Thesis for the Degree of Master of Fisheries Science

The Effect of Fatty Acid Methyl Esters of Oil Extracted from Skipjack Tuna By-products on Fortified Pork Sausage: Microbiological and Sensorial Properties

by

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KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

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가다랑어 부산물에서 추출한 지방산 메틸 에스테르가 돼지고기 소시지 강화에 미치는 영향: 미생물학적 및 관능적 특성

Advisor: Prof. Byung – Soo Chun

by

Abeysinghe Mohottalalage Akalanka

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Abstract

The Study evaluated the efficacy of Fatty Acid Methyl Esters (FAME) derived from Skipjack Tuna (*Katsuwonus pelamis*) head and viscera oil as a novel antimicrobial agent. Soxhlet extraction and esterification by lipase were performed. The functional pork sausage was prepared with FA and FAME-embedded sodium alginate (SA). The highest EPA (4.15%) and DHA (21.96%) were reported in the head oil extracted by ethanol. SEM images and results of the disc diffusion method evidenced FAME's effect on bacterial cell damage and growth inhibition. Compared to control and SA-coated samples, bacterial growth was inhibited in fortified samples starting from the 6^{th} day of storage.

The reduction in colony counts and overall acceptance in sensorial property evaluation were Significantly higher in FAME-fortified sausage (0.72 ± 0.02 log CFU/g), (6.97 ± 0.06) than the FA-fortified sausage (0.60 ± 0.03 log CFU/g), (6.37 ± 0.06). That concludes the FAME's enhanced antimicrobial properties and its possible application in functional foods.

Keywords: Skipjack Tuna, *Katsuwonus pelamis*, by-products, Omega 3, antimicrobial

Introduction

According to the FAO, more than 60% of total world fisheries production was processed to some degree to enhance their preservation capacity (Food and Agricultural Organization of the United Nations, 2002). Because of pre-processing procedures such as beheading, degutting, and skinning a massive quantity of waste material is generated on a daily basis. Especially fish processing industries dispose of 25-50% (on a weight basis) of waste products. The discards comprise the head, viscera, skin, skeletal frames, and scales. Improper handling of fishery waste has become a burden for the public, causing environmental hazards and detrimental health problems (Venugopal, 2021)

Nowadays fish processing industries use processing by-products to recover economically valuable compounds such as fish oil enriched with PUFA, natural pigments (astaxanthin), packaging materials (chitosan), collagen (in cosmetics), etc. It is an approach to sustainable fish processing, where the optimal uses of resources are maximized and the cost of waste disposal is minimized. Also, the processing industry can earn additional revenue by supplying the recovered raw materials for the production lines of other food, pharmaceutical, or cosmetics industries (Arvanitoyannis & Kassaveti, 2008) & (López-Pedrouso et al., 2020).

There are many beneficial polyunsaturated fatty acids (PUFA) contained in fish oil, including eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) that has been an integral part of the human diet for decades. It represents 2% of global fat and oil consumption, and thanks to its curative capabilities, the demand is still increasing (Rizliya & Mendis, 2014). EPA and DHA are two essential fatty acids in human nutrition. Those are well known for their therapeutic properties for cancer, diabetes, and their potential to decrease the risk of depression. Sufficient scientific evidence is available to show their engagement in developing the immune system, and neural functions(Ivanovs & Blumberga, 2017). The results of epidemiological and prospective clinical trials have proven daily intake of 0.5 - 1.8 g of EPA & DHA can decrease the incidence of cardiovascular diseases (Kris-Etherton et al., 2002). The increasing demand for PUFA-rich oils compels the seeking of alternative resources for extraction. The current study suggests that by-products of Skipjack tuna are a potent candidate for that.

Skipjack Tuna (*Katsuwonus pelamis*) is an epipelagic, highly migratory, and top commercial marine fish species that belongs to the family Scombridae. They inhabitant tropical and warm-temperate waters (Kawarazuka & Béné, 2011). The global capture production of 2.8 million tons in 2020 emphasizes the consumer demand for these species worldwide (Food & Agriculture Organization of the United States, 2022). Since the head and viscera of Skipjack tuna account for 30% of total waste yield, (Balogun & Talabi, 1985) this study targeted those two by-products.

Besides their nutritional value and health benefits, fatty acids and their derivatives are well known for their bactericidal and bacteriostatic actions for decades, against a wide range of bacteria, including both Gram's positive and Gram's negative strains (Chandrasekaran et al., 2008). Fatty acid methyl esters (FAME) are economically significant in the food industry and the renewable energy sectors. FAME is often created through the transesterification of vegetable oils, animal fats, and cooking oils using low molecular weight alcohols, most frequently methanol or ethanol. Enzymatic transesterifications, catalyzed using lipases, can substitute these chemical reactions. They may offer benefits like decreased energy consumption, simple biocatalyst removal and reuse, streamlined product purification, and the production of fewer effluents (Poppe et al., 2013).

Although the reports on the antimicrobial properties of Fatty acid (FA) recovered from marine resources are abundant, FAME is less concerned. Therefore, our study evaluated the efficacy of FAME as a novel antimicrobial agent and assessed the preservative capacity and sensory properties of a functional pork sausage prepared with FA and FAME-embedded sodium alginate coating. Soxhlet extraction recovered oil from the head, and viscera of Skipjack Tuna using ethanol, *n*-hexane, and diethyl ether as solvents. Esterification of fatty acid was conducted using lipase enzyme to obtain FAME. The fatty acid composition of the oil was analyzed by gas chromatography (GC). The antioxidant potentiality was evaluated by performing $ABTS^{+}$ and DPPH activities.

The disc diffusion method and SEM analysis of *B. cereus* smear treated with FAME evaluated the antimicrobial capacity. The results of the study will encourage the utilization of Skipjack tuna by-product oil as a source of PUFA and developing novel antimicrobial agents.

Materials and Methods

1. Raw materials and sample preparation

Dongwon Fisheries Co. Ltd, Seoul, South Korea, kindly provided skipjack tuna samples. Samples were transported to the laboratory in frozen conditions. After several rinses with cold water (4 °C) fishes were allowed to thaw for six hours. After thorough cleaning, beheading, degutting, and skinning were performed manually. The separated portions of the head, and viscera, were freeze-dried (Hypercool Freeze drier, HC4110 - Daejeon, South Korea) to eliminate the moisture content. Once enough drying was completed, the samples were crushed in an electric grinder (2500Y-Dong Yi multifunctional grinder, Yongkang Boou Hardware Products Co., Ltd. China). Powdered samples were separately packed in air-tight containers, and stored at -20 °C while maintaining dark conditions until they were obtained for extraction.

2. Chemicals and reagents

Solvents for the extraction such as *n*-hexane (95.0%), ethyl alcohol anhydrous (99.9%), and diethyl ether (99.0%) were brought from Samchun Chemical Co. Ltd., (Seoul, South Korea). Lipase enzyme from *Aspergillus niger* (200u/g) and reagents for antioxidant analysis, such as azino-di, 3-ethylbenzthizoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and standard antioxidants, such as Trolox, Ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Muller Hinton Agar (MHA) and Nutrient Agar (NA) Broth were brought from Becton, Dickinson & Company Ltd. (7 Loveton circle, Sparks, USA). As for all other chemicals and standards used in this study either belonged to the high-performance chromatography (HPLC) or analytical grade categories.

3. Bacterial culture and pork sausage

Through Korean Collection for Type Cultures, in South Korea, *Bacillus cereus* ATCC 14579 (Gram's positive) Was obtained. Bacterial culture was sub-cultured in nutrient agar broth and incubated at 37 °C a day before the experiments. Good quality pork sausages were purchased from the local market (Busan, South Korea) on the experiment day.

4. Extraction of oil

n-Hexane, ethyl alcohol, and diethyl ether were used apart in a Soxhlet extractor to recover oil from the head and viscera. Every extraction procedure was continued for twelve hours. Ten grams of freeze-dried and crushed samples of each by-product were loaded into a paper thimble. Exactly 100 ml of each solvent was poured into a round bottom flask and set up inside the Soxhlet apparatus. Extraction temperatures were maintained at 85 °C, 90 °C, and 50 °C for *n*-hexane, ethyl alcohol, and diethyl ether respectively. At the end of the extraction, rotary evaporation at 42 °C was conducted to remove solvents effectively. It was conducted thrice for each extraction to obtain precise, and reliable data. The extraction yield was calculated according to (Eq.1).

$$Yield\ of\ oil\ (\%) = \frac{weight\ of\ oil}{weight\ of\ the\ sample} \times 100 \quad (1)$$

5. Color analysis of oil

The Lovibond RT series, Portable Reflectance spectrometer, was calibrated with black and white tiles. A glass cuvette was filled up to 1 cm in height with oil. The instrument software automatically generated the colorimetric measurements as L^*, a^*, and b^* values. The total color difference (ΔE^*) was quantified according to Eq.2 (Robertson, 1977). In the equation ΔL^*, Δa^*, and Δb^* represent the differences in lightness/brightness, redness/greenness, and yellowness/blueness, respectively.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

6. Profile of fatty acids in the oil

To evaluate the fatty acid composition of recovered oil, gas Chromatographic analysis was performed. FAME was formed according to the procedure described by(Park et al., 2021). An Agilent 6890 N GC system along with Supelco sp. 2560 fused silica capillary of 100.0 m × 250 μm × 0.2 μm film thickness was used for the analysis. The carrier gas was Helium and the flow rate was maintained at 0.5 ml/min. Inject temperature was 250 °C while the detector temperature was adjusted to 260°C. The oven temperature was maintained at 100 °C for four minutes allowing it to increase by 3 °C /minute and the increment continued for 47 minutes. During the final stage, the temperature inside the oven was stabilized at 240 °C for 20 minutes.

7. Saponification value (SV) & average molecular weight

The SV expresses the necessity of potassium hydroxide in mg for saponification and neutralizing free fatty acids found in an oil sample of 1g. The SV was calculated experimentally according to the AOCS official method cd 3-25. The SVs were obtained based on Eq. 3(American Oil Chemists Society, 2017). An inverse relationship exists between the average molecular weight and the SV of oil samples (Barret, 2018). Please Refer to (Eq.4). The average molecular weight was obtained by replacing the SV with the following equation.

$$Saponification\ Value = \frac{56.1 \times N \times (V2 - V1)}{W} \quad (3)$$

N stands for normality of HCl, v_1 stands for the amount of HCl used in the test, v_2 represents the amount of HCl used in the blank, the weight of oil is denoted by W.

$$\text{Average molecular weight of oil} = \frac{3 \times 56 \times 10^3}{\text{Saponification Value}} \quad (4)$$

8. Preparation of FAME

The process reported by (Natadiputri et al., 2015) was followed in order to prepare the FAME by esterification reaction using methanol stable lipase enzyme, with a few minor adjustments. Recovered oil from the head and viscera was mixed with methanol (94.5%) at a molar ratio of 6:1. Since the microbial lipase enzymes are unstable in highly concentrated methanol solutions, methanol was added to the oil samples in three steps encouraging an efficient enzymatic conversion reaction (Yang et al., 2009) Exactly 1.5 ml of 50mM Tris-HCl buffer (pH 8) was added to the mixture. Afterward, 0.03 g of lipase enzyme was mixed into the solution. The mixtures were placed in a shaking incubator of 30 °C and 300 rpm for 24 h followed by centrifugation at 8000 rpm for 10 minutes. The mixtures were allowed to stand overnight to separate FAME and glycerol into two distinct layers. Finally, the upper layer was carefully obtained and used for antimicrobial studies.

9. Antioxidant activity of recovered oil

Using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-di (3-ethylbenz - thiazoline 6-sulfonate) ($ABTS^{+}$) radical scavenging activity, the antioxidant activity of skipjack tuna by-product oil samples was assessed. With a few minor modifications, experiments were conducted as per the prior study by (Haq et al., 2017). As a comparative study, the absorbance of ethanolic solutions of Trolox and Ascorbic acid solution (300µg/ml) was measured.

9.1 $ABTS^{+}$ radical scavenging activity

A stock solution of ABTS (7mM/L) was prepared in distilled water a day before the experiment. The absorbance of the $ABTS^{+}$ solution was adjusted by adding 94% ethanol until the solution attained the absorbance of 0.7 ± 0.02 at 734nm. Exactly 3950 µl of $ABTS^{+}$ solution was mixed with 50µl of each oil sample. The samples were vortexed for a few seconds immediately after mixing with $ABTS^{+}$ solution. The reaction time was limited to six minutes under dark conditions. All samples were maintained at room temperature during the incubation period. Once the incubation period was completed the absorbance of the samples was measured at 734 nm.

As the control, an ethanolic solution of Trolox (300µg/ml) and a sample blank excluding skipjack tuna by-product oil was evaluated at 734nm. All the tests were performed in triplicates. Eq. 5 was used to determine the proportion of scavenging $ABTS^+$ radical activity.

$$ABTS^+ \ Radical \ Scavenging \ Activity \ (\%) = \left[1 - \left(\frac{A_S - A_b}{A_c}\right)\right] \times 100 \quad (5)$$

A_s - absorbance of the skipjack tuna by-product oil + $ABTS^+$, A_b– absorbance of skipjack. tuna by-product oil (50µl) + methanol (3950 µl), A_c - absorbance of the $ABTS^+$ solution.

9.2 DPPH radical scavenging activity

The ethanolic solution of 0.2mM DPPH sample was freshly prepared. Exactly 3950µl of DPPH solution was added to 50µl of each oil sample. Thirty minutes at room temperature were spent incubating samples that had been thoroughly vortexed. The incubation was carried out in dark conditions. A sample blank was prepared by adding 3950µl of DPPH, excluding the by-product oil. The absorbance was measured at 517nm. As the control, the absorbance of the ascorbic acid solution (300µg/ml in distilled water) was measured following the same procedure. Every sample was tested thrice. DPPH radical scavenging activity was assessed in accordance with Eq. 6.

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \left[1 - \left(\frac{A_s - A_b}{A_c}\right)\right] \times 100 \quad (6)$$

A_s - absorbance of the SKJ Tuna by-product oil + DPPH, A_b - absorbance of SKJ Tuna by-product oil (50µl) + methanol (3950 µl), A_c - absorbance of the DPPH solution.

10. Antimicrobial assay

In vitro antimicrobial activity of FAME was shown against Gram's positive food-borne pathogen, namely *Bacillus cereus* (10^5 CFU/ml), by following the disc diffusion technique as explained by Surendhiran et al., (2014) with slight modifications. FAME (Minimum Bactericidal Concentration – MBC) was impregnated onto the sterile discs at different concentrations of 10µL, 20µL, and 30µL per disc. Ten percent DMSO solution and Ampicillin (10µg/ disc) were used as negative and positive controls, respectively. After 24h of incubation period at 37 °C temperature, the diameter of each inhibition zone was measured. All the experiments were repeated thrice to obtain precise results.

11. Morphological analysis of *B. cereus* strains treated with FAME.

To demonstrate the morphological destructions of bacterial cells that occurred because of the treatment of FAME, a Scanning Electron Microscopic (SEM, JEOL, JEM-2100F, Japan) study was performed for treated and non-treated *B. Cereus* strains. Thin smears of bacteria were prepared according to the method described in (Tyagi & Malik, 2012).

12. Preservation capacity

Four samples of pork sausage (1g) were artificially contaminated by dipping in 24 h old nutrient agar (NA) broth of *B. cereus* (10^5 CFU/ml) for 5 minutes. To make the sodium alginate solution, 0.1g of sodium alginate was dissolved in 10ml of sterile distilled water. The solution was stirred (300rpm) while controlling the temperature at 70 °C. Mixing was continued until the solution became clear. Three samples of sodium alginate were prepared following the same procedure. Two samples of the three coating solutions were placed on a hot surface and applied heat at 40 °C. While agitating vigorously, fatty acids (FA) and fatty acid methyl esters (FAME) of skipjack tuna by-products were added at the concentration level of 6% (v/v) into the two coating solutions separately. After the preparation of FA and FAME-fortified sodium alginate (SA) coating solutions, sausage samples were immersed in the solutions for 120 seconds. The samples were allowed to drip extra solution for 60 seconds. As a control, one sample was wrapped in aluminum foil. Another sausage sample was dipped in sodium alginate solution and devoted as the blank. The remaining samples were dipped in FA-fortified and FAME-fortified SA coatings separately. All the samples were labelled appropriately and placed separately on sterile Petri dishes. The samples were allowed to dry inside a sterile cabinet for 15 minutes, ensuring the adhesion and absorption of FA and FAME.

For 14 days, samples were kept in a refrigerator (4 °C). Samples were removed on 2, 4, 6, 8, 10, 12, and 14 days of storage. On the starting day of the experiment (0^{th} day of storage), 100µl of bacterial suspension was plated on a sterile MHA plate, and calculated CFU value on the following day. Samplings were prepared as a tenfold dilution series of sterile distilled water each day of the experiment. The total plate counts were obtained by spreading 100 µl of each suspension over the Muller Hamilton Agar surface. For 24 h, the plates were left incubating at 37 °C. The colony counts were expressed as Log CFU/g.

13. Sensory evaluation

An evaluation committee of ten members was elected to forecast the customer's preference for FAME-fortified sausages. Four Petri dishes containing 1g of pork sausage were pre-inoculated with *B. cereus* (10^5 CFU/mL). One sample was immersed in SA solution mixed with fatty acids; the other was treated with SA mixed with FAME. The remaining samples were considered as the control and blank without adding oil or FAME. All four samples were placed inside a freezer (4 °C) for 14 days.

On the 15th day evaluators were provided with an unstructured scale where two extremes represent like extremely and dislike extremely of each parameter. The panelists assessed four sausage samples' color, appearance, softness, and overall acceptability and allocated marks for each parameter. The experiment was repeated with three independent groups comprising ten penalizes.

(9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor a dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely)

14. Statistical analysis

Data were statistically assessed using IBM SPSS Statistics version 20.0, followed by Duncan's *post hoc* test. The values are expressed as Mean ± SD, n=3. ($p \leq 0.05$).

Results and Discussion

1. Extraction of oil

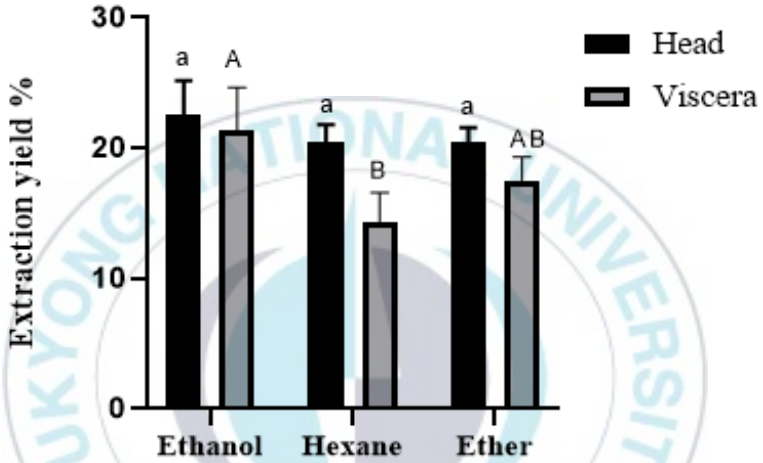

Figure 1. Yield of oil recovered from the head and viscera of skipjack tuna

The values are demonstrated as Mean ± SD, (n=3). The different letters in lowercase and uppercase indicate the significant difference between extraction yields of the head and viscera respectively ($p \leq 0.05$).

The extraction yield of the head and viscera with respect to the solvents is shown in Figure 1. The results indicate that the head portion contains a higher amount of oil compared to the viscera. These results are reasonable because of the unique chemical composition of each body part. A previous salmon fish study(Wu et al., 2011) also concluded that the head and viscera have different chemical profiles. Ethanol recovered more oil from both body parts than the other solvents. There was no significant difference between the extraction yield of the head portion among the three different solvents. But the extraction yields of oil recovered from viscera samples using ethanol and hexane are significantly different ($p \leq 0.05$).

According to the results of this study, the extractability of ethanol was observed to be higher than that of diethyl ether and hexane. This could be possible due to the polarity of the solvent. A previous study by Mitra & Mishra, (2019) described that the extractable lipid portion and quantities can be varied depending on the solvent's polarity.

2. Analysis of the Color of recovered oil

Table 1: Analysis of color of recovered oil from Head and Viscera.

	Head			Viscera		
	Ethanol	Hexane	Ether	Ethanol	Hexane	Ether
L^*	25.45 ± 2.31^{ab}	22.56 ± 2.30^{b}	27.31 ± 0.70^{a}	26.66 ± 1.92^{J}	24.82 ± 2.25^{J}	23.29 ± 2.17^{J}
a^*	0.26 ± 0.08^{d}	2.11 ± 0.40^{c}	2.11 ± 0.35^{c}	0.89 ± 0.36^{K}	0.79 ± 0.46^{K}	0.48 ± 0.33^{K}
b^*	-0.45 ± 0.23^{g}	2.22 ± 0.32^{f}	4.77 ± 0.39^{e}	0.48 ± 0.33^{L}	1.21 ± 0.92^{L}	1.14 ± 0.51^{L}
ΔE	48.68 ± 1.9^{i}	50.04 ± 1.98^{i}	44.65 ± 0.49^{h}	47.24 ± 1.52^{M}	48.51 ± 1.67^{M}	49.89 ± 1.97^{M}

The values are demonstrated as Mean ± SD, (n=3). The different letters in lowercase and uppercase indicate the significant color difference ($p \leq 0.05$) in the oil recovered from Skipjack Tuna head and viscera respectively. (L^*) represents lightness/brightness, (a^*) represents redness/greenness, (b^*) represents yellowness/blueness, (ΔE^*) represents total color difference.

Oil recovered in hexane extraction was darker compared to ethanol and ether extracts. However, there was no significant difference in the color of the oil recovered from viscera concerning the solvent. Impurities, pigments availability, the concentration of oxidized compounds, processing, and extraction parameters are the factors that affect the color of oil recovered from fish byproducts (Franklin et al., 2020). Highest lightness (L^*) value was reported from head oil extracted using ether, while the hexane extracts of the head portion reported the lowest value for lightness.

3. Fatty Acid Profile

The percentages of nutritionally valuable ten fatty acids obtained from the head and viscera oil samples were shown in Table 2. Palmitic acid (C16:0), which belongs to the category of saturated fatty acid (SFA), was the most abundant in both body parts. A study on skipjack tuna flesh also reported palmitic acid as the principal constituent of SFA. Still, the percentage was significantly lower (21.88±1.53%) compared to the composition of palmitic acid in the head portion (33.57-36.99%) (Mahaliyana et al., 2015). In the current study, the major MUFA reported was oleic acid (C18:1), and this finding is in line with the abovementioned study on flesh samples. Among the unsaturated fatty acids, docosahexaenoic acid (C22:6 ω-3) was the main fatty acid, followed by eicosapentaenoic acid (C20:5 ω-3). The DHA levels were detected in a range of 10.85% - 21.96% in this study, whereas the DHA level in flesh samples was reported as 35.66 ± 0.23% by (Mahaliyana et al., 2015). The DHA content was considerably higher in the head portion compared to the viscera. The highest EPA+DHA amount was detected in the ethanol extract of the head portion (26.12%), followed by hexane extracts of the head part (22.18%) Further, (Lu et al., 2018) reported that the DHA and EPA content in the brains of skipjack tuna was 21.1% and 2.4% respectively.

The results indicate that byproducts of skipjack tuna are a rich source of EPA and DHA, which is currently underutilized. Although the result of the fatty acid profile is

compared with previous studies, slight differences were observed in the levels of each fatty acid. This is justifiable because each nutrient's nutritional composition and levels are highly dependent on geographical factors, habitat conditions, sex, and development stages in fish (Ackman, 1982).

Table 2: Fatty acid composition of recovered oil from head and viscera

SN	Fatty Acid	Chemical Formula	Content %					
			Head			Viscera		
			Ethanol	Hexane	Ether	Ethanol	Hexane	Ether
1	Palmitic acid	C16:0	33.57	35.40	36.99	22.97	28.97	23.23
2	Stearic acid	C18:0	12.87	13.93	12.15	13.15	12.87	13.47
3	Methyl behenate	C22:0	0.12	0.05	0.10	0.05	0.06	0.06
4	Oleic acid	C18:1	12.78	13.98	13.35	22.54	22.13	22.62
5	linoleic acid	C18:2	0.17	0.21	0.24	0.14	0.12	0.13
6	linolenic acid	C18:3	0.46	0.49	0.44	0.60	0.56	0.53
7	Eicosadienoic acid	C20:2	1.18	0.63	0.78	0.57	0.53	0.51
8	Eicosatrienoic acid	C20:3	1.00	0.84	0.83	0.23	0.36	0.27
9	Eicosapentaenoic acid	C20:5	4.15	2.79	3.23	2.32	2.12	1.42
10	Docosahexaenoic acid	C22:6	21.96	19.40	18.62	11.33	11.59	10.85
	∑ PUFA		**28.93**	**24.35**	**24.14**	**15.19**	**15.29**	**13.71**
	∑ EPA+DHA		**26.12**	**22.18**	**21.85**	**13.64**	**13.71**	**12.27**

4. Saponification value and average molecular weight of oil

Table 3: Saponification value, and average molecular weight of skipjack tuna head and viscera oil

	Head			Viscera		
	Ethanol	Hexane	Ether	Ethanol	Hexane	Ether
SV (mg KOH)	80.68 ± 0.18^{e}	380.54 ± 0.8^{c}	189.17 ± 0.36^{d}	64.29 ± 0.06^{H}	154.79 ± 0.13^{F}	127.75 ± 0.05^{G}
A.M.W. (g mol^{-1})	2086.00 ± 4.72^{l}	442.27 ± 0.93^{n}	889.69 ± 1.71^{m}	2617.70 ± 2.64^{P}	1087.25 ± 0.93^{R}	1317 ± 0.47^{Q}

The values are demonstrated as Mean ± SD, (n = 3). The different letters in lowercase and uppercase indicate the significant difference ($p \leq 0.05$) of each parameter in the oil recovered from the skipjack tuna head and viscera respectively. SV- Saponification Value, A.M.W – Average Molecular Weight.

The SV itself shows an approximate estimate of the average molecular weight of oil. Higher SVs, such as 255-270 mg KOH in coconut and palm oil, denotes that the oil contains short-chain fatty acids. Lower saponification indexes are acquired for the oils with long-chain fatty acids (Flores et al., 2021). The lowest saponification value was reported for ethanol extracts of viscera, while the highest was obtained in hexane extracts of the head portion and there was a significant difference ($p \leq 0.05$) between the six samples. The average molecular weights were contradictory to the saponification values. Furthermore, average molecular weights of head and viscera oil extracted by three solvents are in descending order with ethanol>ether>hexane.

5. Antioxidant activity

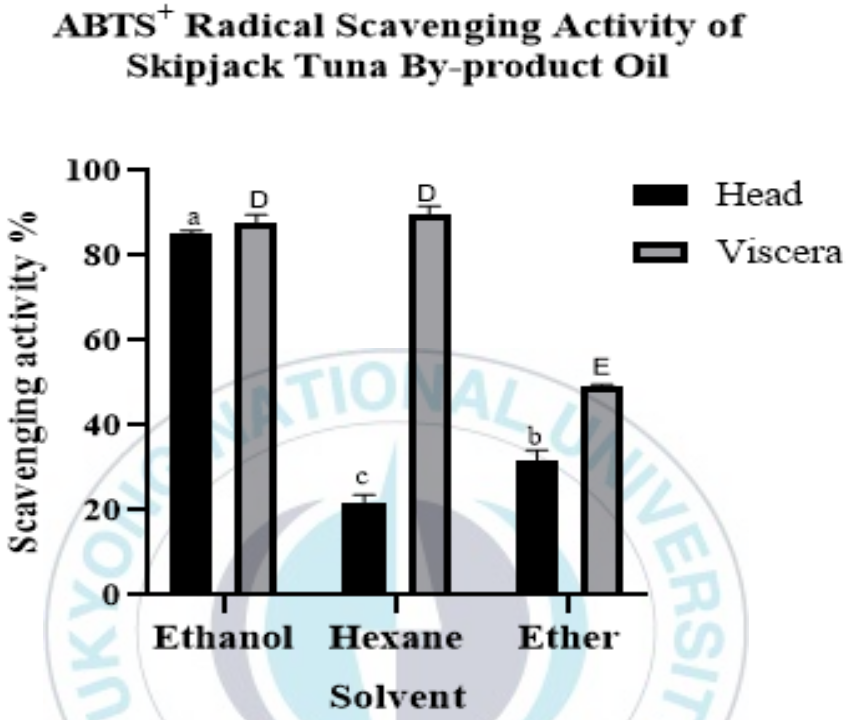

Figure 2. ABTS $^{+}$ radical scavenging activity of recovered oil

The values are demonstrated as Mean ± SD, (n=3). The different letters in lowercase and uppercase indicate the significant difference ($p \leq 0.05$) of scavenging percentages in the oil recovered from Skipjack Tuna head and viscera respectively.

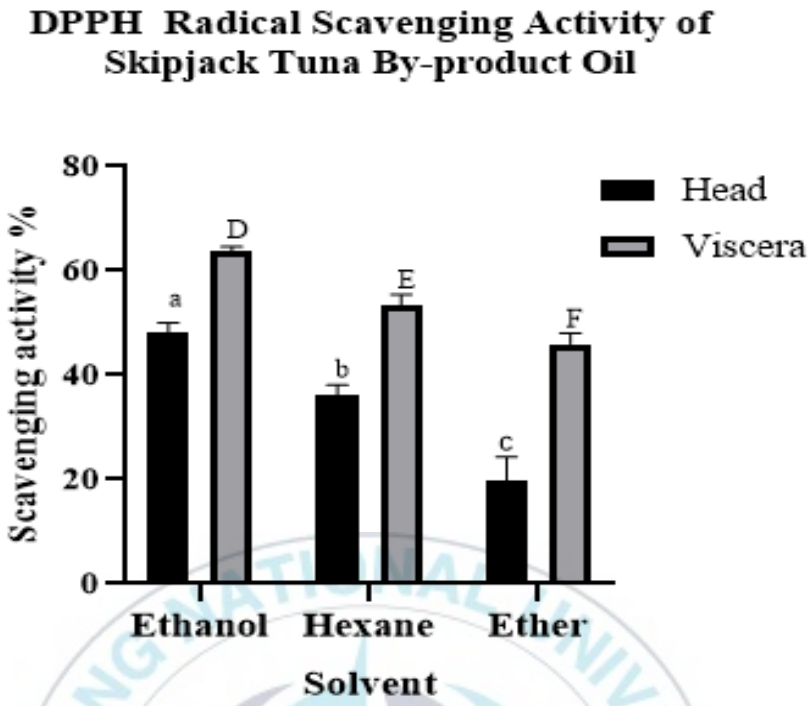

Figure 3. DPPH radical scavenging activity of recovered oil

The values are demonstrated as Mean ± SD, (n = 3). The different letters in lowercase and uppercase indicate the significant difference ($p \leq 0.05$) of scavenging percentages in the oil recovered from skipjack tuna head and viscera respectively.

The possession of antioxidant compounds in different byproducts of skipjack tuna has been recently reported. (Wang et al., 2022), (Qiu et al., 2019), (Zhang et al., 2019), (Chi et al., 2015) have reported the antioxidant capacity of roe, scales, head, and dark muscle of skipjack tuna respectively. Most of those studies experimented on the protein hydrolysates of byproducts. To our best knowledge, this is the first study that assesses the antioxidant capacity of fatty acids recovered from viscera and head portions.

The $ABTS^{+}$ and DPPH radical scavenging activities of head and visceral oil samples are depicted in Fig. 2 and Fig. 3. Compared to the $ABTS^{+}$ and DPPH scavenging percentages of Trolox and Ascorbic acid which were reported as 93.64 ± 1.34%, 91.12 ± 1.23 %, respectively, the oil samples expressed substantially high antioxidant activity in both assays. According to the results, viscera oil was found to be more competent as an antioxidant than the oil recovered from the head portion. This significant difference ($p \leq 0.05$) observed in head and viscera oil may be owing to alterations in the chemical composition of each body part and differences in the quality parameters of the recovered oil (Roy et al., 2022). Ethanol extracts from both segments of the body showed notably high scavenging activity compared to the other two solvents, whereas the ether extracts reported the lowest. These findings confirm that the extractability of antioxidant compounds was higher in ethanol extraction. This might be because of the solvent's effect on the selective recovery of antioxidant compounds out of the biological samples (Zhao et al., 2006).

However, DPPH scavenging activity was comparatively lower than $ABTS^+$ antioxidant activity in this study. Previous research by Floegel et al., (2011) also reported relatively high $ABTS^+$ radical scavenging activity than DPPH scavenging activity by checking the same fruits, vegetables, and beverages samples. These findings are reasonable since a combination of high-pigmented and hydrophilic antioxidant compounds is shown exceptionally by the $ABTS^+$ assay compared to the DPPH assay.

6. Antimicrobial Assay

Previous work by Seo et al., (2014) reported a novel antimicrobial peptide obtained from the skin of skipjack tuna. Our study is the first to report on the antimicrobial properties of FAME obtained from skipjack tuna byproducts. The antimicrobial activity of skipjack tuna FAME (Ethanol extract of head) was evaluated against one of the gram's positive, food-borne pathogenic bacteria, namely *Bacillus cereus* (10^5CFU/ml).

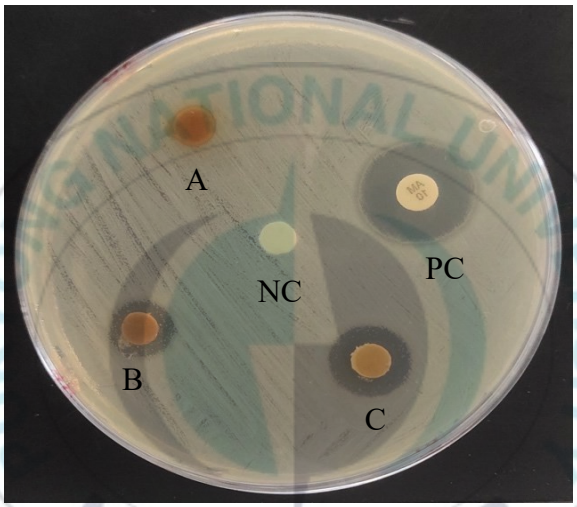

Figure 4. Antimicrobial Properties of FAME [Head (Ethanol)]

NC- Negative control 10% DMSO (10µl), A – 10µl of FAME, B – 20µl of FAME, C – 30µl of FAME, PC – Ampicillin 10µg/disc

Before this experiment, MIC and MBC testing was performed to determine the minimum bactericidal concentration (MBC) of FAME (head, ethanol) dissolved in 10% DMSO. The ethanol-extracted FAME from head oil had an experimental MBC value of 1024 g/ml. The sterile blank discs were impregnated with exactly 10 µl, 20 µl, and 30 µl of FAME (1 MBC), and the development of inhibitory zones was investigated. No clear zone was observed around the disc impregnated with 10% DMSO. Around the disc impregnated with 10µl of FAME, the diameter of the inhibition zone was less than 0.1 mm. By doubling the volume of FAME, the diameter of the clear zone increased up to 9.86 ± 0.12 mm. Further volume expansion led to a zone of inhibition that was 13.96 ± 0.15 mm large. The significant increase in zone measurements revealed a concentration-dependent antibacterial activity of FAME. According to these findings, FAME made from skipjack tuna by-product oil effectively supressed the growth of *B. cereus*.

7. Morphological Analysis of *Bacillus cereus* strains treated with FAME – SEM analysis.

Scanning electron microscopic images (SEM) are strong evidence of the bactericidal action of FAME. Figure 5 is an image of a *B. cereus* smear without treatment. Figure 6 shows an image of *B. cereus* treated using 100µl FAME. In the image of the untreated sample, a uniform layer of curved, slender, rod-shaped cells was observed. Some cells were arranged in a diplobacillus form. Even though they were placed in short chains, the rigid cell wall acted as the separation line between the two cells. The individual cells were barely distinguishable in the treated sample. All the bacterial cells were aggregated and severely distorted. In almost all cells, the structure of the cell wall was completely destroyed due to FAME penetration. The bacterial cell membrane was ruptured, and the cytoplasmic fluid leaked into the intercellular spaces, forming a slurry. Multiple defense mechanisms confer antimicrobial qualities to saturated and unsaturated fatty acids. Disrupting the bacterial membrane, interfering with cell wall biosynthesis, obstacle protein synthesis, and inhibiting DNA/RNA replication are promising mechanisms that whole fatty acids and derivatives to an antimicrobial agent for the next generation (Casillas-Vargas et al., 2021).

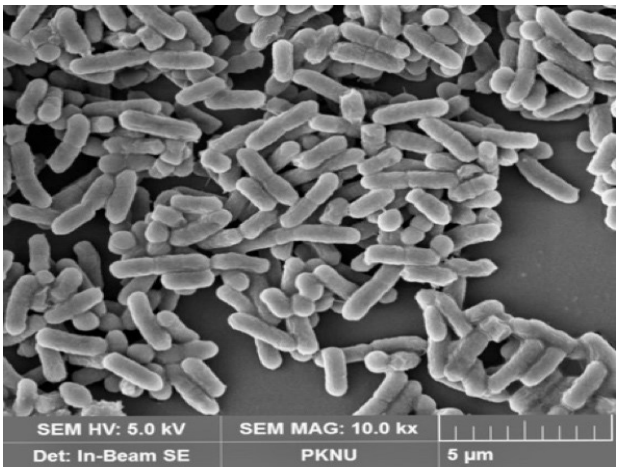

Figure 5. SEM image of the untreated Bacillus cereus smear-control

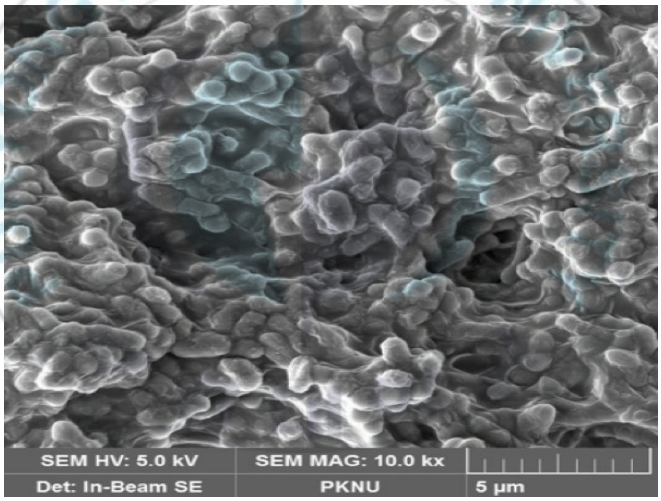

Figure 6. SEM image of the Bacillus cereus smear treated with FAME

8. Preservation capacity of fatty acids and fatty acid methyl esters

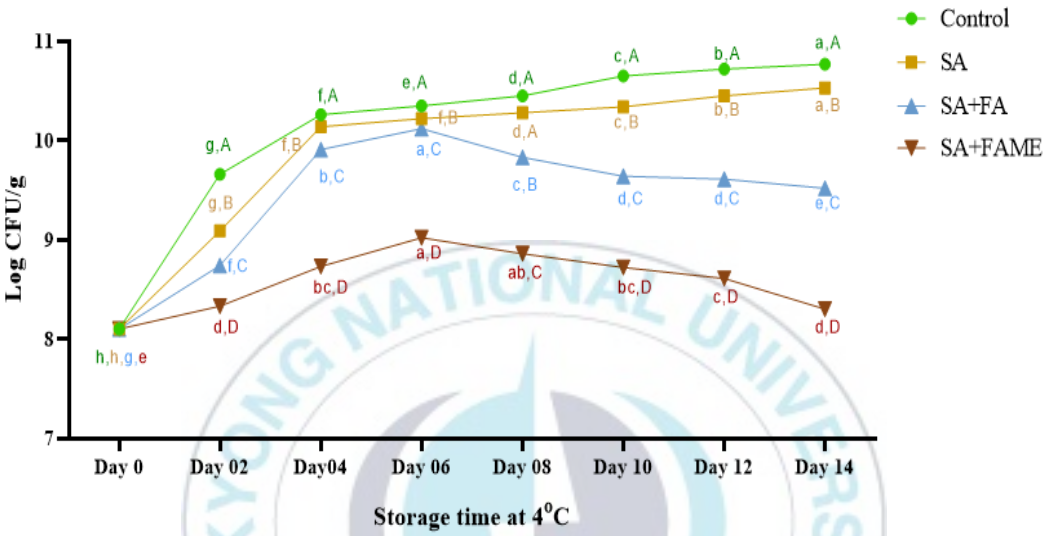

Figure 7: Effect of FAME on the growth of *B. cereus* in pork sausage.

Different large caps indicate the significant difference ($p \leq 0.05$) between 4 treatments in specific storage time and different small caps indicate the significant difference ($p \leq 0.05$) within one treatment over 14 days of storage, Control- wrapped in aluminum foil, SA – only sodium alginate coating, SA+FA – sodium alginate mixed with fatty acids, SA+FAME – sodium alginate mixed with fatty acid methyl esters of skipjack tuna oil.

The bacteriostatic and bactericidal effects of FA and FAME recovered from skipjack tuna byproducts are shown against *B. cereus* over 14 days at cold storage (Fig. 7). All samples were artificially contaminated with bacterial suspension of 8.10 ± 0.01 log CFU/ml (0^{th} day of storage). The control sample wrapped with an aluminum foil showed an increasing trend in colony counts throughout 14 days. The *B. cereus* population in the control sample on day 2 was 9.66 ± 0.02 log CFU/g and increased to 10.77 ± 0.01 log CFU/g on the 14^{th} day. The blank sample coated with sodium alginate also demonstrated an increasing pattern in the colony count. Sodium alginate coatings control the microbial load and increase the shelf life by acting as a packaging material. Therefore, a significant difference was observed between the colony counts of the control and sodium alginate-coated samples. Since the coating did not have any biological activity against the bacterial growth the final colony count was increased to 10.53 ± 0.02 log CFU/g starting from 9.09 ± 0.02 log CFU/g. In the sample coated with SA fortified with the fatty acid, bacterial growth was increased to 10.12 ± 0.03 log CFU/g until day six, starting from 8.74 ± 0.03 log CFU/g. Compared to the control and blank samples the bacterial load was significantly low ($p \leq 0.05$) due to the antimicrobial mechanisms of skipjack tuna byproduct oil. After the 6^{th} day of cold storage, the *B. cereus* population started reducing, showing the bactericidal effect of the oil. The colony count attained 9.52 ± 0.01 log CFU/g on the 14^{th} day, significantly lower than the control and blank samples.

In the case of the FAME fortified sample, 8.33 ± 0.02 log CFU/g was observed on the 2nd day of the cold storage. The number of bacterial colonies increased until day six and reported a maximum colony count of 9.02 ± 0.01 log CFU/g. After that, the bacterial growth was inhibited by antimicrobial mechanisms of FAME against *B. cereus,* causing a declined bacterial load. Compared to the fatty acid the FAME showed a more bacteriostatic effect by significantly inhibiting the growth of *B. cereus*. On the 14th day of the experiment, only 8.30 ± 0.02 CFU/g was obtained, showing the excellent bactericidal capacity of FAME. According to the results, both fatty acids and FAME recovered from skipjack tuna byproducts are effective as antimicrobial agents. Although much research has been conducted on the antimicrobial properties of lipids and fatty acids, very few reports are available on the antimicrobial properties of FAME. From the overall experiment, we can conclude that the FAME is more effective against *B. cereus.* The findings of this study agree with the results of Surendhiran et al., (2014), who stated that the modification of fatty acid into its FAME enhanced the antimicrobial activity.

9. Sensory Evaluation

As shown in table 4, the average scores obtained during three sessions of sensory property evaluation are summarized.

Table 4: Average score of sensorial properties

Sample Type	Color	Appearance	Softness	Overall acceptability
Control	5.83 ± 0.12^{d}	5.40 ± 0.17^{h}	5.63 ± 0.12^{p}	5.37 ± 0.29^{t}
Sodium Alginate coating	6.37 ± 0.06^{c}	5.77 ± 0.03^{g}	6.07 ± 0.12^{o}	5.87 ± 0.06^{s}
Sodium Alginate + Fatty Acid	6.77 ± 0.06^{b}	6.33 ± 0.12^{f}	7.07 ± 0.06^{n}	6.37 ± 0.06^{r}
Sodium Alginate + FAME	7.4 ± 0.17^{a}	7.3 ± 0.17^{e}	7.27 ± 0.06^{m}	6.97 ± 0.06^{q}

The different letters indicate the significant difference ($p \leq 0.05$) among each column.

Compared with the aluminum foil-wrapped control sample and sodium alginate-coated blank samples the panelists rated fatty acid and FAME fortified samples significantly higher ($p \leq 0.05$) based on color, appearance, softness, and overall acceptability in all three sessions of the experiment. According to the average scores perceived for the fortified samples, the ratings were significantly higher for FAME-fortified samples, concluding the higher acceptance level. However, some negative comments were regarding the unpleasant fishy smell in the fortified samples. In industrial-scale applications of omega 3-rich oil recovered from skipjack tuna byproducts, deodorization and purification steps are essential to increase consumer satisfaction.

10. Conclusion

The highest yield was reported from ethanol extraction in the head. Skipjack tuna's head contained a higher amount of oil than the viscera. The maximum DHA content, which was 21.96% was reported in the ethanol extract of the head part. The maximum EPA content, which was 4.15% also reported in the same oil sample. The head portion constituted high amounts of PUFA within a range of 24.14% -28.93 % compared to the viscera part, which reported 13.71-15.19%. The hexane extract of viscera reported the highest $ABTS^{+}$ scavenging activity (89.78 ± 0.20%), whereas the ethanol extract of viscera indicated the highest DPPH scavenging activity (63.68 ± 0.94%). SEM images evidenced FAME's effect on bacterial cell damage. The results of the disc diffusion method showed enhanced growth inhibition capability in FAME. Comparing control and SA-coated samples, bacterial growth was inhibited starting from the 6^{th} day of storage in fortified samples. The decrease in colony counts and overall acceptance in sensorial property evaluation was significantly higher in FAME-fortified sausage (0.72 ± 0.02 log CFU/g), (6.97 ± 0.06) than the FA-fortified sausage (0.60 ± 0.03 log CFU/g), (6.37 ± 0.06). That concludes the FAME's enhanced antimicrobial qualities and its possible application in functional foods.

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Firstly, I owe special gratitude to my supervisor Professor Byung – Soo Chun. Professor Chun has accepted me as his student, and his guidance and support throughout my study were admirable. He provided me with professional and scientific advice starting from the first day of joining the laboratory. I would like to recall memories of the late Dr. Duraiarasan Surendhiran, who guided me to succeed in this research study. May his soul rest in peace. My sincere thanks go to Dr. Truc Cong Ho for his advice and experiences that shaped my research capability. I would like to express my gratitude to all my fellow lab mates for their valuable company and support during this challenging period.

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References

Ackman, R. G. (1982). Fatty acid composition of fish oils. In S. Barlow & M. Stansby (Eds.), *Nutritional Evaluation of Long Chain Fatty Acids in Fish Oil: Proceedings* (p. 25). Academic Press.

American Oil Chemists Society. (2017). *AOCS Official Method Cd 3-25: Saponification value of fats and oils, official methods and recommended practices of the AOCS. American Oil Chemists Society Press, Champaign*. AOCS press.

Arvanitoyannis, I. S., & Kassaveti, A. (2008). Fish industry waste: treatments, environmental impacts, current and potential uses. *International Journal of Food Science & Technology*, *43*(4), 726–745. https://doi.org/https://doi.org/10.1111/j.1365-2621.2006.01513.x

Balogun, A. M., & Talabi, S. O. (1985). Proximate analysis of the flesh and anatomical weight composition of skipjack tuna (Katsuwonus pelamis). *Food Chemistry*, *17*(2), 117–123. https://doi.org/https://doi.org/10.1016/0308-8146(85)90080-9

Barret, R. (2018). 3 - Importance and Evaluation of Lipophilicity. In R. Barret (Ed.), *Therapeutical Chemistry* (pp. 53–78). Elsevier. https://doi.org/https://doi.org/10.1016/B978-1-78548-288-5.50003-2

Casillas-Vargas, G., Ocasio-Malavé, C., Medina, S., Morales-Guzmán, C., del Valle, R. G., Carballeira, N. M., & Sanabria-Ríos, D. J. (2021). Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Progress in Lipid Research*, *82*, 101093. https://doi.org/10.1016/j.plipres.2021.101093

Chandrasekaran, M., Kannathasan, K., & Venkatesalu, V. (2008). Antimicrobial Activity of Fatty Acid Methyl Esters of Some Members of Chenopodiaceae. *Zeitschrift Für Naturforschung C*, *63*(5–6), 331–336. https://doi.org/10.1515/znc-2008-5-604

Chi, C.-F., Hu, F.-Y., Wang, B., Li, Z.-R., & Luo, H.-Y. (2015). Influence of Amino Acid Compositions and Peptide Profiles on Antioxidant Capacities of Two Protein Hydrolysates from Skipjack Tuna (Katsuwonus pelamis) Dark Muscle. *Marine Drugs*, *13*(5), 2580–2601. https://doi.org/10.3390/md13052580

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in

popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, *24*(7), 1043–1048. https://doi.org/10.1016/j.jfca.2011.01.008

Flores, M., Avendaño, V., Bravo, J., Valdés, C., Forero-Doria, O., Quitral, V., Vilcanqui, Y., & Ortiz-Viedma, J. (2021). Edible Oil Parameters during Deterioration Processes. *International Journal of Food Science*, *2021*, 1–16. https://doi.org/10.1155/2021/7105170

Food & Agriculture Organization of the United States. (2022). *The State of World Fisheries and Aquaculture 2022*. FAO. https://doi.org/10.4060/cc0461en

Food and Agricultural Organization of the United Nations. (2002). *FAO Fisheries Statistical Yearbook 2002*. FAO.

Franklin, E. C., Haq, M., Roy, V. C., Park, J., & Chun, B. (2020). Supercritical CO_2 extraction and quality comparison of lipids from Yellowtail fish (*Seriola quinqueradiata*) waste in different conditions. *Journal of Food Processing and Preservation*, *44*(11). https://doi.org/10.1111/jfpp.14892

Haq, M., Ahmed, R., Cho, Y.-J., & Chun, B.-S. (2017). Quality Properties and Bio-potentiality of Edible Oils from Atlantic Salmon By-products Extracted by Supercritial Carbon Dioxide and Conventional Methods. *Waste and Biomass Valorization*, *8*(6), 1953–1967. https://doi.org/10.1007/s12649-016-9710-2

Ivanovs, K., & Blumberga, D. (2017). Extraction of fish oil using green extraction methods: a short review. *Energy Procedia*, *128*, 477–483. https://doi.org/https://doi.org/10.1016/j.egypro.2017.09.033

Kawarazuka, N., & Béné, C. (2011). The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence. *Public Health Nutrition*, *14*(11), 1927–1938. https://doi.org/DOI: 10.1017/S1368980011000814

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2002). Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Circulation*, *106*(21), 2747–2757. https://doi.org/10.1161/01.CIR.0000038493.65177.94

López-Pedrouso, M., Lorenzo, J. M., Cantalapiedra, J., Zapata, C., Franco, J. M., & Franco, D. (2020). Chapter Five - Aquaculture and by-products: Challenges and opportunities in the use of alternative protein sources and bioactive compounds. In J. M. Lorenzo & F. J. Barba (Eds.), *Advances in Food and Nutrition Research* (Vol. 92, pp. 127–185). Academic Press. https://doi.org/https://doi.org/10.1016/bs.afnr.2019.11.001

Lu, H., Zhang, L., Zhao, H., Li, J., You, H., Jiang, L., & Hu, J. (2018). Activation of Macrophages <i>in vitro</i> by Phospholipids from Brain of

<i>Katsuwonus pelamis</i> (Skipjack Tuna). *Journal of Oleo Science*, *67*(3), 327–333. https://doi.org/10.5650/jos.ess17181

Mahaliyana, A. S., Jinadasa, B. K. K. K., Liyanage, N. P. P., Jayasinghe, G. D. T. M., & Jayamanne, S. C. (2015). Nutritional Composition of Skipjack Tuna *(Katsuwonus pelamis)* Caught from the Oceanic Waters around Sri Lankae. *American Journal of Food and Nutrition*, *3*(4), 106–111. https://doi.org/10.12691/ajfn-3-4-3

Mitra, M., & Mishra, S. (2019). A comparative analysis of different extraction solvent systems on the extractability of eicosapentaenoic acid from the marine eustigmatophyte Nannochloropsis oceanica. *Algal Research*, *38*, 101387. https://doi.org/10.1016/j.algal.2018.101387

Natadiputri, G. H., Suwanto, A., & Kim, H. K. (2015). One-step transesterification reaction using methanol-stable lipase for omega-3 fatty acid ethyl ester production. *Journal of the Korean Society for Applied Biological Chemistry*, *58*(2), 195–202. https://doi.org/10.1007/s13765-015-0032-z

Park, J., Kim, S., Lee, S., Jeong, Y., Roy, V. C., Rizkyana, A. D., & Chun, B. (2021). Edible oil extracted from anchovies using supercritical CO_2 : Availability of fat-soluble vitamins and comparison with commercial oils. *Journal of Food Processing and Preservation*, *45*(5). https://doi.org/10.1111/jfpp.15441

Poppe, J. K., Garcia-Galan, C., Matte, C. R., Fernandez-Lafuente, R., Rodrigues, R. C., & Ayub, M. A. Z. (2013). Optimization of synthesis of fatty acid methyl esters catalyzed by lipase B from Candida antarctica immobilized on hydrophobic supports. *Journal of Molecular Catalysis B: Enzymatic*, *94*, 51–56. https://doi.org/10.1016/j.molcatb.2013.05.010

Qiu, Wang, Yang, Zhao, Chi, & Wang. (2019). Gelatin and Antioxidant Peptides from Gelatin Hydrolysate of Skipjack Tuna (Katsuwonus pelamis) Scales: Preparation, Identification and Activity Evaluation. *Marine Drugs*, *17*(10), 565. https://doi.org/10.3390/md17100565

Rizliya, V., & Mendis, E. (2014). Biological, Physical, and Chemical Properties of Fish Oil and Industrial Applications. In S.-K. Kim (Ed.), *Seafood Processing By-Products: Trends and Applications* (pp. 285–313). Springer New York. https://doi.org/10.1007/978-1-4614-9590-1_14

Robertson, A. R. (1977). The CIE 1976 Color-Difference Formulae. *Color Research & Application*, *2*(1), 7–11. https://doi.org/10.1002/j.1520-6378.1977.tb00104.x

Roy, V. C., Park, J.-S., Ho, T. C., & Chun, B.-S. (2022). Lipid Indexes and Quality Evaluation of Omega-3 Rich Oil from the Waste of Japanese Spanish

Mackerel Extracted by Supercritical CO2. *Marine Drugs*, *20*(1), 70. https://doi.org/10.3390/md20010070

Seo, J.-K., Lee, M. J., Go, H.-J., Kim, Y. J., & Park, N. G. (2014). Antimicrobial function of the GAPDH-related antimicrobial peptide in the skin of skipjack tuna, Katsuwonus pelamis. *Fish & Shellfish Immunology*, *36*(2), 571–581. https://doi.org/10.1016/j.fsi.2014.01.003

Surendhiran, D., Vijay, M., Razack, A., Subramaniyan, T., Shellomith, A. S., & Tamilselvam, K. (2014). A green synthesis of antimicrobial compounds from marine microalgae Nannochloropsis oculata. *Journal of Coastal Life Medicine*, *2*, 859–863. https://doi.org/10.12980/JCLM.2.2014APJTB-2014-0138

Tyagi, A. K., & Malik, A. (2012). Morphostructural Damage in Food-Spoiling Bacteria due to the Lemon Grass Oil and Its Vapour: SEM, TEM, and AFM Investigations. *Evidence-Based Complementary and Alternative Medicine*, *2012*, 1–12. https://doi.org/10.1155/2012/692625

Venugopal, V. (2021). Valorization of Seafood Processing Discards: Bioconversion and Bio-Refinery Approaches. *Frontiers in Sustainable Food Systems*, *5*. https://doi.org/10.3389/fsufs.2021.611835

Wang, J., Wang, Y.-M., Li, L.-Y., Chi, C.-F., & Wang, B. (2022). Twelve Antioxidant Peptides From Protein Hydrolysate of Skipjack Tuna

(Katsuwonus pelamis) Roe Prepared by Flavourzyme: Purification, Sequence Identification, and Activity Evaluation. *Frontiers in Nutrition*, *8*. https://doi.org/10.3389/fnut.2021.813780

Wu, T. H., Nigg, J. D., Stine, J. J., & Bechtel, P. J. (2011). Nutritional and Chemical Composition of By-Product Fractions Produced from Wet Reduction of Individual Red Salmon (Oncorhynchus nerka) Heads and Viscera. *Journal of Aquatic Food Product Technology*, *20*(2), 183–195. https://doi.org/10.1080/10498850.2011.557524

Yang, K. S., Sohn, J.-H., & Kim, H. K. (2009). Catalytic properties of a lipase from Photobacterium lipolyticum for biodiesel production containing a high methanol concentration. *Journal of Bioscience and Bioengineering*, *107*(6), 599–604. https://doi.org/https://doi.org/10.1016/j.jbiosc.2009.01.009

Zhang, L., Zhao, G.-X., Zhao, Y.-Q., Qiu, Y.-T., Chi, C.-F., & Wang, B. (2019). Identification and Active Evaluation of Antioxidant Peptides from Protein Hydrolysates of Skipjack Tuna (Katsuwonus pelamis) Head. *Antioxidants*, *8*(8), 318. https://doi.org/10.3390/antiox8080318

Zhao, H., Dong, J., Lu, J., Chen, J., Li, Y., Shan, L., Lin, Y., Fan, W., & Gu, G. (2006). Effects of Extraction Solvent Mixtures on Antioxidant Activity Evaluation and Their Extraction Capacity and Selectivity for Free Phenolic

Compounds in Barley (*Hordeum vulgare* L.). *Journal of Agricultural and Food Chemistry*, *54*(19), 7277–7286. https://doi.org/10.1021/jf061087w