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Evaluation of fatty acid quality and antioxidant activity of supplement tablets from tuna eyes oil and *spirulina platensis*

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Abstract

There are many supplements in tablet form on the market with various ingredients and benefits. However, previously, fish oil-based tablet supplements with other ingredients have not been widely found in the market, presumably due to stability issues during storage. Therefore, an alternative that can be considered is combining tuna eye oil with antioxidant-rich ingredients such as *Spirulina platensis* in the form of tablets. Tuna eyes oil comes from a by-product of the tuna processing industry which contains fatty acids that are beneficial for health. Besides that, *Spirulina platensis* is a microalgae that has antioxidant potential for the human body and can maintain product quality to prevent oxidation. This study aimed to evaluate the quality of fatty acids and antioxidant activity of supplement tablets from tuna eyes oil and *Spirulina platensis*. The design used in this research was a completely randomized design (CRD) with three treatments and three replications. Treatment includes different formulas F0 = (tuna eyes oil without *Spirulina platensis*), F1 = (tuna eyes oil + *Spirulina* culture), and F2 = (tuna eyes oil + *Spirulina* commercial). The research results show that supplement tablets that have optimal shelf-life stability are those with a formula tuna eyes oil +*Spirulina* culture IC_{50} value obtained amounted to 119.63 ppm, atherogenicity index (AI) 0.38, thrombogenicity index (TI) 0.17, and total fatty acids identified were 99.95%. A combination of tuna eyes oil and *Spirulina platensis* has a more complete fatty acid quality than without the combination.

Keywords: Antioxidant Activity, Fatty Acids, *Spirulina platensis*, Supplement Tablets, Tuna Eyes Oil

Full length article **Corresponding Author*, e-mail: wtrilaksani@apps.ipb.ac.id

1. Introduction

Food supplements are health products that are often a popular topic and the center of attention of consumers in the world. Currently, research on food supplements is still limited even though the prevalence of the use of food supplements has increased drastically over the last 20 years and it is estimated that demand will increase by $272.4 billion in 2028 [1–3]. Food supplements have various forms, one of which is solid or tablet form which is a dosage form containing active ingredients and is the most widely used because it is practical, easy to carry, easy to find, and easy to produce [4–6]. Apart from that, food supplements have also been circulating on the market with various ingredients and benefits. One alternative supplement in tablet form that has potential is easy to obtain and has shelf-life stability, namely a combination of tuna eyes oil and *Spirulina platensis*. Tuna eyes oil is a fishery product that comes from the by-products of the tuna processing industry, generally processed into fillet, sashimi, and canned products that still contain head, eye, skin, innards, and bone by-products [7]. Tunas eye oil contains polyunsaturated fatty acids omega-3 in particular docosahexaenoic acid (DHA) by 35% [8–9]. Tuna eyes has DHA content is higher than other fish oils, one of which is sardines, 6.90% and the DHA content has benefits for forming brain nerves, reducing the risk of various degenerative diseases and mental disorders [10–14]. Besides that, *Spirulina platensis* is a type of microalgae with blue-green, lives in waters, is easy to cultivate on a large scale due to that were high photosynthetic efficiency, easy to adapt to the environment, a protein content of 63% and other bioactive compounds [15–17]. *Spirulina platensis* has antioxidant potential for the human body and maintains product quality hence that it does not oxidize [18–19]. Combined dietary supplement tablets *Spirulina platensis* and virgin fish oil from tuna eyes which has been approved [20], particularly has the disadvantage of studying the fatty acid profile and limited antioxidant activity because it was only tested with the best treatment. Therefore, the author

conducted research related to evaluating the quality of fatty acids and antioxidant activity of supplement tablets from tuna eyes oil and *Spirulina platensis*.

2. Materials and methods

2.1 Materials

The ingredients used consist of tuna eyes oil, *Spirulina platensis* culture, *Spirulina* commercial, beta cyclodextrin, gum arabic, vitamin C, avicel 102, silicon dioxide, talc, and magnesium stearate. The tools used consist of food processor (Panasonic MK-5086M), high-speed refrigerated centrifuge (Himac CR 21G), magnetic stirrer, analytical balance, electronic pipette, glass stirrer, blender, caliper (smallest scale 0.1 cm), Spray drayer (Holes 190 Ø nozzle 0.7 mm), homogenizer, tablet printing machine (Rimek mini press-II) and tools for cultivation *Spirulina platensis*.

2.2 Method

This research was carried out experimentally using a completely randomized design (CRD) with three treatments and three replications. A different formula is F0 = (tuna eyes oil without *Spirulina platensis*), F1 = (tuna eyes oil + *Spirulina* culture), and F2 = (tuna eyes oil + *Spirulina* commercial).

2.3 Tunas eye oil extraction

Extraction of tuna eyes oil refers to [9]. Tunas eye samples were obtained from the Muara Baru tuna loin company, North Jakarta. Tuna eyes preparation is done by cutting the tunas eye into three parts to remove the sclera and lenses. The tuna eyes meat is then crushed using a blender until it becomes a paste, then low-temperature centrifugation is carried out to separate tuna eyes oil (10.000 rpm, 30 minutes, 4 ˚C). The tunas eye oil that is formed is taken using a dropper pipette and put into a coated glass bottle of aluminum foil to avoid oxidation.

2.4 Microencapsulation of tuna eyes oil

Microencapsulation of tuna eyes oil refers to [21] using beta-cyclodextrin and gum arabic coating materials in a ratio of 1:2. The ratio of coating material to tunas eye oil is 2:1 and the homogenization time is 10 minutes. The coating mixture is dissolved using 15% water and heated at a temperature of 60 °C. The solution was cooled to a temperature of 45 °C and homogenized (13.000 rpm for 2 minutes). Tunas eye oil was added to the mixture gradually which was then homogenized (13.000 rpm for 10 minutes). The resulting emulsion is dried using a spray dryer.

2.5 Cultivation Spirulina platensis

Cultivation *Spirulina platensis* refers to [22]. *Spirulina platensis* has grown on technical Walne media at 1 mL/L at a temperature of 25 ˚C with a light intensity of 3,250 lux, lighting for 24 hours, and seawater salinity of 15 ppt. The seeds used are 20% of the culture volume. *Spirulina platensis* harvested on the 14th day after reaching optical density (OD) ≥ 0.5. Harvesting *Spirulina platensis* was carried out by filtering nylon mesh size 20 µm to separate biomass and filtrate. The biomass was dried using an oven at 40 ˚C for 24 hours and ground into powder.

2.6 Supplement tablet formula

The manufacture of supplement tablet formulas is required using the compression method according to [20]. Provide all the ingredients, namely tuna eyes oil microcapsules, *Spirulina platensis*, vitamin C, avicel 102, silicon dioxide, talc, and magnesium stearate. Next, homogenization is prescribed and printing of supplement tablets is conceded. The supplement tablet formula in this study can be seen in Table 1. The supplement tablet formula will be analyzed for the fatty acid profile using the method [23], the fatty acid ratio of the supplement tablet [24], the atherogenicity index (AI), and the thrombogenicity index (TI) in supplement tablets [25] and the antioxidant activity of supplement tablets [26].

2.7 Variables

The parameters measured were the fatty acid profile of supplement tablets, the fatty acid ratio of supplement tablets, the atherogenicity index (AI) and thrombogenicity index (TI) of supplement tablets, and the antioxidant activity of supplement tablets.

2.8 Data analysis

The data obtained will be admitted analysis of variant (ANOVA) with a 95% confidence interval, when results show a significant influence (p<0.05) then further testing is done Duncan. The applications used for data analysis are Software Microsoft Excel 2013 and Statistical Process for Social Science (SPSS) version 22.0.

3. Results and discussions

3.1 Fatty acid profile of supplement tablets

The fatty acid profile of supplement tablets consists of eleven saturated fatty acids (SFA), nine monounsaturated fatty acids (MUFA), and nine polyunsaturated fatty acids (PUFA). The fatty acid profile of supplement tablets can be seen in Table 2.

This research produced a supplement tablet fatty acid profile, namely two SFA and two MUFA which were not detected. Apart from that, nine SFA were detected, seven MUFA and nine PUFA (Table 2). The total SFA in this study experienced an increase, namely F0 of 29.13%, F1 of 32.69%, and F2 of 33.27%. The increase that occurred in SFA was caused by the supplement tablets originating from tuna eyes oil combined with *Spirulina platensis*. This is following research [27] that the addition *Spirulina platensis* Honey was able to increase the SFA content as indicated by the addition of palmitic fatty acid (C18:0) to the combination product, which was not detected in the control, increasing by around 11% after the combination. [28] added that the fat content, especially SFA, namely palmitic and stearic, increased in the added bread *Spirulina*.

The total MUFA in this study produced various values, namely F0 of 30.80%, F1 of 32.25% and F2 of 32.20%. Total PUFA in this study produced various values, namely F0 of 36.08%, F1 of 35.01% and F2 of 34.45%. In the F1 treatment (MUFA) there was an increase as well as in the F0 treatment (PUFA). This is because the supplement tablets in this study were combined with *Spirulina platensis* which can increase its nutritional and fatty acid (MUFA) content. Research [29] reported that *Spirulina* contains 32.94% MUFA where oleate is dominant so the addition of *Spirulina* to the product can increase its nutritional content (MUFA).

Table 1: Supplement tablet formula

Material type	Formula (mg)		
	F0	**F1**	**F2**
Tuna eyes oil microcapsules	300	300	300
Spirulina platensis	-	140	140
Vitamin C	140	-	-
Avicel 102	30	30	30
Silicon dioxide	15	15	15
Talc	10	10	10
Magnesium stearate	5	5	5
Weight per tablet	± 500 mg	± 500 mg	± 500 mg

Table 2: Fatty acid profile of supplement tablets

Fatty acids	Formula (%)		
	F0	**F1**	**F2**
Lauric acid, C12:0	nd	nd	nd
Tridecaenoat acid, C13:0	0.25	0.18	0.13
Myristic acid, C14:0	2.63	2.68	2.79
Pentadecanoic acid, C15:0	0.87	0.92	0.89
Palmitic acid, C16:0	19.39	22.66	23.07
Heptadecanoic acid, C17:0	1.14	1.18	1.21
Stearic acid, C18:0	4.20	4.33	4.37
Arachidic acid, C20:0	0.33	0.34	0.35
Heneicosanoic acid, C21:0	0.12	0.60	0.13
Bahenic acid, C22:0	nd	nd	nd
Lignoceric acid, C24:0	0.09	0.25	0.24
Total SFA	**29.13**	**32.69**	**33.27**
Myristoleic acid, C14:1	0.08	0.11	0.13
Pentadecanoic acid, C15:1	0.11	0.11	0.13
Palmitoleic acid, C16:1	6.64	7.01	7.03
Cis-10-Heptadecanoate acid, C17:1	1.19	1.18	1.23
Olaidic acid, C18:1n-9c	20.92	21.74	21.68
Eicosenoic acid, C18:1n-9t	nd	nd	nd
Cis-11-Eicosenoic acid, C20:1	1.71	1.76	1.80
Erucic acid methyl ester (C22:1 n-9)	0.20	0.24	0.19
Nervonoic acid, C24:1	nd	nd	nd
Total MUFA	**30.80**	**32.25**	**32.20**
Linoleic acid, C18:2n-6c	1.50	1.97	2.20
Linolenoic acid, C18:3n-3	0.39	0.26	0.83
ɣ- Linolenoic acid, C18:3n-6	0.07	0.66	0.51
Cis-11,14-Eicosadinoic acid, C20:2	0,29	0.50	0.19
Cis-8,11,14-Eicosantrienoic acid, C20:3n-6	0.22	0.18	0.13
Arachidonic acid, C20:4n-6	2.62	2.49	2.41
Cis-13,16-Docosadinoic acid, C22:2	0.18	1.08	0.89
Eucosapentanoic acid (EPA), C20:5n-3	5.69	5.20	5.12
Dokosahexaenoic acid (DHA), C22:6n-3	25.02	22.61	21.87
Total PUFA	**36.08**	**35.01**	**34.45**
Total fatty acids identified	**91.90**	**99.95**	**99.92**

Notes: nd (not detected)

Table 3: Fatty acid ratio of supplement tablets

Formula	Total fatty acids (%)			Ratio
	Omega-3	**Omega-6**	**Omega-9**	
F0	31.27	4.31	21.12	7.26:1:4.9
F1	28.28	5.23	21.98	5.41:1:4.2

F2	27.50	5.69	21.92	4.83:1:3.8

Table 4: Antioxidant activity of supplement tablets

Formula	IC_{50} value (ppm)
F0	184.07±3.52[c]
F1	119.63±1.53[a]
F2	130.45±2.13[b]

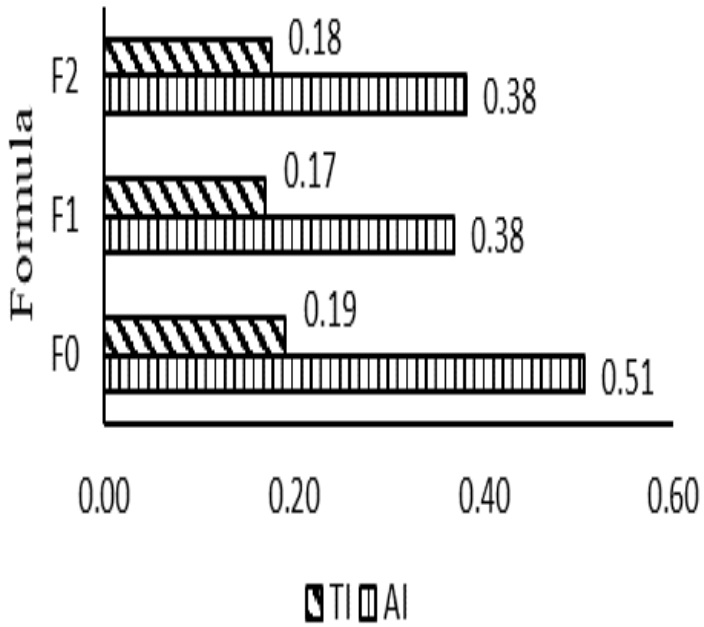

Figure 1: Atherogenicity index (AI) and thrombogenicity index (TI)) of supplement tablets

Research [30] reported that *Spirulina platensis* is rich in PUFA fatty acids, especially α-linolenic acid (ALA) and γ-linolenic acid (GLA).

Apart from that, the fatty acid profile components of supplement tablets dominate the DHA content, namely F0 of 25.02%, F1 of 22.61%, and F2 of 21.87% which will later play a role in health, one of which is as a nutrient for brain nerve development and bone growth. This is by research [31] that DHA is good at helping brain development and optimal functional development of the central nervous system. [32] added that DHA also plays a role in increasing bone growth through increasing calcium absorption in the intestine, as well as encouraging mineral deposits for bone development.

3.2 Fatty acid ratio of supplement tablets

The fatty acid ratio of supplement tablets in formulas F0, F1 and F2 has different values which can be seen in Table 3. The fatty acid ratio of supplement tablets produces different omega-3, omega-6, and omega-9 in different formulas (F0, F1, and F2). F0 produces 31.27% omega-3, 4.31% omega-6 and 21.12% omega-9 with a ratio of 7.26:1:4.9. F1 produces 28.28% omega-3, 5.23% omega-6 and 21.98% omega-9 with a ratio of 5.41:1:4.2. F2 produces 27.50% omega-3, 5.69% omega-6 and 21.92% omega-9 with a ratio of 4.83:1:3.8.

This research shows that with higher levels of omega-3, omega-6, and omega-9, the resulting ratio is also higher. This is because the supplement tablet formula has various types, hence omega-3, omega-6, and omega-9, and their ratios will have different results. Research [33] reported that different ingredients and combinations will produce different ratios of omega-3, omega-6, and omega-9. Omega-6 in this study increased due to the linoleic acid content in *Spirulina* and omega-3 from tuna eyes oil. This is by what was reported [34] that the content of fatty acids (omega-6) in *Spirulina* contains linoleic acid. Apart from that, it is necessary to pay attention to the balanced ratio of fatty acids in supplement tablets which is beneficial for health. This is to the statements [35] and [24] that it is very important to pay attention to the balanced ratio of fatty acids in products to

support health such as maintaining the function of the heart, brain, and nervous system.

3.3 Atherogenicity index (AI) and thrombogenicity index (TI) of supplement tablets

The atherogenicity index and thrombogenicity index of supplement tablets have varying values which can be seen in Figure 1. The AI index of supplement tablets produces namely F0 (0.51), F1 (0.38) and F2 (0.38). The TI indices of supplement tablets produced were F0 (0.19), F1 (0.17) and F2 (0.18). Figure 1 shows the IA and TI of different supplement tablet preparations and with additions *Spirulina platensis* can reduce the AI value from 0.51 to 0.38 and the TI value from 0.19 to 0.17. However, the AI and TI values in this study are still classified as good fatty acids because they have an index below 0.50. This is by the statement [25] that good fatty acids have an index below 0.50 for both AI and TI values.

The low TI value in this study was caused by low levels of myristic fatty acid. This is by the statement [36] that low myristic fatty acid levels will influence low TI values. Apart from that, the addition of *Spirulina platensis* resulted in lowering the value of AI and TI. This is by the statement [37] that *Spirulina platensis* has a greater yield of unsaturated fatty acids (PUFA) compared to saturated fatty acids (SFA), so the addition of *Spirulina platensis* can reduce the value of AI and TI.

3.4 Antioxidant activity of supplement tablets

Antioxidants are compounds that can prevent the oxidation process from occurring if they react with free radicals. The effectiveness of a sample to ward off free radicals in the DPPH method is described by an inhibitory concentration of 50% (IC_{50}). The smaller the IC_{50} value the stronger the antioxidant activity. IC_{50} value Supplement tablets can be seen in Table 4.

The antioxidant activity of supplement tablets produces IC_{50} values which vary, namely F0 of 184.07 ppm, F1 of 119.63 ppm, and F2 of 130.45 ppm. The results of the supplement tablet antioxidant activity test showed that the IC_{50} values F1 and F2 have better antioxidant activity and are in the medium category than F0. Based on the analysis of various antioxidant activities, supplement tablets had a significant effect (p<0.05). This is by [38] that antioxidant activity is classified as moderate due to the IC_{50} range of 100–150 ppm. [20] reported that the antioxidant activity of tablets that had a greater concentration of tuna eyes oil had better antioxidant activity.

Low IC_{50} value supplement tablets will have an impact on stable shelf-life so that supplement tablets will not easily experience oxidation. This is supported by the statement [39] that IC_{50} Low levels in a product can increase shelf life and not easily experience oxidation. Besides that, *Spirulina* has antioxidant potential, oxidative reduction power, high solubility, and heat stability and is reported to reduce the level of nitric oxide and lipid peroxide in the product [40].

4. Conclusions

Based on the research results, it shows that supplement tablets that have optimal shelf-life stability are those with a formula tuna eyes oil + *Spirulina* culture IC_{50} value obtained amounted to 119.63 ppm, atherogenicity index (AI) 0.38, thrombogenicity index (TI) 0.17, and total fatty acids identified were 99.95%. A combination of tuna eyes oil and *Spirulina platensis* has a more complete fatty acid quality than without the combination.

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