Extraction and microencapsulation of tuna virgin fish oil with mangrove fruit extract fortified into extrusion cereals

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Abstract. Long-chain omega-3 fatty acids (LCn-3FA) originally from fish have been recognized plays an important role in improving health status. Fortification these LCn-3FAs into foodstuffs will enhance their nutritional and functional value, however, their vulnerability, particularly to oxidation, requires extra protection. The goals of the research were to produce tuna eyes extra virgin fish oil (EVFO) microcapsules rich in DHA protected by mangrove fruit extract as a natural antioxidant and to obtain an extruded cereal with EVFO microcapsules. EVFO was extracted using cool centrifugation, microencapsulated by maltodextrin and arabic gum, and the cereal was formulated as extrusion flake products. The proportion of EVFO extracted from tuna eyes was 10.3596±0.73%. Mangrove fruit extract had a strong antioxidant activity with IC50 of 53.28 ppm. The best microcapsules were gained from arabic gum-maltodextrin coating material and the addition of 4000 ppm of mangrove fruit extract, which had an efficiency value of 93.71%, and round shape with size 6.26 µm. Cereal with 35 g serving sizes and the addition of 3.6% EVFO microcapsules contributed approximately 134 kcal, 100 mg DHA, and 125 mg omega-3 increasing the great nutritional value.

1. Introduction
Omega-3 fatty acids play an important role in fetal brain development, infant motor skills and visual acuity, children lipid metabolism and cognitive development. The source of fish oil that rich in long-chain omega-3 fatty acids which are currently being used extensively are the by-products of tuna industry [1]. The biggest part of tuna by-products was head (19.7%) [2], and tuna eyes contained high levels of eicosapentaenoic acids (EPA) and docosahexanoic acids (DHA) at 7% and 35% [3], however, their vulnerability need additional protection to inhibit oxidation.

Fish oil extraction is generally carried out with wet rendering method [4], including cooking, pressing, and centrifugation, followed by adding synthetic antioxidant such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) reported could be carcinogen. Cold extraction is an important method expected to maintain quality, reduce production costs and reduce devouring chemicals (environmentally friendly) [5]. Fish oil protection can be prepared by adding Rhizophora mucronata mangroves extract. The R. mucronata extracts showed very strong antioxidant activity with IC50 6.69-58.61 mg/mL [6-8]. Microencapsulation can also be prepared to protect omega-3 from oxidation. Microencapsulation technology has been developed as a technique for coating fish oil with materials that can form a shield from exposure to oxygen. Microencapsulation techniques convert liquid fish oil into a powder so that it is easily fortified to food products [9].
The Food and Agriculture Organization (FAO 2010) recommends consumption omega-3 as much as 250 mg per day and this omega-3 requirement can be fulfilled through a healthy (fortified with omega-3) breakfast cereals. Westernization of food habits in the middle-class population, tourist population, and high public health awareness contributed significantly to the increasing of breakfast cereal needs, especially in the Asia Pacific. Research of virgin fish oil microcapsules rich in omega-3 with antioxidant protection of mangrove fruits for breakfast cereal fortification has not been conducted, the result obtained from this study should provide valuable information for recovering EVFO, selecting and extracting mangrove fruits as an antioxidant source, and formulating the EVFO fortified breakfast cereal as a functional food.

2. Objectives
The goals of these research were to recover and characterize tuna eyes EVFO and mangrove’s fruits natural antioxidant, to characterize the EVFO microcapsules protected by natural antioxidants from mangroves fruit crude extracts, and to examine the quality changes of breakfast cereal products fortified by EVFO microcapsules.

3. Materials
The materials used were tuna eyes (Thunnus sp.) transported with cold chain system (0-4°C) to laboratory, including tuna eyes (A) taken in May 2018 from Muara Baru packed with polyethylene plastic packaging, tuna eyes (B) received in February 2018 from Bitung wrapped with polyethylene plastic vacuum packaging (Frozen), and tuna eyes (C) gotten in February 2018 from Muara Baru with polyethylene plastic packaging. The three types of eyes were stored at -20 °C. Other materials included mangrove fruit R. micronata, based on the description of FAO (2000) classified to adult mangroves (size 39-70 cm) taken from Jakarta mangrove conservation center, arabic gum (80 mesh), maltodextrin (90 mesh, DE 10-15), sodium caseinate, sorghum flour (Numbu ©), bran flour (Gasol ©), tapioca flour (Gunung agung ©), soto flavorings (turmeric, candlenut, ginger, onion, garlic, pepper, salt), and standard FAME (Supelco 37 component fatty acid methyl ester mix ). The tools used included the Philips HR 2115 blender, centrifuge Hitachi Model No. No. R12A6904357D0, Rayleigh Vis-723G spectrophotometer, drum dryer (Bufovak double drum), homogenizer Wiggen Hause, spray dryer (Büchi 190 nozzle 0.7 mm), Scanning Electron Microscope (Phenom ProX), and Gas Chromatography Shimadzu GC 2010 Plus.

4. Method
The research procedures comprised preparation, extraction, and characterization of virgin tuna eye oil and mangrove fruits antioxidant, microencapsulation and characterization of EVFO microcapsules, formulation and fortification EVFO into breakfast cereal. Preparation of tuna eye was done by separated the eye meat from hard parts such as lenses and sclera. The meat and liquid of tuna eye were mixed and crushed using a homogenizer. The paste then extracted by centrifugation (11200 xg, 30 minutes, 4°C) to separate the oil from other eye components, such as eye meat, blood, and water. Characterization of EVFO included weighing proportions of each tuna eye, analyzing EVFO quality and fatty acid composition. Extracted mangrove fruit was completed by boiling mangroves for 30 minutes with a ratio of mangroves and water was 1:5 (w:v). Description of mangrove fruits antioxidant covering phytochemical and antioxidant activity analysis using 2,2-Azinobis (3-ethylbenzotiazolin)-6-Sulfonic acids (ABTS) method. Microcapsule formulation consisted of arabic gum-maltodextrin coating with 2000 ppm mangrove antioxidant (A01), arabic gum-maltodextrin with 4000 ppm mangrove antioxidant (A02), sodium caseinate with 2000 ppm mangrove antioxidant (B01), and sodium caseinate with 4000 ppm mangrove antioxidant (B02). Microcapsules A01 and A02 were made by homogenization (18928 xg, 10 minutes) and dried with T_{inlet} = 160°C and T_{outlet} 95°C and airflow rate 73 m3/hour and feed rate 5.3g/minute. B001 and B002 microcapsules created by homogenization (448 xg, 1 hour) were dried with T_{inlet} = 120°C and T_{outlet} = 80°C and airflow rates 73 m3/hour and feed rate 5.3g/minute. Characterization of EVFO microcapsules comprised
Microencapsulation efficiency, peroxide numbers, microcapsule microstructure, and composition of fatty acids. The formulation of extra virgin fish oil microcapsules protected by mangrove fruit extracts are summarized in Table 1.

Table 1. Formulation of extra virgin fish oil microcapsules protected by mangrove fruit extracts.

| Ingredients                        | Formulation (%) |
|------------------------------------|-----------------|
|                                   | A01  | A02  | B01  | B02  | Control (+) | Control (-) |
| Water                              | 70   | 70   | 70   | 70   | 70           | 70           |
| Extra Virgin Fish Oil (EVFO)       | 10   | 10   | 10   | 10   | 10           | 10           |
| Arabic gum                         | 12   | 12   | -    | -    | 12           | 12           |
| Maltodextrin                       | 8    | 8    | -    | -    | 8            | 8            |
| Sodium caseinate                   | -    | -    | 20   | 20   | -            | -            |
| Mangrove fruit extract             | 0.4  | 0.8  | 0.4  | 0.8  | -            | -            |
| Ascorbic acid                      | -    | -    | -    | -    | 0.4          | -            |

Breakfast cereal production was conducted using a drum dryer with ingredients consisting of bran flour: sorghum flour: tapioca = 2:7:1. The process of making breakfast cereal through the boiling stage until thickening, steaming (70-80°C, 15 minutes), and drying. Breakfast cereal was served by adding soto flavorings. Fortification was conducted by adding microcapsules 6% (b/b) each (selected microcapsules). The characterization of EVFO breakfast cereal covered hedonic testing and comparison of partners (30 panelists), physical characteristics, chemical characteristics, and percentage contributions to the recommended daily intake (RDI).

5. Results
The results of this research consist of organoleptic freshness and chemical composition of tuna eye, visualization of separation form and proportion of tuna eye fish oil, characteristics of extra virgin fish oil (EVFO), Visual, morphometric, phytochemie, and antioxidant activity of mangrove extract, characteristics of EVFO microcapsule, characteristics sensory of fortified EVFO cereals, nutrition and energy donations of cereal products toward the recommended daily intake (RDI).

5.1. Organoleptic freshness and chemical composition of tuna eye
Tuna eyes used to consist of 3 types, namely eyes (A), eyes (B), and eyes (C). All three types of tuna eyes were organoleptic tested by 30 panelists based on SNI 2729:2013 about fresh fish to know the freshness level of all three types of tuna eyes. Organoleptic results of tuna eyes are shown in Figure 1.

![Figure 1](image-url)  
**Figure 1.** Organoleptic results of 3 types of tuna eyes ( Eyes A, Eyes B, Eyes C refer to SNI 2729:2013 about fresh fish.
The eyes A used had an average weight of 162.86±40.07 g with an average diameter of 8.07±0.34 cm. The proportion of each part of the eye was 92% of the meat and liquid, while the lens and sclera were 7-8%. The extracted eye belonged to the medium curved eye. Tuna eyes A contained a water 73.32±1.40 %, ash 1.03±0.07%, protein 4.03±0.14%, carbohydrate 3.58%, and fat 18.03±0.58%.

5.2. Visualization of separation form and proportion of tuna eye fish oil
Cold centrifugation formed 4 layers where the oil was on the top layer. The proportion of tuna eye fish oil was 10.3596±0.73%. Visualization of separation form and tuna fish oil are exhibited in Figure 2.

![Figure 2](image)

**Figure 2.** Visualization of extraction and centrifugation forms (a) separation of cold centrifuged layers (b) tuna eye fish oil.

5.3. Characteristics of extra vurgin fish oil (EVFO)
The quality characteristics of tuna eye fish oil include free fatty acids (%FFA), acidity value, peroxide value, p-anisidin value, and total oxidation. The value of %FFA, acidity value, and p–anisidin value meets the provisions of CODEX 329-2017 about standard for fish oil. The quality characteristics of EVFO are summarized in Table 2.

| Parameter                  | A                | B                | C                | Codex (2017) |
|---------------------------|------------------|------------------|------------------|--------------|
| Free fatty acid (%)       | 1.26±0.06        | 1.34±0.10        | 2.30±0.13        | -            |
| Acid value (mg KOH/g)     | 2.16±0.11        | 2.28±0.16        | 3.92±0.22        | < 3          |
| Peroxide value (meq/kg)   | 94.11±0.16       | 45.16±2.90       | 225.27±9.69      | < 5          |
| p-Anisidin value (meq/kg) | 7.31±1.08        | 1.73±1.07        | 11.66±2.65       | < 20         |
| TOTOX (meq/kg)            | 195.53±1.40      | 92.05±4.73       | 462.19±16.72     | < 26         |

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

5.4. Visual, morphometric, phytochemie, and antioxidant activity of mangrove extract
Mangroves in this study had straight hypocotyls, brownish-green, and grainy with a weight of 56.07±2.51 and length 49.39±2.02. The yield of mangroves obtained was 9.33±1.76. The IC value of 50 mangrove *R. mucronata* fruits was 52.9645 ± 0.42 ppm and belongs to a powerful antioxidant activity. The high antioxidant activity in the water extracted mangrove fruit correlates with the results of phytochemical testing. Phytochemical tests on mangrove fruits showed that mangrove extracts contained phenols, triterpenoids, tannins, flavonoids, and saponins, and negative alkaloids both by testing for Dragendorf, Mayer, and Wagner.
5.5. Characteristics of EVFO microcapsules

Characteristics of EVFO microcapsules comprise the efficiency value, peroxide value, microstructure, and performance of fatty acids. The A01 and A02 formulas used a coating of arabic gum and maltodextrin, while B01 and B02 used a sodium caseinate coating. The Efficiency value of tuna eye fish oil microcapsules is recapitulated in Table 3.

Table 3. The Efficiency values of tuna eye fish oil microcapsules with different coating materials and antioxidant concentrations.

| Formula | Microcapsules efficiency value |
|---------|--------------------------------|
| A01     | 91.63±0.78^c                  |
| A02     | 93.71±0.81^d                  |
| B01     | 70.78±0.31^a                  |
| B02     | 73.20±0.42^b                  |

*the numbers followed by different superscript (a, b, c, d) show significantly different (P <0.05).

Peroxide value is the number of peroxide in the active oxygen milliequivalent contained in 100 g of compounds. Detection of peroxide gives the initial evidence of rancidity. Analysis of peroxide value conducted for the tuna eye fish oil microcapsules added with natural antioxidants, complemented with control antioxidants (ascorbic acid), and without addition of antioxidants. The peroxide value of tuna eye fish oil and the microcapsules are condensed in Table 4. It gives a measure of the extent to which an EVFO and the microcapsules samples have undergone primary oxidation, nevertheless, the addition of mangrove fruits extracts was effective enough to inhibit the secondary oxidation process.

Table 4. The Peroxide value of tuna eye fish oil and tuna eye fish oil microcapsules.

| Formula                  | Peroxide Value (meq/Kg) |
|--------------------------|--------------------------|
| Tuna eye fish oil        | 94.11±0.16^a             |
| A01                      | 107.81±1.55^c            |
| A02                      | 97.68±0.39^b             |
| Control (+) Ascorbic acid| 96.30±0.72^b             |
| Control (-)              | 125.93±0.61^d            |

*the numbers with superscript letters that are different (a, b, c, d) show significantly different (P <0.05).

Microcapsules microstructure analysis was performed using a Scanning Electron Microscope (SEM). The observation results indicate that the size of microcapsules was 6.26 µm. The Microstructure of tuna eye fish oil microcapsules can be seen in Figure 3.

Figure 3. Microstructures of tuna eye fish oil microcapsules. *Scanning Electron Microscope* (a) 10000x magnification, (b) 5000x magnification.
Performance analysis of microcapsules and tuna EVFO fatty acids were performed using Gas Chromatography (GC). The EVFO fatty acids content was dominated by Polyunsaturated Fatty Acids (PUFA). Performance of microcapsules and tuna eye fish oil fatty acids are presented in Table 5.

| Fatty Acids                  | EVFO Fatty Acids (%b/b) | EVFO Microcapsules Fatty Acids (%b/b) | Decrease (%) |
|------------------------------|-------------------------|---------------------------------------|--------------|
| Lauric Acid, C12: 0          | 0.09                    | 0.03                                  | 66.67        |
| Myristic Acid, C14: 0        | 2.08                    | 1.98                                  | 4.81         |
| Pentadecanoic Acid, C15: 0   | 0.63                    | 0.61                                  | 3.17         |
| Palmitic Acid, C16: 0        | 14.50                   | 14.03                                 | 3.24         |
| Heptadecanoic acid, C17: 0   | 0.52                    | 0.55                                  | -7.77        |
| Stearic Acid, C18: 0         | 1.80                    | 1.89                                  | -5.00        |
| Arachidic Acid, C20: 0       | 0.20                    | 0.19                                  | 5.00         |
| Behenic Acid, C22: 0         | 0.11                    | 0.11                                  | 0.00         |
| Trichosanoic Acid, C23: 0    | 0.04                    | 0.05                                  | -25.00       |
| Lignoseric Acid, C24: 0      | 0.11                    | 0.11                                  | 0.00         |
| Total SFA (Saturated Fatty Acid) | 20.08                  | 19.04                                 | 5.18         |
| Miristoleic Acid, C14: 1     | 0.10                    | 0.10                                  | 0.00         |
| Palmitoleic Acid, C16: 1     | 6.07                    | 6.32                                  | -4.12        |
| Cis-10-Heptadecanoic Acid, C17: 1 | 0.07               | 0.99                                  | -1314.29     |
| Elaidic Acid, C18: 1n-9t     | 0.15                    | 0.18                                  | -20.00       |
| Oleic Acid, C18: 1n-9c       | 16.98                   | 18.28                                 | -7.66        |
| cis-11-Eikosenoic Acid, C20: 1 | 1.59               | 0.16                                  | 89.94        |
| Nervonic Acid, C24: 1        | 0.60                    | 0.62                                  | -3.33        |
| Total MUFA (Monounsaturated Fatty Acid) | 25.56              | 26.65                                 | -4.26        |
| Linoleic Acid, C18: 2n-6c    | 0.87                    | 0.85                                  | 2.30         |
| Linolenic Acid, C18: 3n-3    | 0.03                    | 1.88                                  | -6166.67     |
| γ-Linolenic Acid, C18: 3n-6  | 0.04                    | 0.03                                  | 25.00        |
| cis-11,14-Eikosadinoic Acid, C20: 2 | 0.37            | 0.34                                  | 8.11         |
| cis-8,11,14-Eikosantrinoic Acid, C20: 3n-6 | 0.10 | 0.11 | -10.00 |
| Arachidonic Acid, C20: 4n-6  | 3.03                    | 0.19                                  | 93.73        |
| cis-13,16-Docosadinoic Acid, C22: 2 | 0.06            | 0.04                                  | 33.33        |
| Eicosapentanoic Acid (EPA), C20: 5n-3 | 6.19         | 4.58                                  | 26.01        |
| Docosahexaenoic Acid (DHA), C22: 6n-3 | 34.96      | 25.30                                 | 27.63        |
| Total PUFA (Polyunsaturated Fatty Acid) | 45.65             | 33.32                                 | 27.01        |

5.6. Characteristics sensory of fortified EVFO cereals
Sensory analysis was completed for the cereal fortified by tuna eye fish oil microcapsules and control products. The tested characteristics include appearance, color, aroma, taste, texture, and aftertaste. The Cereal sensory results are displayed in Figure 4.
5.7. Physical and chemical characteristics of fortified cereals
The physical characteristics of fortified cereals include water density, water absorption, and rehydration power. The fortified cereal has a density value 0.49±0.02 g/mL, water absorption 3.05±0.03 mL/g and rehydration power 123±1.41 seconds. The cereal products fortified EVFO microcapsules contained 4.36±0.01% water, ash 4.94±0.02%, protein 8.77±0.01%, fat 3.67±0.01%, and carbohydrate (by difference) 78.26±0.02. The product also has a water activity value of 0.46±0.00.

5.8. Nutrition and energy donations for cereal products the recommended daily intake (RDI)
The cereal fortified product donated energy amounting to 134 kcal in a 35 g per serving size. Cereal with fortification 3.6% tuna eye fish oil microcapsules contained 125 mg omega-3, 100 mg DHA, and 1 mg AA. The nutrition value of cereal fortified extra virgin fish oil is performed in Table 6.

Table 6. The nutritional value of cereals fortified extra virgin fish oil microcapsules.

| Parameter          | Amount of energy (kcal) | %AKG<sup>2</sup> |
|--------------------|-------------------------|------------------|
| Total energy       | 134                     |                  |
| Total energy from  | 12                      |                  |
| fat                |                         |                  |

| Parameter          | Amount of nutrient content per serving dose | %AKG<sup>2</sup> |
|--------------------|--------------------------------------------|------------------|
| Carbohydrate       | 28 g                                       | 11 %             |
| Protein            | 4 g                                        | 7 %              |
| Fat                | 2 g                                        | 2 %              |
| Per Dish Contains: |                                            |                  |
| DHA                | 100 mg                                     |                  |
| AA                 | 1 mg                                       |                  |
| Omega-3            | 125 mg                                     |                  |

AKG percent based on the population energy needs Indonesia ages 7-9 years per day 18 50 kcal.
6. Discussions
Fat content of tuna eyes was 18.03±0.58%. Fat content of tuna eye affected by tuna eye size, where tuna eye at levels small eyes (d = 3-6 cm, weight 100 g) was 12.67±0.95%, medium-sized eyes (d = 6-9 cm, weight 180 g) was 15.41±0.23%, and large eyes (d>9 cm, weight 380 g) was 21.86±1.16% [10]. Fat content was also affected by season, environmental conditions, stage of gonadal maturity, nutrient state, and age of fish [11].

Free fatty acids are formed due to the hydrolysis reaction of triglycerides during storage and extraction processes. Hydrolysis will occur if oil comes into contact with water and heat [12]. The value of free fatty acid is directly proportional to the acidity values. Acidity value indicate the quality of fish during the process or storage of oil [13]. The three tuna eye fish oils have a low value of free fatty acids indicating that the oil is in good quality.

Tuna fish oil had peroxide value and total oxidation that have not met the CODEX standard (2017). The high peroxide and total oxidation value in fish oil are caused by the high content of long-chain unsaturated fatty acids, especially EPA and DHA which are very susceptible to oxidation [14]. Increases in peroxide value also occur during storage so that the longer the storage time the higher the peroxide numbers [15]. Oil B had the lowest peroxide value because the raw material was stored at -20°C in vacuum packaging. Vacuum packaging prevents the formation of ice crystals which can damage tissue and limit contact with oxygen so that oxidative reactions can be inhibited [16].

The IC₅₀ value of mangrove fruits using the ABTS method was 52.96±0.42 ppm and belongs to a powerful antioxidant activity. Previous research mentions that IC₅₀ value of mangrove fruits R. mucronata extract was 25.07 ppm [7]. The high antioxidant activity in the water extracted mangrove fruit correlates with the results of phytochemical test. Phenol hydroquinone compounds and flavonoids were positively identified, and they have long been recognized to be very potential as an antioxidant that able to inhibit oxidation in some sensitive oxidative material so that their shelf life can be extent longer [17].

Microcapsules A02 with arabic gum-maltodextrin coating material and an addition of 4000 ppm mangrove fruit antioxidants have the highest efficiency of microcapsules. Maltodextrin plays a role in the formation of viscoelastic films in microcapsules, therefore, it can improve the efficiency of microcapsules [18], whereas Arabic gum has good properties as encapsulation agents, namely high solubility, low viscosity, and good emulsification characteristics [19]. The combination of both was able to form microcapsules with a higher efficiency value than sodium caseinate coating material. Sodium caseinate was not able to protect fish oil efficiently because it was unable to form dense skin in the early stages of drying. The spray drying process can also cause protein denaturation and b-lactoglobulin aggregation [20].

Peroxide in encapsulated EVFO has increased. The increasing of peroxide number in tuna eye fish oil microcapsules is due to oil and air contact in the homogenization process [21]. The use of high temperatures during the drying process can also increase peroxide numbers [22]. Microcapsules with the addition of 4000 ppm fruit antioxidants have peroxide values that are not significantly different from the control (+) ascorbic acid. Antioxidant activity in mangroves is caused by the presence of polyphenols. Polyphenols function as antioxidants with a radical binding mechanism [23].

The result of the fatty acids showed that the content of omega-3 in microcapsules of tuna fish oil decreased by 22.87%. The contents of EPA and DHA respectively also decreased by 26.01% and 27.63%. A decrease in the percentage of fatty acids occurs during the microencapsulation process. Most tuna EVFO consists of n-3 components. Omega-3 is a compound unsaturated fatty acid that has many double bonds so it is very susceptible to heat [24]. The homogenization and spray drying process in the microencapsulation development used high temperature, consequently, some breaking of double bonds on long-chain fatty acids occurs and the microcapsules being oxidized [25].

Bulk density is also influenced by the drying method. Drying with a drum dryer using high temperatures causes termination of the branch chain of starch so that the amylose content increases and the solubility value gets higher [26]. Water absorption is influenced by particle size. The smaller particle sizes have higher water absorption because of the larger surface area and so they have more
space to absorb water [27]. Rehydration time is related to the gelatinization process of starch contained in the flour. Pragelatinization is a partial gelatinization process which causes the formation of porous structures in starch granules during drying. This structure makes it easier for water to concentrate on the granule when it is rehydrated. Perfectly gelatinized starch will be difficult to be rehydrated because starch granules have been damaged or broken, while starch which does not undergo gelatinization causes water to be difficult to penetrate when it is rehydrated [28].

The recommended Daily Intake (RDI) is an average of daily nutrition for all people based on age group, gender, body size, and body activities to achieve optimal health status. The average energy sufficiency for the Indonesian population aged 7-9 years based on PERMENKES (2013) is 1850 kilocalories (kcal). Fortified healthy cereal products contribute 134 kcal of energy with serving sizes 35 g. Cereals with fortification 3.6% microcapsules of tuna fish oil contain 125 mg of omega-3 fatty acids, 100 mg DHA, and 1 mg AA. This value already fulfills half of the omega-3 requirement per day. FAO (2010) recommends intake of omega-3 (EPA and DHA) per day of 150-200 mg for children aged 4-6 years and 200-250 mg for children aged 6-10 years.

7. Conclusion
Tuna eye Extra Virgin Fish Oil extracted using cool centrifugation rich in Poly Unsaturated Fatty Acids dominated by DHA. Mangroves fruits extract have strong antioxidant activity which is very potential as EVFO protective agent. Microcapsules with maltodextrin and Arabic gum coatings have higher microcapsule efficiency values than the sodium caseinate coatings. Cereals fortified by EVFO have a preferred appearance, color and texture than a commercial one hence can be a healthy breakfast alternative.

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