

Effect of cinnamon masking and nanoencapsulation on fatty acid profile and health lipid indices (AI, TI) of tuna eye oil

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Abstract. Tuna eye oil is rich in omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), yet its utilization is limited by a strong fishy odor and low oxidative stability. This study evaluated the effects of cinnamon masking and nanoencapsulation using gum arabic and Tween 80 on the fatty acid profile, atherogenicity index (AI), and thrombogenicity index (TI) of tuna eye oil. Pure tuna eye oil contained saturated fatty acids (SFA) 30.23%, monounsaturated fatty acids (MUFA) 26.93%, polyunsaturated fatty acids (PUFA) 42.76%, with EPA 6.94%, DHA 30.27%, AI 0.43, and TI 0.20. Cinnamon masking increased SFA and PUFA while decreasing MUFA, maintaining low AI and TI values due to the protective effect of cinnamon antioxidants. Nanoencapsulation reduced PUFA, particularly DHA, likely due to oxidation during processing, yet still resulted in low AI and TI values. These findings demonstrate that both treatments can modify the lipid profile and maintain favorable cardiovascular health indices, supporting the development of stable, consumer acceptable omega-3 rich products

1 Introduction

Tuna eye is a by-product of tuna processing that contains a high proportion of polyunsaturated fatty acids (PUFA), notably the long-chain omega-3 such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The PUFA content in oil obtained from tuna eye ranges from 36–48%, in which EPA generally occurs at 4–6% and DHA can reach 25–37% of total fatty acids. [1]. PUFAs, especially those belonging to the n-3 group, are known to have positive health effects, including lowering the possibility of cerebrovascular and coronary complications, helping to decrease circulating triglycerides, contributing to blood pressure regulation, improving insulin sensitivity, supporting neural growth and maintaining visual system performance [2].

Despite its high nutritional value, tuna eye oil has limitations, such as a strong fishy odor and low oxidative stability. The fishy odor can reduce consumer acceptance, while its susceptibility to oxidation accelerates quality deterioration. One approach to overcome these issues is through masking with natural ingredients and nanoencapsulation. Masking is a method of adding strong-flavored ingredients to cover the fishy odor of fish oil. Previous studies have shown that cinnamon as a masking agent can effectively reduce fishy odor and improve overall sensory acceptance [3]. Nanoencapsulation is an encapsulation technique with a particle size of <100 nm, which serves to protect bioactive compounds from

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degradation caused by exposure to oxygen, light, and temperature [4]. In addition to enhancing oxidative stability, this processing technique can also influence the composition of fatty acids in the oil [5].

Changes in the proportion of fatty acids may affect health lipid indices such as the atherogenic index (AI) and thrombogenic index (TI). These indices help determine the potential risk or benefit of lipids to cardiovascular health. PUFAs including linoleic acid (LA), α -linolenic acid (LNA), EPA, and DHA have antithrombogenic effects, while saturated fatty acids (SFAs) such as lauric, myristic, and palmitic acids contribute to an increased risk of atherosclerosis and thrombosis [6]. This study aims to seek the leverage of cinnamon masking and nanoencapsulation treatments on the fatty acid profile, AI, and TI of tuna eye oil. The findings are expected to add value to tuna by-products while enhancing their economic potential as a more stable and consumer-acceptable source of functional lipids.

2 Methods

2.1 Extraction

Tuna eye samples (*Thunnus albacares*) were obtained from a tuna processing company in Denpasar, Bali. The samples were collected fresh, directly stored at -20°C , and kept under frozen conditions prior to processing. The extraction method followed the procedure described by [7]. The preparation stage involved thawing the tuna eyes, followed by cutting each eye into three parts to remove the sclera and lens. The remaining tuna eye tissue was then homogenized into a paste. The paste was subjected to ultrasonic treatment and subsequently centrifuged at a low temperature (4°C) at 10,000 rpm for 30 minutes. Centrifugation was conducted to separate the virgin fish oil (VFO) from other components (eye tissue, blood, water, and others). The extracted tuna eye oil was stored in dark bottles at -20°C .

2.2 Masking

The masking method was modified from [8]. The process was carried out by adding cinnamon powder at a concentration of 15% (w/w) of oil. Specifically, 7.5 g of cinnamon powder was added to 50 g of tuna eye oil, and the mixture was homogenized using a magnetic stirrer at 750 rpm for 15 minutes. The concentration of 15% was selected based on a previous study [3].

2.3 Nanoencapsulation

Nanoencapsulation of tuna eye oil followed the method described by [9], using gum arabic (25%) and Tween 80 (4%) as wall materials. Gum arabic was dissolved in distilled water using a magnetic stirrer at 500 rpm and 60°C for 1 hour, then stored in a refrigerator for 24 hours to achieve maximum hydration. Tween 80 was then added as an emulsifier, followed by the gradual addition of fish oil at a concentration of 6%. The mixture was immediately stirred for 30 minutes at 500 rpm using a magnetic stirrer, and subsequently subjected to size reduction using a homogenizer at 25,000 rpm for 3 minutes. The resulting emulsion was then sonicated at 24 kHz, 5 minutes.

2.4 Fatty acid profile

The analytical method was based on transforming fatty acids into their derivative forms, namely methyl esters, to enable detection by chromatography. Separation of components was performed by detecting peaks on the chromatogram corresponding to specific retention times, which were then compared with standards. The procedure began by weighing 25 mg of fish oil sample and mixing it with 1 mL of NaOH solution in methanol, followed by heating for 20 minutes. Subsequently, 2 mL of 20% BF₃ solution along with an internal standard was added, and the mixture was reheated for another 20 minutes. After cooling, 2 mL of saturated NaCl solution and 1 mL of isooctane were added, mixed, and the isooctane layer was separated and dried over anhydrous sodium sulfate prior to injection into the instrument. A 1 µL aliquot of the fatty acid methyl esters (FAME) was injected in split mode into an HP-88 capillary column (100 m × 0.25 mm × 0.20 µm). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector and detector temperatures were set at 250°C and 280°C, respectively. The oven temperature program was set at 120°C (held for 2 min), increased by 10°C/min to 180°C (maintained for 10 min), and further increased by 5°C/min to 220°C (held for 5 min). Identification of fatty acids was achieved by matching the chromatographic retention times of sample peaks with certified FAME references

2.5 Atherogenicity index (AI) and thrombogenicity index (TI)

The atherogenicity index (AI) and thrombogenicity index (TI) derived from the proportion of principal saturated fatty acids relative to key unsaturated fatty acid groups. The indices were computed using equations previously outlined by [6] the AI and TI values:

$$AI = \frac{C12:0+(4xC12:0)+C16:0}{\Sigma MUFA+\Sigma n6\ PUF A+\Sigma n3\ PUF A} \quad (1)$$

$$TI = \frac{C14:0+C16:0+C18:0}{(0.5x\Sigma MUFA)+(0.5x\Sigma n6\ PUF A)+(3x\Sigma n3\ PUF A)+\left(\frac{\Sigma n3\ PUF A}{\Sigma n6\ PUF A}\right)} \quad (2)$$

2.6 Data analysis

The dataset was processed through one-way analysis of variance (ANOVA) to evaluate differences among the three types of fish oils: virgin fish oil (VFO), masked fish oil (MFO), and nanoencapsulated fish oil (NFO). Although these samples represent different forms of fish oil rather than treatment levels, ANOVA was applied to statistically compare their physicochemical characteristics. Prior to analysis, data were tested for normality and homogeneity of variances at a 95% confidence level ($\alpha = 0.05$). When significant differences were found ($p < 0.05$), Duncan's multiple range test was conducted to identify pairwise differences among sample types. Statistical analyses were performed using SPSS version 22.0.

3 Result and discussion

The results of the analysis indicate that processing methods, such as masking and nanoencapsulation, exert a notable influence on the fatty acid profile of tuna eye oil (Table 1). The fatty acid profile data for the masking treatment were obtained from a previous report [3]. In the masking treatment, an increase was observed in SFA, EPA, DHA, and PUFA values, while MUFA values decreased. This reduction in MUFA may be attributed to the heating step during the masking process, conducted at 50 °C. Such a temperature has the

potential to cause degradation of monounsaturated fatty acids (MUFA), as MUFA contain double bonds that are susceptible to heat-induced damage.

Different heat treatments can affect both the type and quantity of fatty acids produced. High temperatures are likely to break certain double bonds in fatty acids and replace them with single bonds, thereby altering the fatty acid composition. The increase in SFA, EPA, DHA, and PUFA in the masking treatment may be related to the decrease in MUFA, given that the percentage composition is recalculated based on the total fatty acids after processing. SFA are more stable due to their saturated carbon chains, making them more likely to remain unchanged or increase proportionally during processing. The rise in PUFA levels could be attributed to higher proportion of EPA and DHA, which in turn may be linked to the presence of antioxidants in cinnamon used as a masking agent, protecting these fatty acids. [10] noted that antioxidants can remove prooxidant impurities. This finding is consistent with [11] who reported that fish oil supplemented with tocopherol antioxidants contained higher EPA, DHA, and PUFA levels compared to oil without antioxidant addition.

Table 1. Fatty acid profile.

| Fatty acid (%) | Samples | | |
|----------------|-------------------------|-------------------------|-------------------------|
| | VFO | MFO | NFO |
| C12:0 | 0.01 | 0.15 | 0.03 |
| C13:0 | 0.03 | 0.03 | 0.02 |
| C14:0 | 3.02 | 3.05 | 2.65 |
| C15:0 | 0.86 | 0.94 | 0.79 |
| C16:0 | 19.47 | 18.60 | 17.44 |
| C17:0 | 1.26 | 0.99 | 1.09 |
| C18:0 | 4.92 | 4.74 | 4.34 |
| C20:0 | 0.40 | 0.35 | 1.00 |
| SFA | 30.20±0.07 ^b | 31.60±0.00 ^c | 26.95±0.01 ^a |
| C14:1 | 0.08 | 0.08 | 0.04 |
| C16:1 | 6.56 | 5.67 | 5.03 |
| C17:1 | 1.13 | 1.08 | 0.93 |
| C18:1 | 17.81 | 16.55 | 28.39 |
| C20:1 | 1.23 | 1.35 | 1.20 |
| MUFA | 26.94±0.05 ^b | 24.88±0.03 ^a | 36.21±0.04 ^c |
| C18:2 | 1.29 | 1.21 | 1.32 |
| C18:3 | 0.45 | 0.39 | 0.38 |
| C20:2 | 0.31 | 0.36 | 0.26 |
| C20:3 | 0.17 | 0.14 | 1.52 |
| C20:4 | 3.08 | 3.05 | 2.58 |
| EPA, C20:5n-3 | 6.95±0.04 ^b | 7.31±0.01 ^c | 6.31±0.01 ^a |
| DHA, C22:6n-3 | 30.24±0.11 ^b | 30.42±0.01 ^c | 25.56±0.01 ^a |
| PUFA | 42.76±0.13 ^b | 43.46±0.04 ^c | 36.84±0.05 ^a |

Notes: Numbers followed by different superscript letters within columns (a–c) indicate significant differences ($p < 0.05$). VFO: Virgin Fish Oil; MFO: Masking Fish Oil; NFO: Nanoencapsulation Fish Oil.

In the nanoencapsulation treatment, fatty acid contents decreased compared to the masking sample. The DHA value in this treatment was 25% lower than the 42–43% observed in VFO and MFO. This reduction is most likely associated with the encapsulation stages, such as homogenization, ultrasonication, and freeze-drying. During freeze-drying, unsaturated fatty acids may come into contact with oxygen, triggering oxidation and lipid degradation [12]. Homogenization may also reduce DHA content due to increased exposure to prooxidant compounds and oxygen, induced by cavitation and localized heating during processing [13]. Ultrasonication has a similar impact, reducing fatty acid levels, including DHA, in nanocapsules.

The predominant MUFAs in tuna eye oil are oleic acid and palmitoleic acid, whereas the dominant SFAs are palmitate and stearate. In the PUFA group, the most abundant fatty acids are EPA and DHA. Variations in the percentage of fatty acids in fish oil influence health-related lipid indices, which are represented by the atherogenic (AI) and thrombogenic index (TI). The AI values are presented in Figure 1.

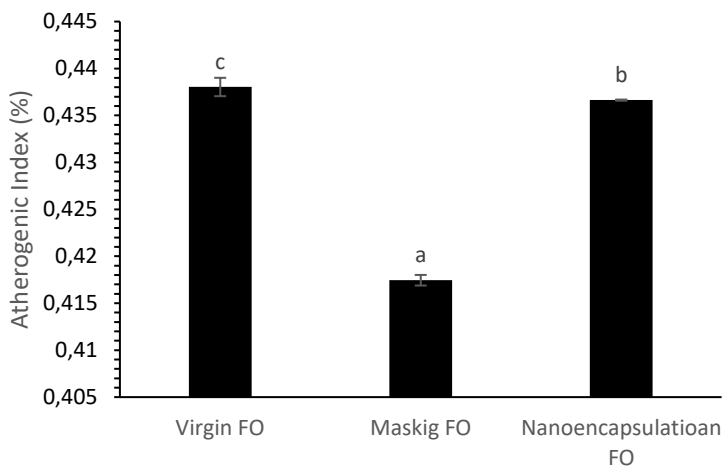


Fig. 1. Atherogeneity index tuna eye oil. Different letters in the graph indicate significantly different results ($p < 0.05$).

Indices relating to atherogenesis (AI) and thrombogenesis (TI) can be calculated to describe the effects of fatty acid variability on human health. AI and TI indicate the potential for atherosclerotic plaque formation, thrombus development, and blood clotting. The TI values are presented in Figure 2.

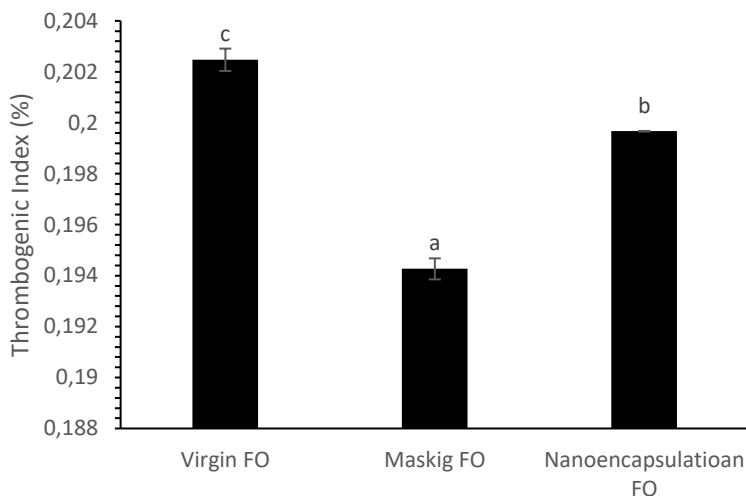


Fig. 2. Thrombogenicity index. Different letters in the graph indicate significantly different results ($p < 0.05$).

The AI and TI values for each type of tuna eye oil showed significant differences among treatments ($P < 0.05$). Variations in AI and TI values in tuna eye oil may be influenced by the extraction method, processing temperature, pretreatment, and the degree of interaction between the material and the oil [14]. Moreover, the lipid composition of the oil further influences AI and TI indices. These indices are determined by the balance between pro-thrombogenic fatty acids (saturated fatty acids) and anti-thrombogenic fatty acids (MUFA and PUFA). A high unsaturated fatty acid content in the oil reduces AI and TI values. Low index values in tuna eye oil reflect its high PUFA content, where a high proportion of PUFA indicates good nutritional quality of the studied fish oils.

In this study, AI values were recorded between 0.41 and 0.43 (Fig. 1), whereas TI values were observed between 0.19 and 0.21 (Fig. 2). These results indicate that tuna eye oil has a low potential risk for cardiovascular disease. This finding is consistent with [1], who reported an AI value of 0.47 and a TI value of 0.20 for medium-sized tuna eye oil. High AI and TI values are associated with a heightened likelihood of cardiovascular complications due to the consumption of certain fatty acids. Lipid sources exhibiting AI scores under 1 and TI levels below 0.5 are regarded as highly suitable for dietary use [15].

4 Conclusion

Cinnamon masking and nanoencapsulation significantly affected the fatty acid profile, AI, and TI of tuna eye oil. Masking increased SFA, EPA, DHA, and PUFA levels, while reducing MUFA, and maintained low AI and TI values due to the protective effect of cinnamon antioxidants. Nanoencapsulation reduced PUFA, particularly DHA, likely due to processing-induced oxidation. All treatments produced tuna eye oil with low AI (0.41–0.4) and TI (0.19–0.21) values.

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