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The characteristics of PUFAs-rich virgin fish oil as affected by size of tuna eye

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Abstract. *Trilaksani W, Riyanto B, Ramadhan W, Sinulingga F, Fauziah S. 2023. The characteristics of PUFAs-rich virgin fish oil as affected by size of tuna eye. Biodiversitas 24: 6545-6556.* The food supplement market expects growth in demand for long-chain omega-3 fatty acids, particularly Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA), which play crucial for brain development and can lower the chances of health issues like depression, Myocardial Infarction (MI), thromboembolism, and cardiac arrhythmias, and have anti-viral effects, boosting the immune system during the pandemic. Therefore, to meet this demand, exploring new sources is crucial. Tuna eyes have emerged as a potential source of DHA; however, quality and standardized sizes pose a challenge as they are by-products. The purpose of this research was to determine how the eye size of tuna affected the quality and yield of fish oil, which is a novel source of omega-3 fatty acids. This study determined the virgin oil profile of tuna eyes, which met the quality standards. The investigation covered morphology, chemical composition, heavy metal content, oil yield and quality, fatty acid composition, and related health lipid indices (Atherogenicity Index (AI) and Thrombogenicity Index (TI)). For the size specifications, tuna eyes were classified into three groups based on their diameter, namely 'small' (<6 cm), 'medium' (6-9 cm), and 'large' (>9 cm). The results showed that eye size directly influenced the weight, fat content, and oil yield, with negligible disparity in chemical composition except for the fat content; the larger the eyes, the higher the oil yield. Tuna eye oil had a safe composition for consumption with low or undetected heavy metals, the oil's oxidative level met CODEX standards, and the fatty acid profile revealed DHA as the most abundant fatty acid, reaching 36.95%. The predominance of n-3 series values in the fatty acid composition of tuna's eye was 9-12 times greater than that of n-6 fatty acid values. The values of AI and TI were 0.33 and 0.12; 0.47 and 0.20; 0.55 and 0.20 for large, small, and medium eyes, respectively. The study highlights tuna eyes' unique characteristics and potential applications in the food supplement industry.

Keywords: Docosahexaenoic acid, fatty acid composition, food supplement, oxidative level, yield

INTRODUCTION

Fish oil serves as the primary source of Polyunsaturated Fatty Acids (PUFAs), mainly composed of four to six double bonds fatty acids, such as eicosapentaenoic acid (EPA C20:5 n3) and docosahexaenoic acid (DHA C22:6 n3). Polyunsaturated fatty acids are essential for cognitive function as they are involved in the growth and development of brain nerves (Kuratko et al. 2013), reducing the risk of various degenerative diseases such as cardiovascular conditions and arteriosclerosis (Echeverría et al. 2017; Yamagata 2017). Adults should consume 250 mg of EPA and DHA on a daily basis to protect against Coronary Heart Disease (CHD). To achieve optimal brain development in children, the recommended daily intake is 150 mg (FAO 2013). The European Food Safety Authority (EFSA) (2011) recommends daily consumption of DHA supplementation of 100-200 mg per day for pregnant and nursing women. Formerly, recommendations for the consumption of n-3 Polyunsaturated Fatty Acids (PUFAs) in one's diet were primarily distressed by mitigating deficiencies. Nevertheless, current research mostly focuses on identifying the optimal level of consumption in order to reduce the likelihood of developing chronic diseases. There is no consensus among scientists regarding the optimal intake of n-3 PUFAs in their entirety. Various reputable organizations, such as the EFSA, the UK Committee on Medical Aspects of Food Policy, the World Health Organisation, and the American Heart Association, have provided distinct dietary guidelines on the consumption of n-3 PUFAs.

A new study on the health advantages of omega-3 fatty acids has shown that consuming EPA and DHA is essential for the growth and maturation of brain neurons and the retina. Following this dietary diet additionally decreases the probability of suffering from diseases of aging, such as cardiovascular problems, arteriosclerosis, obesity, cancer, inflammation, Alzheimer's disease, and mental disorders. (Mason et al. 2020; Stando et al. 2020; Balakrishnan et al. 2021; Wei et al. 2021; Khalid et al. 2022; Sittiprapaporn et al. 2022; Zhang et al. 2023). In addition, several studies conducted during the COVID-19 pandemic have demonstrated that regular intake of omega-3 can enhance the immune system (Hathaway et al. 2020; Alagawany et al. 2021; Shakoor et al. 2021; Fadiyah et al. 2022; Motti et al. 2022). Data on the DHA's role in preventing mental diseases is becoming increasingly clear. This is especially crucial in light of the fact that brain diseases are escalating dramatically across the globe; the cost of mental disorders

now exceeds the combined cost of cardiovascular disease and cancer. The intake of DHA through supplements and food items becomes essential since it requires a precursor fatty acid, especially Alpha-Linolenic Acid (ALA), with a conversion rate of less than 5% (Khoshnoudi-Nia et al. 2022). In addition, frequent occurrence of fish overprocessing results in PUFAs damage in fish meat tissues, so direct consumption of fish meat becomes less significant in terms of PUFA intake. Another consideration is that the FDA recommends the consumption of large-sized fish only two times a week in limited portions due to the bioaccumulative of heavy metals in sizeable fish such as tuna, marlin, etc. Furthermore, the increase in chronic diseases and health issues during the COVID-19 pandemic has heightened consumer awareness of the benefits of omega-3 fish oil (EPA/DHA) in various forms, including functional foods and health supplements (Luthfiah et al. 2014; Calder et al. 2020). Consequently, these factors have increased demand for omega-3 fatty acids.

The global demand for Polyunsaturated Fatty Acids (PUFAs) is expected to experience substantial growth, with a projected annual growth rate of 8.6% from 2021 to 2028 (GVR 2021). In 2019, imported fish oil reached $19.9 million; domestically only produced $370,000 (OEC 2019). Generally, fish oil can be obtained from the body and liver of fish through recovery during canning processing (Mkadem and Kaanane 2019). However, this conventional approach presents sustainability and quality concerns, mainly regarding the decline of oil quality during processing. Green technology has revealed that the tuna eye can be the new source of PUFAs-rich oil extracted in low temperatures using cool centrifugation (Jeong et al. 2016; Trilaksani et al. 2020a; La Dia et al. 2022). According to the standard for Fish Oils CODEX Stan 329-2017, The term 'rich' is used to define concentrated fish oil that includes 35 to 50 w/w % fatty acids, namely the sum of C20:5 (n-3) Eicosapentaenoic Acid (EPA) and C22:6 (n-3) Docosahexaenoic Acid (DHA). Moreover, as mentioned earlier, the term 'virgin' in relation to oil refers to oil that is extracted without any changes to its natural state, utilizing mechanical methods such as pressing or expelling and applying heat only. Purification can be achieved through a series of steps, including water washing, settling, filtering, and centrifugation (CODEX-STAN 210-1999). Thus, we scientifically followed the required procedure to extract the oil from fish and termed it 'Virgin Fish Oil,' as reported in our previous report (Trilaksani et al. 2020a, 2020b).

Tuna processing industries commonly generate by-products of 40% of the fish's body weight, comprising skin, skeleton, tail, viscera, and head. From tuna weighing over 50 kg, the eyeballs average 200 g each, constituting 0.8% of the body weight and 2% of the by-product weight. The percentage of PUFAs in tuna eyes reached 48.75% of total fat, while EPA and DHA were 7% and 35%, respectively (Gamarro et al. 2013; Renuka et al. 2016). This value is much higher than oil produced from other fishes, such as sardines which contain 6.9±1.5% DHA (Mohanty et al. 2016), Catfish 4.78% (Mustapha et al. 2014), salmon 17.1±0.47% (Soltan and Gibson 2008), herring 11.50% (Aitta et al. 2021), and tuna body oil 24.56% (Suseno et al. 2014).

The size of a fish affects various attributes of its metabolism and biochemical composition. For instance, levels of amino acids alanine, arginine, and aspartate significantly increased with the size of fish (Gam et al. 2005). Pratama et al. (2020) showed that larger fish have higher albumin levels. Asikin and Kusumaningrum (2018) reported that the extractable fat content of fish is influenced by its weight; the larger the fish, the higher the fat content. These findings indicate that the size of fish influences its biochemical properties. The relationship between the age and size of tuna is still a matter of discussion. Many studies present only preliminary results, indicating that the estimated growth rate of these species is uncertain and is not definitively linked to the aging of tuna. Ku et al. (2021) investigated the age and growth patterns of the Southern Bluefin Tuna (*Thunnus maccoyii*) by analyzing the microstructure of its otoliths. Similarly, the FAO released a preliminary study on determining the age of Bluefin Tuna (*Thunnus thynnus*), while another study has provided a more recent estimation of the growth pattern of Western Atlantic Bluefin Tuna (Restrepo et al. 2010). In addition, Lu et al. (2023) reported the influence of age-related uncertainty on the computation of growth functions for notable tuna species. The work shows a study that used models to investigate the influence of aging errors and sampled age ranges on the estimation of Von Bertalanffy growth curves for five important tuna species: *Thunnus maccoyii* (southern bluefin tuna), *Thunnus alalunga* (albacore tuna), *Thunnus albacares* (yellowfin tuna), *Thunnus obesus* (bigeye tuna), and *Katsuwonus pelamis* (skipjack tuna). The extent to which the size of the tuna's eye influences the quantity and quality of fish oil produced remains uncertain in this instance. Moreover, our initial study is required to consider fundamental studies relating to the characteristics and chemical composition of tuna and could potentially serve as an initial hypothesis. Therefore, the aim of this research was to investigate the impact of tuna eye size on the quality and productivity of fish oil enriched with Polyunsaturated Fatty Acids (PUFA) as a novel resource.

MATERIALS AND METHODS

Materials

The utilized material was tuna eyeball (*Thunnus albacares*), a by-product of the tuna industry in Bitung, North Sulawesi, Indonesia. The chemical reagents used were chloroform, phenolphthalein indicator, p-anisidine reagent, glacial acetic acid, KOH 0.1 N, saturated KI solution, and Whatman paper. The chemical reagent was an analytical grade acquired from Merck, Darmstadt, Germany. Equipment used included digital camera (Nikon Coolpix l820, Sendai, Japan), high-speed J2-21 cold centrifuge (Beckman Coulter J2-21, Beckman, United State), microwave oven (Sharp R-230R, Kameyama, Japan), water bath shakers (Julabo SW22, Baden-Württemberg, Germany), vortex thermocline (Ependorft, Hamburg, Germany), gas chromatography (Shimadzu GC 2010 Plus, Kyoto, Japan), thermometer (Tecpal Co.Ltd,

Taipei, Taiwan), mixer (Thermoscientific, Waltham-Massachusetts, USA), blender (Philips HR 211, Amsterdam, Netherland), centrifuge (Hitachi Model Part No. R12A6904357D0, Chiba, Japan), spectrophotometer (UV-Vis 2500 Shimadzu, Tokyo, Japan), digital water bath (18-ONE, New Jersey, USA), atomic absorption spectrophotometer (Shimadzu AA-7000, Kyoto, Japan), oven (Blue-M Thermal Product Solution, New Columbia, USA), furnace (Yamato Scientific Co.Ltd., Tokyo, Japan), Destilator (Buchi R-215, New Castle, USA).

Procedures

Tuna eye preparation

As a by-product of the tuna industry, tuna eyes originate from Bitung City, North Sulawesi, Indonesia. It was transported to Muara Baru Port, North Jakarta, using a cargo equipped with a freezer (tight cardboard and ice around to maintain freezing temperatures -20°C). Initial grouping of Tuna's eye (*T. albacares*) based on the part, size, and weight of the tuna eye (Figures 1 and 2). The freshness level of tuna eyes based on the sensory assessment of tuna eyes (i.e., eyeball, cornea, and pupil) refers to the Indonesian National Standard (SNI) 2729:2013 concerning the healthy fish specifications. The handling method of removing the eye from the tuna head was adopted by Murado et al. (2012). Tuna eye preparation separates the hard tissue parts (lens and sclera) from the soft tissue parts (eye muscles, liquid phase, and fat). Each part of the eye was calculated as a percentage of its mass by the whole eye. The main part used for extraction and analysis was the soft tissue. As for the size specifications, tuna eyes were classified into three groups based on their diameter, namely 'small' (<6 cm), 'medium' (6-9 cm), and 'large' (>9 cm). The tuna eyeballs were kept at a temperature of -20°C until they were ready for further examination. The tuna eye was subjected to chemical component tests, which included the determination of moisture, fat, protein, ash, and carbohydrates (by difference). These analyses were conducted in accordance with the guidelines provided by AOAC (2005).

Tuna eye oil extraction

The method of extracting oil from tuna eyes used cool centrifugation. Samples of eye muscle, liquid phase, and tuna eye fat mixtures were crushed to create a paste using a blender for approximately 3 minutes. The tuna paste was centrifuged (11,200×g, 4°C) for 30 minutes. The separation method of virgin fish oil from tuna eyes was referred to Clodoveo and Hbaieb (2013) with modifications of sample type. Virgin fish oil was separated from impurities by vacuum pipetting. The separated virgin fish oil was stored in a glass bottle, covered by aluminum foil in a freezer at -20°C.

Characteristics of tuna eye oil

Tuna eye proportion. Tuna eye proportion analysis was determined by measuring the weight of the vitreous humor, lens, and other parts compared to the weight of the whole tuna's eyes. The tuna eye proportion was calculated using the following formula:

$$\text{Tuna eye Proportion (\%)} = \frac{\text{Weight of tuna eyes part (g)}}{\text{Weight of whole tuna eyes (g)}} \times 100\%$$

Chemical composition analysis of tuna eyes. The chemical composition analysis of tuna eye was performed using a modified AOAC method (2005). Moisture content analysis was determined using the method 925.45. The protein content was assessed using the Kjeldahl technique according to method 920.152, while ash content was referred to method 940.26, and fat content was measured using the soxhlet extraction method 963.16. Carbohydrate content was calculated by difference.

Heavy metals analysis of tuna eyes. The heavy metals Cd, Pb (SNI 2354:5:2016), and Hg (SNI 2354:6:2016) were analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Thermo scientific 7900, Waltham-Massachusetts, USA). The measurement of As (SNI 2354:15:2017) was performed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Agilent Technologies 700, Santa Clara, USA).

The yield of virgin fish oil. The yield of fish oil extract from tuna eyes is presented as a percentage. The analysis compared the amount of extracted fish oil with the fish eye total weight used. The formula applied was as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of fish oil (g)}}{\text{Total of fish eye weight (g)}} \times 100\%$$

Oxidation analysis. Extracted fish oil was subjected to get yield and determined the quality, including analysis of peroxide number (AOAC 2005 No. Method Ca 5a-40), free fatty acids (AOCS, 1998), p-anisidine (IUPAC 1987 No.2504), total oxidation (TOTOX) (AOCS, 1997).

Fatty acid profile. The evaluation of the fatty acid performance of tuna eye was conducted using the AOAC 2005 No. 969.33 technique. An oil sample weighing 20 mg was placed within a Teflon tube cap. Then, it was mixed with 1 mL of a solution containing 0.5 N NaOH in methanol. Subsequently, the sample was subjected to heating in a water bath for a duration of 20 minutes. The combination was subjected to the addition of 2 mL of a 20% BF_3 solution and 5 mg/mL of the standard, followed by heating for a duration of 20 minutes. Subsequently, the liquid was chilled and combined with 2 mL of saturated NaCl and 1 mL of isooctane, followed by vigorous shaking. The layer of isooctane was extracted using a pipette and transferred into a tube containing 0.1 g of anhydrous Na_2SO_4. The mixture was left undisturbed for a duration of 15 minutes. The separated liquid phase was extracted, while the resultant oil phase was introduced into the FAME standard mixture in a 1 µL injection (using Supelco 37 component fatty acid methyl ester mix). A volume of 1 µL was injected into the GC instrument (Shimadzu GC 2010 Plus, Kyoto, Japan). The nitrogen (N_2) flow rate on the Gas Chromatography (GC) instrument was 20 mL/minute, while the water (H_2O) flow rate was 30 mL/minute. The injector temperature was set at 200°C, and the detector temperature was set at 230°C. The retention times and peaks of each component were measured and compared with standard retention times in order to gather information regarding the types and constituents present in the sample.

Atherogenicity Index (AI) and Thrombogenicity Index (TI) of tuna eye oil

The Atherogenic Index (AI) and Thrombogenic Index (TI) are important factors used to assess the nutritional value of oil. These indices are calculated using the method developed by Ulbricht and Southgate (1991). The lipid quality index was determined using many factors, including the Atherogenicity Index (AI), which quantifies the correlation between primary saturated fatty acids and the principal classes of unsaturated fatty acids. The equation provided can be used to calculate the atherogenic index analysis.

$$AI = \frac{[(4xC14:0) + C16:0 + C18:0]}{[\sum MUFA + \sum PUFA + n6 + \sum PUFA - n3]}$$

The Thrombogenicity Index (TI) shows an activity that can form clots in blood vessels. The following equation can estimate thrombogenic index analysis.

$$TI = \frac{(C14:0 + C16:0 + C18:0)}{(0.5\,MUFA + 0.5\,PUFA - n6 + 3\,PUFA - n3 + PUFA - n3/PUFA - n6)}$$

Data analysis

The data were analyzed by descriptive analysis, while the experiments were designed using a completely randomized design. The data was analyzed using a one-way Analysis of Variance (ANOVA) with a 95% confidence interval ($p<0.05$). Duncan's post-hoc test was employed to further investigate the factors influencing the response. The data analysis was conducted using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 22.0.

RESULTS AND DISCUSSION

Characteristics of tuna eyes

Visualization and specification of tuna eyes part

The eye of tuna used in this research is the yellowfin tuna. As for the diameter size specifications, tuna eyes are grouped into three, namely small (<6 cm), medium (6-9 cm), and large (>9 cm). Visualization of the small, medium, and big tuna eyes can be seen in Figure 1.

The result exhibited the variations and trends of the size effect of tuna eye on several quality parameters. Firstly, Figure 1 visually compares tuna eye characteristics across different sizes: large tuna eyes with a diameter >9 cm, medium tuna eyes with a diameter of 6-9 cm, and small tuna eyes with a diameter <6 cm. Morphometric measurements include lens and sclera weight, flesh and liquid weight, flesh-to-liquid ratio, and lens-to-sclera ratio, as detailed in Table 1. Table 1 exhibits the increase in the weight of tuna eyes with variations in eye size (large, medium, and small). The differences in eye size significantly and linearly impact weight (g) for each size. However, there is no significant difference in meat and liquid (lens and sclera) proportion percentage (%). Figure 2 illustrates the specifications for the appearance of tuna eyes, particularly whole ones (Figure 2A). After preparation, the tuna eye's extraocular muscles, cornea, vitreous humor, sclera, and lens were also visualized. The extraocular muscle is the main part of the tuna eye that will be harnessed as the source of tuna eye fish oil. Understanding the proportion of this ratio will significantly affect the industry's consideration of using this product. Interestingly, we can conclude that the ratio of the extraocular muscle will increase linearly with the size of the tuna eye.

Tuna eye chemical composition

The muscle and liquid parts of the tuna eye were analyzed for their chemical composition, which had been prepared as a paste. The results of the chemical composition analysis revealed statistically significant variations ($p<0.05$) in the fat content among the tuna with varying eye sizes. The bigger the eye size of the tuna, the greater the fat content of the tuna eye produced. Table 2 shows the chemical characteristics based on the different eye sizes of tuna.

The chemical analysis showed that the small, medium, and large eyes contained percentages of moisture, ash, protein, and carbohydrates, which were not significantly different. Interestingly, for the parameters of fat, they were significantly different. Further tests show that the larger the eye size of the tuna, the higher the fat content produced.

Heavy metal in tuna eye

Heavy metal analysis was conducted to ensure that tuna eye oil meets the safety standards for consumption. The analysis of heavy metals in tuna eye oil was performed based on the size of the eyes (small, medium, large), comprising cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As). The result of heavy metals analysis in tuna eye oil is presented in Table 3.

The heavy metal content of tuna eye oil was detected as cadmium (Cd) 0.02 mg/kg (large-eye tuna) and lead (Pb) 0.08 mg/kg (large-eye tuna). The heavy metal content detected in medium tuna eyes was cadmium (Cd) 0.03 mg/kg. The heavy metal contents still comply with the standards set by the Codex Alimentarius Commission (CAC) (2017) regarding fish oil standards and testing limits, ensuring their safety for consumption.

Table 1. Tuna eye specifications

Parameter	Eye size		
	Small (<6 cm)	**Medium (6-9 cm)**	**Large (>9 cm)**
Lens and sclera weight (g)	9.25±3.34[a]	15.50±1.00[b]	27.75±1.50[c]
Weight of meat and liquid (g)	90.50±5.05[a]	167.75±13.25[b]	363±17.17[c]
Meat and liquid proportion (%)*	92.35±0.08[a]	91.56±0.23[a]	92.90±0.05[a]
Lens and sclera proportion (%)*	7.65±0.08[a]	8.47±0.26[b]	7.10±0.05[a]

Notes:*The % of each eye part based on the whole eye

Table 2. Tuna eye chemical composition

Parameters	Small	Medium	Large
Moisture (%)	68.56±7.29[a]	71.52±2.54[a]	68.93±0.08[a]
Ash (%)	2.12±1.11[a]	1.05±0.03[a]	0.92±0.04[a]
Fat (%)	13.89±1.96[a]	18.17±0.19[b]	22.21±0.27[c]
Protein (%)	11.86±4.99[a]	6.50±3.49[a]	4.80±0.16[a]
Carbohydrate (%)	2.75±1.86[a]	1.09±0.00[a]	3.12±0.33[a]

Note: Significant differences are indicated by distinct superscript letters in the same row ($p<0.05$)

Table 3. Heavy metal in tuna eye

Parameters	Size			CODEX 193-1995
	Small	Medium	Large	
Arsen (As) (mg/kg)	nd	nd	nd	0.10
Mercury (Hg) (mg/kg)	nd	nd	nd	0.10
Lead (Pb) (mg/kg)	nd	nd	0.08	0.10
Cadmium (Cd) (mg/kg)	-	0.03	0.02	0.10

Note: *nd: not detected

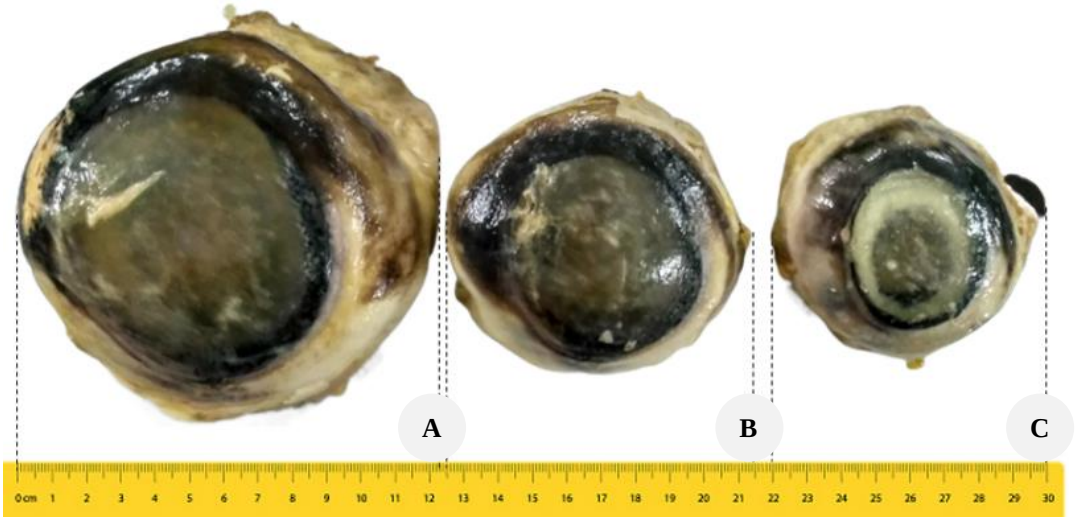

Figure 1. Visual comparison of tuna eye characteristics across different sizes, A: Big tuna eye, B: Medium tuna eye, C: Small tuna eye

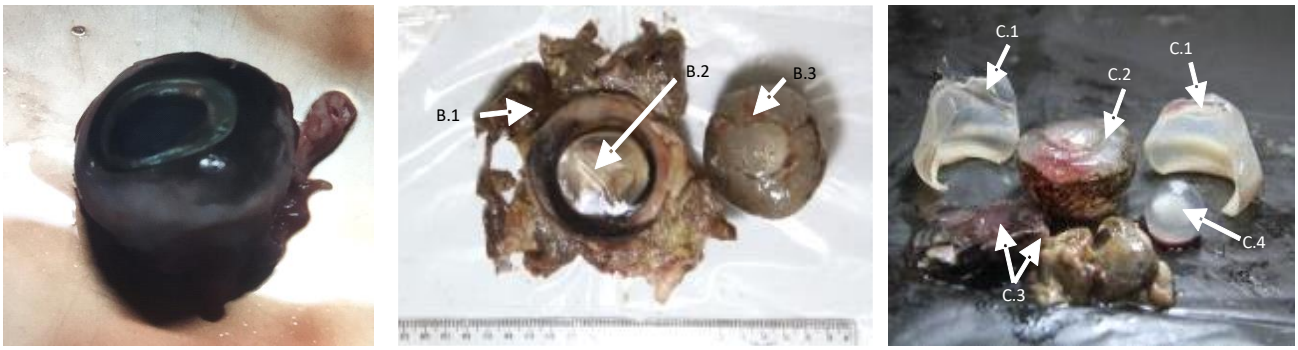

Figure 2. Specifications for the appearance of tuna eyes part. A. Whole tuna eye, B. Tuna eye after preparation: B.1: Extraocular muscles, B.2: Cornea, B.3: Vitreous humor. C. Components of the tuna eye: C.1: Sclera, C.2: Vitreous humor, C.3: Extraocular muscles, C.4: Lens

Characteristics of tuna eye oil

Yield and oxidative level of tuna eye oil

Yield is the portion of the primary raw materials that can generate the finished product or the correlation between the primary raw materials and the finished product. Initially, we checked the moisture content of small, medium, and large tuna eyes and revealed 0.06±0.02%, 0.10±0.01%, and 0.22±0.01%, respectively. The greater the yield, the higher the percentage of oil produced. The results of the analysis of variance indicated that the yield of tuna eye oil produced was significantly influenced (p-value<0.05) by eye size. The percentage of small tuna eye yield was 3.33±0.58%; medium tuna eye 5.67±0.09%; and big tuna eye 12.82±0.18%. The percentage is directly proportional to the eye size; the more significant the eye size of the tuna, the higher the percentage of oil. The yield of tuna eye oil from extraction can be seen in Figure 3.

Following this, a comparison was made between the results of the tuna oil oxidation parameter analysis and the International Fish Oil Standard (IFOS) (2014). The IFOS (2014) utilized the subsequent parameters: Free Fatty Acid

content (FFA) not exceeding 3.5%, acid value (3 mgKOH/g), peroxide value below 5 meq/kg, p-anisidine value below 20 meq/kg, and total oxidation below 26 meq/kg. The purity of fish oil is ascertained by utilizing the parameters listed in Table 4. The results of the analysis of variance indicated that the eye size had a statistically significant impact on all parameters associated with the quality of the fish oil generated ($p<0.05$). Duncan's posthoc test indicated that the small, medium, and large eye types exhibited significantly different values for free fatty acids, acid numbers, and peroxide numbers.

Fatty acid profile of tuna eye oil

The constituent fatty acids and their proportions in the irises of small, medium, and large tuna were determined by analyzing the fatty acid profiles of these fish. The fatty acid profile of tuna eye oil was evaluated, revealing fluctuations in the saturated fatty acid (SFA) content. The SFA total exhibited variations of 27.6%, 32.1%, and 19.66% for small, medium, and large eye sizes, respectively. Palmitic and stearic acids constituted the major components of SFA, exhibiting a similar trend to the total SFA values. In contrast, the lowest SFA numbers were detected for lauric and tridecanoic fatty acids. Meanwhile, the monounsaturated fatty acid profile demonstrated a linear decreasing trend with increasing tuna eye size. The primary components of monounsaturated fatty acids were oleic acid and palmitoleic acid. In contrast with the SFA and MUFA, PUFA demonstrated a linear increase in the total amount of fatty acids with the increase in tuna eye size. Docosahexaenoic Acid (DHA) occupied the highest percentage among the fatty acids, followed by palmitic acid (C16:0). The percentages of fatty acids in tuna eye oil are presented in Table 5.

No significant difference in the EPA content between tuna eye oil extracted from medium and large-sized eyes is observed. However, the amount of DHA increased significantly with increasing eye size. This result indicated that DHA content in tuna eye oil positively correlates with eye size. DHA, an essential fatty acid, may also have anti-inflammatory and cardiovascular properties and is vital for the development of the brain and ocular retina. In general, the concentrations of EPA, DHA, and total polyunsaturated fatty acids were found to be substantially higher in the eyes of large tuna as compared to those of small and medium-sized eyes. The fatty acid concentration in tuna's eye exhibited a prevalence of n-3 series values that were nine to twelve times greater than those of n-6 fatty acid. Figure 4 illustrates the percentages of EPA, DHA, and PUFA fatty acids in small, medium, and large-eye tuna.

Atherogenicity Index (AI) and Thrombogenicity Index (TI) of tuna eye oil

The composition of fatty acids in Tuna's eyes was investigated, along with the associated health lipid indices (AI, atherogenic, and TI thrombogenic). The Atherogenicity Index/AI (Equation 1) provides information regarding the predominant categories of unsaturated fatty acids as well as the overall quantity of saturated fatty acids. The values of AI and TI were calculated using the proportion of unsaturated to saturated fatty acids. As the detailed fatty acid profile in Table 5 revealed, the MUFA contents decreased with the increase in eye size, indicating a proportional content balance with the PUFA content. These two components were attributed to the final AI and TI indices. A high PUFA content is indicated by a lower value, which is preferable. The results showed that the large tuna eye has the lowest value compared to the small and medium ones; the values of AI and TI are 0.33 and 0.12; 0.47 and 0.20; 0.55 and 0.20 for large, medium, and small eyes, respectively (Figure 5).

Discussion

Tuna is a promising fishery commodity because the Indonesian sea population of tuna is assigned to the significant Pacific and Indian Oceans (Pertiwi et al. 2017). The tuna processing industry leaves valuable by-products, especially the eye, as a source of oil rich in PUFAs. The results reported in Table 2 demonstrate a positive correlation between the size of a tuna's eye and the concentration of chemical components, specifically an increase in fat content. Specifically, small tuna eyes (<6 cm) showed a fat content of 13.89±1.96%, lower than that observed in large tuna eyes (at 22.21±0.27%). Conversely, the protein content appeared to exhibit the opposite trend. Small eyes recorded a higher protein content of 11.86%, whereas medium and large tuna eyes registered protein contents of 6.50% and 4.80%, respectively. Following further investigation, it is feasible to propose that a larger size of the eye organ in tuna could contribute to an increased formation of adipose tissue in the eye area.

Table 4. Oxidative level of tuna eye oil

Oxidative level	Small	Medium	Large
FFA (%)	0.46 ± 0.09^{a}	0.66 ± 0.05^{b}	1.07 ± 0.08^{c}
Acid value (mg KOH/g)	0.20 ± 0.04^{a}	0.28 ± 0.02^{b}	0.46 ± 0.03^{c}
Peroxide value (meq/kg)	8.18 ± 0.16^{c}	5.57 ± 0.66^{b}	4.26 ± 0.06^{a}
Anisidine value (meq/kg)	4.45 ± 0.14^{a}	5.45 ± 0.28^{b}	4.15 ± 0.11^{a}
TOTOX (meq/kg)	20.82 ± 0.25^{b}	16.60 ± 1.57^{b}	12.67 ± 0.05^{a}

Note: Significant differences are indicated by distinct superscript letters in the same row ($p<0.05$)

Table 5. Fatty acid profile tuna-eye oil

Fatty acid	Small (%)	Medium (%)	Large (%)
Saturated Fatty Acid			
Lauric acid, C12:0	0.02±0.00	0.05±0.01	0.02±0.00
Tridecaenoic Acid, C13:0	0.02±0.00	0.02±0.01	-
Myristic Acid, C14:0	2.78±0.02	2.90±0.19	1.70±0.02
Pentadecanoic Acid, C15:0	0.83±0.10	1.00±0.06	0.50±0.01
Palmitic Acid, C16:0	18.71±0.47	20.40±0.83	14.41±0.63
Heptadecanoic Acid, C17:0	0.65±0.05	1.32±0.13	0.49±0.01
Stearic Acid, C18:0	4.08±0.87	5.01±0.01	2.09±0.01
Arachidic Acid, C20:0	0.20±0.05	0.40±0.00	0.17±0.00
Heneicosanoic Acid, C21:0	0.03±0.01	0.14±0.03	0.02±0.00
Behenic Acid, C22:0	0.18±0.02	0.35±0.02	0.12±0.02
Tricosanoic Acid, C23:0	0.05±0.02	0.16±0.00	0.04±0.00
Lignoseric Acid, C24:0	0.16±0.04	0.36±0.01	0.10±0.00
Total	27.60±0.13^{b}	32.1±0.83^{c}	19.66±0.71^{a}
Monounsaturated Fatty Acid			
Myristoleic Acid, C14:1	0.10±0.01	0.06±0.01	0.06±0.00
Pentadenoic Acid, C 15:1	-	0.07±0.02	-
Palmitoleic Acid, C16:1	7.15±0.48	6.33±0.2	5.05±0.13
Cis-10-Heptadecanoic Acid, C17:1	0.65±0.49	1.15±0.03	0.73±0.03
Elaidic Acid, C18:1n-9t	0.12±0.02	-	0.11±0.00
Oleic Acid, C18:1n-9c	23.15±0.26	16.55±0.03	16.29±0.92
Cis-11-Eicosenoic Acid, C20:1	1.35±0.23	0.87±0.01	1.36±0.13
Erucic Acid Methyl Ester, C22:1n9	0.28±0.06	0.11±0.00	-
Nervonic Acid, C24:1	0.63±0.07	-	0.51±0.01
Total	34.40±0.51^{b}	25.17±0.26^{ab}	24.61±0.70^{a}
Polyunsaturated Fatty Acid			
Linoleic Acid, C18:2n-6c	1.26±0.04	1.31±0.015	0.75±0.05
Linolenic Acid, C18:3n-3	0.25±0.04	0.54±0.01	0.1±0.07
ɣ- Linolenic acid, C18:3n-6	0.13±0.11	0.12±0.01	0.03±0.00
Eicosadinoic Acid, C20:2	0.19±0.01	-	-
Cis-11,14-Eicosadinoic Acid, C20:2	0.08±0.01	0.28±0.00	0.27±0.01
Eicosatrienoic Acid, C 20:3n-3	-	0.25±0.04	-
Cis-8,11,14-Eicosantrinoic Acid, C20:3n-6	2.67±0.11	0.13±0.00	0.09±0.01
Arachidonic Acid, C20:4n-6	0.14±0.09	2.69±0.05	2.67±0.09
Cis-13,16-Docosadinoic Acid, C22:2	0.46±0.03	-	0.04±0.01
Eicosapentanoic Acid (EPA), C20:5n-3	4.97±0.63	6.23±0.11	6.19±0.23
Docosahexaenoic Acid (DHA), C22:6n-3	25.57±0.91	31.07±0.9	37.45±0.87
Total	36.63±0.80^{a}	43.10±0.57^{ab}	48.10±0.51^{b}

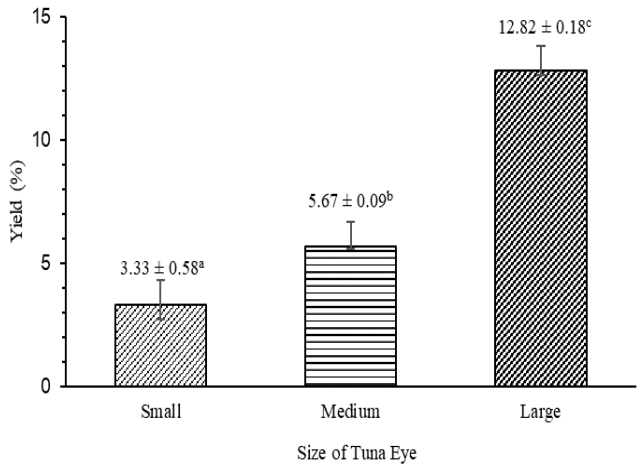

Figure 3. The yield percentage of tuna eye oil. Note: Significant differences are indicated by distinct superscript letters in the same row ($p<0.05$)

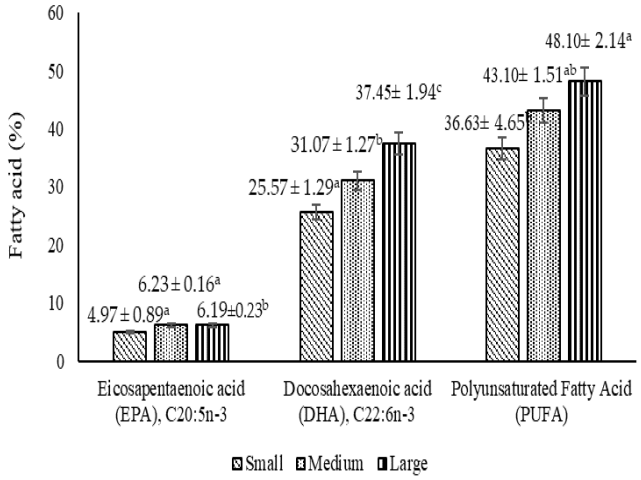

Figure 4. The composition of EPA, DHA, and total of PUFA extracted from different sizes of tuna eye. Note: Different superscript letters indicate that there are significant differences ($p<0.05$)

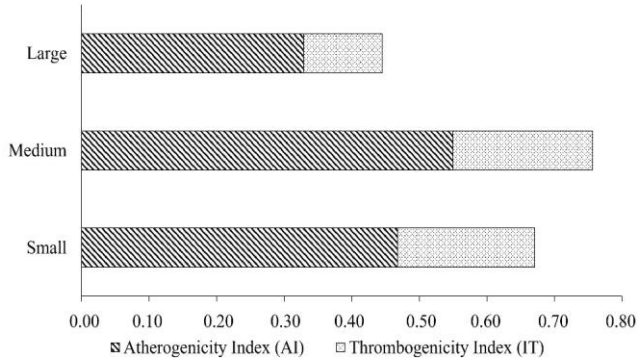

Figure 5. Atherogenicity Index (AI), Thrombogenicity Index (TI) tuna eye oil

The observed growth of the eye size may result in an increased availability of adipose tissue for extraction, increasing the fat content and oil yield in the extraction process. The finding mentioned above aligns with the basic concept that a positive correlation exists between the size of adipose tissues in organisms and the volume of fat that can be extracted, as Sardenne et al. (2016) reported. Furthermore, increased fat content in tuna is often linked with variations in weight parameters (Tables 1 and 2), highlighting the interconnectedness of size and fat accumulation. Importantly, the examination of fat composition reveals that the eyes of larger tuna possess the highest capacity for fish oil content due to their increased fat levels.

Tuna exhibit a voracious grazing pattern throughout its life cycle, consuming prey that comprises cephalopods, crustaceans, and small fish at both night and day. The nutritional intake of tuna will have an impact on the chemical composition of its anatomical structures, such as its eyes. Season, environmental conditions, gonadal maturity stage, nutritional state, and fish age are additional factors that influence fat content (Verheyen et al. 2019; Ruilova et al. 2022). This lipid content will have an effect on the fish oil obtained; the majority of fish, similar to humans, must consume EPA and DHA. This assertion holds particular validity with regard to fish originating from marine ecosystems, where the primary producers of these advantageous fatty acids are phytoplankton, consisting of marine algae, diatoms, and other photosynthetic organisms. Fish ingest these oils, which subsequently accumulate and enter our food chain. For their typical development, marine finfish necessitate Essential Fatty Acids (EFA), including n-3 High Unsaturated Fatty Acids (HUFA) like Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) (Carr et al. 2023). As a migratory species, tuna expands its nomadism as it reaches maturity, necessitating substantial fat deposition for energy storage. Hence, the greater the opportunity to ingest varied, high-quality feed with accumulated fat content while foraging in open water, the higher the quality of plankton as a food source in the marine food chain. This condition also permits the accumulation of heavy metals in waters contaminated with industrial refuse when tuna migrate. As the weight of the tuna increases (from 50 kg onwards), industrialists have observed that greater attention must be paid to heavy metal contamination.

Heavy metal accumulation is a common occurrence among aquatic biota inhabiting waters contaminated with such substances. In the context of apex predators, such as large pelagic fish, particularly tuna, the dynamics shift upon exposure to metal contamination. The risks are heightened due to the propensity of various heavy metals to accumulate in their systems at concentrations exceeding those found in the environment and in the organisms they consume (Araújo and Cedeño-Macias 2016). Heavy metals enter the body of aquatic organisms can be through food, gills, and diffusion through the body surface. Fish that live in waters containing heavy metals will passively absorb heavy metals. Heavy metals enter the fish body and are diffusely absorbed by the gills and then distributed throughout the body through the blood, resulting in heavy metals in the flesh. Heavy metal accumulation in fish is most concentrated in the gonads and cranium, according to Annabi et al. (2013). Since heavy metals are typically accumulative in the tissues of aquatic animals, larger/older fish have a higher probability of accumulating heavier heavy metals. The heavy metal content of tuna eye oil was detected as cadmium (Cd) 0.02 mg/kg (large-eye tuna) and lead (Pb) 0.08 mg/kg (large-eye tuna). The heavy metal content identified in medium tuna eyes was cadmium (Cd) 0,03 mg/kg, and no heavy metal was found in small size of tuna eyes. Heavy metals can accumulate in an organism's body and remain for a long time as poisonous substrates. Heavy metals can be distributed to the human body, and some will accumulate through intermediaries in the form of food contaminated with heavy metals, which can endanger human health if consumed for a long time (Maurya et al. 2019).

Table 4 shows the effect of the tuna eye size compared to their oxidative parameters. The large tuna eye exhibited the highest free fatty acids and acid numbers values at 1.07±0.08% and 0.46±0.03 mg KOH/g, respectively. The small and large tuna eyes showed significant differences from the medium-sized eyes regarding the p-anisidine value parameter. Additionally, the small and medium-sized eyes differed significantly from the large eyes in terms of the TOTOX value. Based on the oxidation parameter values, the small tuna eyes demonstrated a higher total oxidation value than the medium and large ones.

Furthermore, the hydrolysis reaction of triglycerides that occurs during the storage and extraction procedures results in the formation of free fatty acids. When oil reacts with heat and water, an autocatalytic mechanism is triggered (Nazir et al. 2017). The oil of small, medium, and large-eye tuna contains few free fatty acids. The minimal concentrations of free fatty acids observed in the three tuna eye oils are hypothesized to result from the extraction process not involving thermal treatment and the consequent low moisture content. The acid value denotes the quantity of potassium hydroxide needed to neutralize Free Fatty Acids (FFA) in oil or fat, measured in milligrams. Consequently, an association can be observed between the acid number and free fatty acids; more precisely, the acid

value is directly proportional to the quantity of free fatty acids (García-Moreno et al. 2013). Fish oil with a low acid number indicates good quality. A low acid number suggests minimal damage to the oil during processing or storage (Mahesar et al. 2014). In this study, the FFA percentage content of large tuna eyes differed significantly from that of medium and small eyes. An autocatalytic mechanism forms the primary oxidation product in the form of hydroperoxide.

The oxidative level of the produced eye oil met the standards for free fatty acids and acid value. According to the Codex Alimentarius Commission (CAC) standards from 2017 regarding fish oil standards/testing limits, the maximum values for free fatty acids and acid numbers are set at <3.5% and <3 mg KOH/g, respectively. The oil from tuna eyes complied with these standards. However, a trend indicated that as the size of the tuna eye increased, so did the values of these parameters, potentially correlating with eye size. Possible consequence: elevated concentrations of free fatty acids in the oil; an increase in eye size could stimulate a more pronounced discharge of fat from the tissue. As the size increases, there is a possibility that more fatty acid molecules are exposed to oxygen, which could elevate the acid value. Similar results were reported by Zhang et al. (2021).

The peroxide amount indicates the presence of early-stage oxidation damage. Medium and large-eye tuna oil has a standard peroxide value, while small-eye fish have a value that exceeds the standard. The variability in oxidation values observed in fish oil can be attributed to its high concentration of long-chain unsaturated fatty acids, particularly EPA and DHA, which are exceptionally vulnerable to oxidation (Kaushik et al. 2015). Furthermore, while the acid value and FFA levels remained below the established standard, we acquired supplementary data indicating that the moisture content of small, medium, and large tuna eyes increased by 0.06±0.02, 0.10±0.01, and 0.22±0.01, respectively. A trend indicated that the moisture content in larger tuna eyes was 3-4 times higher than in smaller eyes. An increased level of moisture can stimulate additional lipid hydrolysis, resulting in the liberation of free fatty acids.

The p-anisidine number indicates a secondary oxidation product resulting from the hydroperoxide decomposition as a further oxidation process. The measured secondary oxidation products are not always directly proportional to the peroxide amount, but a high peroxide amount can cause a high p-anisidine value, allowing further deteorization processes to occur (Baek 2012). The p-anisidine value of small, medium, and large tuna eye fish oil is low. The low p-anisidine value of the three tuna eye oils indicated that the fish oil extraction process did not cause further oil degradation. Determining the formation of primary and secondary oxidation products is how the TOTOX value, which is utilized to quantify oil rancidity, was computed. The TOTOX index gauges the extent of oil rancidity and was computed to assess the creation of both initial and subsequent oxidation by-products. This index is derived by doubling the primary and secondary oxidation by-products summary (Chew and Nyam 2019). Importantly, the fish oil extracted from small, medium, and large tuna eyes all met the quality standards for fish oil.

The tuna's eye size is directly related to the amount of eye muscle and meat it produces, which are rich sources of oil. The more oil produced, the higher the percentage of essential fatty acids such as EPA, DHA, and total PUFA. The percentage of Polyunsaturated Fatty Acids (PUFA) increased significantly from 34.13% in small tuna eyes to 47.10% in large tuna eyes (p0.05), as shown in Table 5. Notably, there was a significant positive trend in the levels of DHA, with proportions of 25.57%, 31.07%, and 36.95% found in small, medium, and large tuna eyes, respectively. In addition, Figure 4 illustrates the distribution of EPA, DHA, and PUFA fatty acids among tuna eyes of varying sizes: small, medium, and large. It showcases the obvious trend in this correlation phenomenon between tuna eye size and its PUFA contents.

There is a positive correlation between an increase in eye dimensions and a rise in fat content, according to one hypothesis. A positive correlation was observed between ocular lipid content and oil yield, leading to increased production and availability of Polyunsaturated Fatty Acid (PUFA) and Docosahexaenoic Acid (DHA) compounds. The higher fat content corresponded naturally with the crucial role and metabolic processes of lipids in the ocular function of tuna. According to Nag and Wadhwa (2012), lipids located in the orbital region of the eye significantly facilitate orbital circulation by assisting the eye muscles. Furthermore, lipids and lipid-soluble components are essential constituents of the cells and tissues that make up the eye. There was an apparent correlation between eye size and retinal weight, with larger eyes having a more expansive retinal surface area. An expanded retinal area increased the synthesis of Docosahexaenoic Acid (DHA) and Polyunsaturated Fatty Acids (PUFA), which functioned as essential precursor fatty acids necessary for light capture. DHA fatty acids were notably present, especially in the orbital area's outer membranes of photoreceptor cells. According to Lad et al. (2015), these DHA fatty acids facilitate rhodopsin's function during photoreception. Consequently, larger eyes were considered more desirable for oil extraction enriched in DHA and PUFA.

According to Renuka et al. (2016), yellowfin tuna contains a significant amount of DHA, making up 35% of its fatty acid content. In another study, Jeffrey et al. (2001) suggested that DHA is crucial in the retina as a photoreceptor. Among various sources of DHA, low-temperature extracted tuna eye oil contains the highest concentration of this fatty acid, with 30.00±0.27% PUFA and 16.85±0.90% DHA compared to sardine fish oil (Renuka et al. 2016) and farmed Atlantic salmon (Blanchet et al. 2005). This high level of PUFA, especially DHA, in tuna eye oil is likely due to the protective function of DHA in the retina against oxidative stress and its critical role in retinal development (Van-Leeuwen et al. 2018). As a meso-carnivorous predator, tuna obtains its PUFA primarily from small fish, squid, and microorganisms that act as synthesizers. As a result, the fatty acid content of tuna, especially DHA, is more dominant than other fatty acids due to its dietary intake (Sidhu 2003). A high level of

palmitic acid in tuna eyes is believed to contribute to the production of mucin, which helps retain moisture in the retinol palmitate (Diao et al. 2017). Previous research conducted by Gamarro et al. (2013) and Renuka et al. (2016) also reported the highest percentage of DHA in mature tuna.

AI and TI are widely employed for analyzing fatty acid composition due to their substantial implications and the conclusive evidence they offer. EPA and DHA are prevalent choices for evaluating the nutritional value of marine animal products. Each index has its own advantages and disadvantages, underscoring the importance of a well-considered selection. The AI is a significant marker for oils capable of preventing cardiac illnesses. Meanwhile, the TI provides insights into the formation of blood vessel clots (Chen and Liu 2020). The TI signifies the development of occlusions in the blood vessels. The correlation between prothrombogenic (saturated) fatty acids and anti-thrombogenic fatty acids (MUFA, n-6 PUFA, and n-3 PUFA) is determined by TI (Equation 2). An essential oil quality index is derived from the ratio of n-3 to n-6 PUFA (DiNicolantonio and O'Keefe 2018). Ghaeni et al. (2013) state that goatfish and ponyfish contain a mixture of saturated and unsaturated fatty acids during both fishing seasons. The proportion of Polyunsaturated Fatty Acids (PUFA) is greatest in both species of fish. The potential incorporation of the elevated concentrations of EPA and DHA fatty acids found in the three varieties of tuna eyes that were assessed for their fatty acid composition into dietary food products exists. They help in preventing several ailments.

The current study and subsequent discussion have revealed significant findings regarding the impact of tuna eye size on their chemical composition, specifically their fat content. In conclusion, this study comprehensively examined the quality of fish oil, encompassing various parameters, including the maximum levels of heavy metals, fish oil quality and oxidation parameters, and thrombogenicity and atherogenicity indexes. Remarkably, these parameters meet the expected standards for virgin fish oil extracted from tuna eyes. This indicates that the fish oil obtained from tuna eyes can be considered high quality, fulfilling the criteria for purity and stability. Importantly, the results demonstrate a strong correlation between larger tuna eye size and a higher concentration of PUFA-rich fish oil in tuna, particularly regarding their DHA and EPA content. Future investigations should focus on exploring tuna species and assessing the feasibility of utilizing their oils as standardized raw materials within the fish oil industry.

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