to recover and characterize extra virgin fish oil (EVFO) from tuna eyes, and avoid deterioration and loss of EVFO functionality during extraction, formulation and storage of EVFO emulsion supplement. Research comprised extraction, characterization, and formulation of emulsions with addition of mangrove (Rhizophora mucronata) natural antioxidant extracts (0%, 2%, 4% and 8%), ascorbic acid (0.2%) and combination of mangrove extract and ascorbic acid (0.2%:0.2%). The extraction of EVFO used cool centrifugation technology and antioxidant activity of mangroves was measured by the ABTS method. Emulsion stability was verified for 2 weeks (25°C). The best formula was determined by using Bayes method. About 10% EVFO was isolated from tuna eye but its peroxide value was still high. The EVFO had a proportion of DHA up to 34.96%. Mangrove extract had an antioxidant activity of 52.96±0.42 ppm. The best preferred emulsion formula closest to the standard of stability was the formula added with mangrove fruit extract 0.8%. This EVFO emulsions supplement formula with serving sizes of 2 tea spoon (10 mL) donated 0.38 g of DHA which was approximately 32% of the average omega-3 requirement for the first trimester period of pregnancy.

Keywords: antioxidant; DHA; extraction; EVFO; pregnancy.

1. Introduction
Long chain omega-3 fatty acids docosahexaenoic Acid (DHA) supplementation has an important role during pregnancy and lactating period due to the functional properties in supporting the synthesis of structural lipids in normal fetus growth. High intake of long chain omega-3 fatty acids can improve cognitive development, speech and social interaction on infant less than two years [1]. Tuna eye rich in virgin fish oil (VFO) dominated by DHA, however the thermolabile characteristic of this long-chain omega-3 fatty acid needs extra protection to maintain the quality during extraction, formulation and storage as well. Tuna head especially the eye could be a new source of LCN-3FA with DHA ratio reaching 36.72% [2]. The use of chemicals and high temperatures in extraction can reduce the value of LCN-3FA [3]. Cold extraction of LCN-omega-3FA could be done without heat process, with only fermentation, chilling and thawing, or centrifugation and enzymatic treatment [4]. The oil obtained without changing its physico-chemical properties only by mechanical treatment is called pure oil or
6. Extra virgin oil is extracted without altering the physico-chemical properties and does not undergo any form of purification. Susan et al. [6] stated that fish oil in the form of emulsions had higher LC n-3 FA bioavailability than soft capsules form. Emulsion is unstable combination of two phase solutions, therefore oxidation of oil in water emulsion is unavoidable and causes deterioration. Emulsified fish oil is necessary to be stabilized preferably using natural antioxidants and encapsulation. Rhizophora mucronata can be an alternative substitute of synthetic antioxidant to stabilize fish oil emulsion. Its extracts (IC₅₀ 6.69 ppm) were able to stabilize squalene based cosmetic during five weeks storage [7]. Saifullah and Aziz [8] stated that fish oil emulsions generally use orange flavor which is only aimed to increase the emulsion palatability. Nausea and vomiting occurred in more than 80% pregnant women, and affect significantly on their quality of life. Ginger as a herbal medicine has long been used as an antiemetic, motion sickness prevention and has lighter side effect than other antiemetic drugs [9]. Formulation of food supplement EVFO and natural antioxidants using ginger as flavor diversification is expected to improve the life quality of pregnant women and overcome the prolonged effects of stunting indirectly.

2. Material and Methodology

Material

There were two samples of tuna eyes used, first tuna eyes taken on May 22nd, 2018 in Muara Baru, Jakarta stored using Polyethylene (PE) plastic at a temperature of -20°C (A), and second tuna eyes taken on February 23rd, 2018 in Muara Baru, Jakarta stored using PE plastic at a temperature of -20°C (C). Fresh mangrove used from the species Rhizophora mucronata Lamk. was obtained from Mangrove Tourism Area, Pantai Indah Kapuk, Jakarta. Ginger (Zingiber officinale Roscoe) added in emulsion formula was identified by Center of Plant Conservation LIPI-KRB, and whey protein concentrate 34 (L’Nutrition©) also guar gum were added as emulsifier and stabilizer. The apparatus operation were homogenizer (Wiggen Hause), centrifuge (Hitachi Model Part No. R12A6904357D0), Gas Chromatography (Shimadzu GC 2010 plus), Dino eye microscope, and spectrofotometer (UV-Vis 2500 Shimadzu).

2.1. Method

Extraction of mangrove fruit was prepared by commuting (±10 minutes) and boiling in the distilled water (1:5) for 30 minutes and filtering using calico. The filtrate was centrifuged (10 minutes, 11200 xg) and the supernatant was evaporated (80 °C). Characterization of mangrove extract includes analysis of phytochemical compounds and antioxidant activity. Tuna eye extraction was initiated by thawing frozen tuna eyes with running water, followed by separation of hard phases such as lenses and sclera from soft phases (meat) and liquid phase (water soluble substances and fat). The soft phases together with liquid phase were crushed (for ±3 minutes) then centrifuged (11200 xg, 30 minutes, 4 °C). The extra virgin fish oil (EVFO) produced then was placed in dark glass bottles coated with aluminium foil and stored at a temperature -20°C. Characterization was carried out on EVFO in the form of analysis of oil quality and fatty acids profile.

The emulsion was formulated using 5 different antioxidant treatments (Table 1). The ginger water was prepared by dissolving the triturated dried ginger into the water. The initial stage of composing emulsion was by dispersing the emulsion materials, mangrove extract and ginger water using stirrer (10108 xg, 1.5 minutes). The oil phase, which was composed by extra virgin fish oil and virgin coconut oil was added to the stirrer then homogenized (11200xg, 3 minutes). Emulsions were then stored in glass jar coated with aluinium foil at room temperature (25°C) for 15 days. Analysis of Emulsions comprised viscosity, acidity (pH) on day 1st, day 8th and day 15th. Antioxidant effectiveness against inhibition of peroxide determined on day 15th. Formula with the best storage stability was selected using Bayes method by weighting the objective parameters (pH, Viscosity and effectiveness of PV inhibition). Emulsion with the highest acceptance score as the best formula was analyzed for
chemical composition and fatty acid content to determine the nutrient calculation and contribution toward Indonesian recommended daily intake (RDI).

**Table 1.** Emulsion formulation of extra virgin fish oil (EVFO) food supplement with natural antioxidant mangrove extracts.

| Ingredients                      | Control + | Control - | F1 | F2 | F3 |
|----------------------------------|-----------|-----------|----|----|----|
| EVFO (extra virgin fish oil)     | 12.0      | 12.0      | 12.0 | 12.0 | 12.0 |
| VCO (virgin coconut oil)         | 3.0       | 3.0       | 3.0    | 3.0    | 3.0 |
| Whey protein                     | 0.5       | 0.5       | 0.5    | 0.5    | 0.5 |
| Guar gum                         | 1.5       | 1.5       | 1.5    | 1.5    | 1.5 |
| Mangrove extract                 | -         | -         | 0.2    | 0.4    | 0.8 |
| Ascorbic Acid                    | 0.2       | -         | 0.2    | -      | -   |
| Sodium Benzoate                  | 0.1       | 0.1       | 0.1    | 0.1    | 0.1 |
| Ginger water                     | 82.7      | 82.9      | 82.7   | 82.5   | 82.1 |
| Total                            | 100.0     | 100.0     | 100.0  | 100.0  | 100.0 |

* in 100g of the final emulsion weight.

3. Results

3.1. Visual, Morphometric, Phytochemical and Antioxidant Activity of Rhizophora mucronata Lamk. Mangroves

Mangroves used in this study were mature propagules with an average length of 53.73±12.95 cm and an average weight of 47.39±4.15 g. Mangroves were extracted using hot water (100 °C, 30 minutes) with the yield of 9.33 ± 1.76 %. The results of phytochemical analysis showed that *R. mucronata* Lamk extracts contained tannins, saponins, phenols, flavonoids and steroids. Alkaloid compounds in the extracts were not detected. The antioxidant activity of *R. mucronata* Lamk. mangrove extracts was IC$_{50}$ 52.96±0.42 ppm (strong antioxidant).

3.2. Characteristics of Tuna Eyes and Extra Virgin Fish Oil Quality

Two types of tuna eyes (A and C) were analyzed for freshness degree (SNI 2729:2013) in 10 August 2018. The organoleptic value of tuna eye A (80 days frozen storage) was 6.33, while the organoleptic value of tuna C (168 days frozen storage) was 4.23. The tuna eyes used were classified into a medium-sized category with a diameter of 6-9 cm. The meat and liquid phase of tuna eyes proportion reached 92%, while the proportion of sclera and lens parts ranged from 7-8% of the whole eye tuna weights. The chemical composition analysis resulting in a very high proportion of tuna eyes moisture which was around 73.32±1.40%. The proportion of fat content was 18.04±0.58% as the second dominant component of tuna eyes. The protein and ash proportion of tuna eyes were 4.03±0.14% and 1.03±0.07%. Centrifugation of tuna eyes soft phase resulting 4 layers in the tube. The first layer was extra virgin fish oil, the second layer was light lipid, the third was liquid protein hydrolisates and the last was heavy sludge residual of tuna eyes tissue. The oil produced from tuna eyes A had a light transparent yellow color while tuna eyes C produced darker yellow color. The quality characteristics of tuna eyes extra virgin fish oil can be seen in Table 2. The stability characteristics of EVFO emulsion with addition of mangrove extracts comprised acidity changes of food supplement emulsion during storage (Figure 1) and viscosity and Inhibition of peroxide value (PV) of EVFO food supplement emulsions with the addition of antioxidants during storage (Table 3).
Table 2. Quality Characteristics of Tuna Eyes Extra Virgin Fish Oil.

| Parameter                        | A     | C     | Codex (2017) |
|----------------------------------|-------|-------|--------------|
| Free fatty acid (%)              | 1.05 ± 0.05<sup>a</sup> | 1.09 ± 0.11<sup>b</sup> | -            |
| Acid value (mgKOH/g)             | 2.09 ± 0.00<sup>a</sup> | 3.77 ± 0.00<sup>b</sup> | < 3          |
| Peroxide value (meq/kg)          | 62.48 ± 13.98<sup>a</sup> | 225.27 ± 76.03<sup>b</sup> | < 5          |
| Anisidine value (meq/kg)         | 7.31 ± 1.08<sup>a</sup> | 11.66 ± 2.65<sup>b</sup> | < 20         |
| Total Oxidation (meq/kg)         | 132.27 ± 0.00<sup>a</sup> | 462.19 ± 0.00<sup>b</sup> | < 26         |

* the numbers followed by different superscript letters (a, b) show significantly different (p <0.05).

3.3. Stability Characteristics of EVFO Emulsion with Addition of Mangrove Extracts

![Acidity changes of food supplement emulsion during storage (15 days, 25°C)](image)

Figure 1. Acidity changes of food supplement emulsion during storage (15 days, 25°C) (ሽ) positive control, (ශ) negative control, (්) F1, (ෙ) F2, (ේ) F3.

Table 3. Viscosity and Inhibition of peroxide value (PV) of EVFO food supplement emulsions with the addition of antioxidants during storage.

| Treatment                             | Viscosity (cP) | % Inhibition |
|---------------------------------------|----------------|--------------|
|                                       | day-1 | day-8 | day-15 |                  |
| K- (without antioxidant)*             | 25300±0.00 | 1413.5±0.00 | 214.5±0.00 | -                |
| K+ (ascorbic acid 0.2%)               | 12220±0.00 | 633.0±0.00  | 102.5±0.00 | 100.00           |
| F1 (ascorbic acid 0.2% : mangrove extracts 0.2%) | 10360±0.00 | 1800.0±0.00 | 122.5±0.00 | 100.00           |
| F2 (mangrove extracts 0.4%)           | 31520±0.00 | 1616.5±0.00 | 127.0±0.00 | 64.05            |
| F3 (mangrove extracts 0.8%)           | 29040±0.00 | 680.0±0.00  | 188.5±0.00 | 73.41            |

* % inhibition calculation was not performed.

The selected food supplement emulsion formula was determined using performance index (Bayes method), and the emulsion stability parameters covered pH, viscosity and inhibition of peroxide values on different types and concentrations of antioxidants. The performance index test results show that F1 emulsion (combination of ascorbic acid with mangrove fruit extract (1:1)) had the best stability during storage. Formula F3 (0.8% mangrove fruit extract) was then selected as a comparison for sensory and total microbial counts determination. Table 4 shows the microbial total on food supplement emulsion of EVFO during three weeks storage.
Table 4. Microbial total on food supplement emulsion of EVFO during three weeks storage.

| Treatment | Total Plate Count (CFU/mL) |
|-----------|----------------------------|
|           | week-1 | 2 | 3 |
| F1        | 1.2x10⁴ | 1.6x10⁸ | 6.3x10⁸ |
| F3        | 1.2x10⁴ | 1.6x10⁸ | 1.4x10⁸ |

3.4. Chemical Composition of The Best Formula of EVFO Food Supplement (F3) and its Fatty Acid Contribution on Recommended Daily Intake (RDI)

Extra virgin fish oil food supplement emulsion (F3) contained water 80.05±0.04 %, ash 0.29±0.00%, protein 0.42±0.02%, carbohydrate 4.61±0.00%, and fat 14.64±0.03%. One serving size of EVFO food supplement emulsion (2 tsp) contained 1.5 g of fat and contributes 1.8% of trimester 1 pregnant woman’s fat requirements per day. One serving of EVFO food supplement emulsion provided 32.36% omega-3 intake on pregnant woman with the amount of EPA 0.074g and DHA 0.38g.

4. Discussion

Strong antioxidant activities of mangroves extracts can be influenced by its natural ecosystem characteristics. Mangroves live in estuary swamps with high salinity, temperature, radiation and are low in nutrients. Exposure to these stress conditions results in the formulation of reactive oxygen species (ROS) in mangroves, therefore to reduce the adverse effects, mangroves producing enzymes, antioxidants and various defence compounds including polyphenolic [10].

Stored tuna eyes at freezing temperatures will still experience quality deterioration. This is due to the protein denaturation, lipid hydrolysis and autooxidation. Nucleation or forming and growing of ice crystal during frozen storage causes damage to the tuna eye tissue [11]. The color of fish oil comes from its natural pigment or changes during storage and after processing. The color of fish oil can show its quality indicators. The higher FFA value of fish oil resulting the darker it color appeared [12]. The two extracted fish oils have not met the Codex 329-2017 Standard for Fish Oil requirements on peroxide value and total oxidation (Table 2). This was allegedly because the raw material used had been damaged during frozen storage. The highest fatty acid content of tuna eye EVFO was DHA (PUFA) reaching 34.96%, followed by oleic acid (MUFA) of 16.98% and palmitic acid (SFA) 14.50%. The high PUFA especially DHA in tuna eye was predicted because DHA plays a role in the process of light catching by the retina. Reported by Hodge et al., DHA fatty acids are found to be very high in the retina, especially in the outer membrane of photoreceptor cells (orbital region) [13].

There was a tendency on decreasing pH during room storage of all emulsions treatments (Figure 1). The decrease in acidity is thought to be due the decomposition of fatty acids in the emulsion into volatile compounds and organic acids. Pak [14] stated that characteristics organoleptic that can be detected such as acids are formed as a result of peroxide decomposition products such as aldehydes, ketones and acids. The decreased pH in the emulsion can also caused by the growth of microorganisms during storage at room temperature.

The high viscosity during early stage of storage was due to the use of 1.5% guar gum. Guar gum has high viscosity even at low concentrations. The use of guar gum more than 1% increases thixothropic properties, causing a decrease in viscosity over time [15]. Emulsions that are freshly made have small globules that are scattered homogeneously. Thus the dispersed phase increased high viscosity at the beginning of observation. Decreased viscosity in all treatments during storage indicates a changes in the emulsion system. This changes was marked by formation of flocculation on the globule observed on the 8th and 15th days storage. Significant changes in the emulsion globule were observed in the negative control (K-) where the emulsion globule was enlarged due to flocculation and coalescence that occurred.
The use of antioxidant extracts of mangrove fruit (0.4% and 0.8%) was not able to inhibit food supplement emulsion peroxide numbers up to 100% (Table 3). Increasing the concentration of mangrove fruit enlarged the inhibition of peroxide numbers obtained. Inhibition of peroxide numbers up to 100% was obtained using ascorbic acid as antioxidant on formula (F1 and F3). Ascorbic acid is an electron donor that has a low reduction potential (282 mV) so that it is easily related to the propagation chain or decomposition process in oxidation [16].

The F3 emulsion had a sorrel color that was preferred by panelists compared to F1 emulsion that had a light brown color. Emulsions F1 and F3 had the same acceptance value for taste (2.75), although they produced different tastes. Formula F3 had a bitter taste due to the use of mangrove extracts while formula F1 had a distinctive taste of fish oil. Liquid drinks specifically for pregnant and or nursing mothers have a maximum contamination limit (30 °C, 72 hours) of 1x10^5 colonies/ml [17]. Food supplement emulsions of formula F1 and F3 (Table 5) met the criteria for the maximum limit of microbial contamination in the first week of storage based on BPOM 2009 concerning the maximum determination of microbial and chemical contamination in food [17].

Ashes and food supplement emulsion proteins contents were low because guar gum as a stabilizer and thickener in the formulation does not contain high minerals and protein. Mudgil et al. [15] reported that guar gum ash content was 0.5-1% and protein content was 5-6%. Meanwhile, whey protein concentrate used in the formulation as an emulsifier was only 0.5%. The high content of carbohydrates in the emulsion was suspected to be originated from guar gum which has a galactomannan content of 75-85%. Fat content in food supplement emulsions was obtained from the use of EVFO and VCO used.

Saturated fatty acids (SFA) dominated the proportion of emulsion fatty acids (37.78%), followed by unsaturated fatty acid compounds (PUFA) (34.44%) and monounsaturated fatty acid compounds have the lowest proportion (23.29%). Docosahexaenoic acids had the highest proportion (25%) of overall proportion of emulsion fatty acids, because of the EVFO used had a high DHA proportion (34.96%). The use of virgin coconut oil (VCO) in the formulation increased the total SFA in the emulsion. Women in 19-29 age in the first trimester of pregnancy was recommended to consume omega 3 fatty acids of 1.4 g/day. One serving size of EVFO emulsion contributed to meet one-third of the omega 3 requirements of first-trimester pregnant women [17].

5. Conclusions
Extra virgin fish oil produced from cold centrifugation of tuna eye rich in PUFA dominated by DHA (34.96%). Mangrove extract (IC50 52.96 ± 0.42 ppm) was able to stabilize the emulsion of food supplements produced during 15 days storage. The best preferred emulsion formula having a good stability during storage was formula with 0.8% mangrove extract addition (F3). One serving size of EVFO food supplement emulsion was sufficient for one-third of the omega 3 intake on recommended daily average of first-trimester pregnant women.

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