

Ensure that "*Oreochromis Niloticus*" is Formatted Correctly with a Space Between the Genus and Species

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Abstract

In the fisheries industry, the consumption of residue and fish waste allows effective uses of the potential fish wastes as food additives, such as omega-3 fatty acids. Oil extraction from tilapia wastes and by-products were carried out by supercritical carbon dioxide (SC-CO₂) at 45 °C, 250 bar, for 3 h and n-hexane at 45 °C, 250 rpm, for 16 h. Hydrolysis of de-oiled tilapia residues was done by subcritical water at 160 °C and 190 °C. The overall oil yield obtained through SC-CO₂ was 34.3% ± 1.12% and through n-hexane was 63.5% ± 0.69%. Tilapia by-products were observed to have a higher proportion of total saturated fatty acids (SFAs) than monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The acid content of SC-CO₂ for the skin, head and viscera was 5.46 ± 0.25, 3.35 ± 0.28, and 7.19 ± 1.06 mg KOH/g, respectively, and the peroxide values (PVs) were 1.77 ± 0.43, 1.34 ± 0.46, and 4.48 ± 0.17 meq/kg, respectively. The free fatty acid (FFA) obtained via SC-CO₂ yields 3.62% ± 0.10% (skin), 1.79% ± 0.09% (head), and 4.29% ± 0.41% (viscera), and that via n-hexane yields 3.19% ± 0.35% (skin), 1.69% ± 0.02% (head), and 4.44% ± 0.55% (viscera) within a reasonable value range 1%–7%. Hydrolysate values ranged from 2.04% ± 0.01% to 7.44% ± 0.01% of samples. The maximum and minimum values of protein were 40.42 ± 0.79 and 31.28 ± 0.92 mg BSA/g and that of total sugar were 30.06 ± 4.07 and 28.51 ± 0.83 mg Glu/g, respectively. SC-CO₂ and n-hexane are the favored techniques in the valorization of tilapia waste containing substantial bioactive compounds that are essential for human and animal health.

Keywords: Biomass Valorization, Pressurized Liquid, Tilapia Waste, Polyunsaturated Fatty Acids, Hydrolysis

1. Introduction

Among the most important cultured aquatic animal, tilapia take the greatest part. Tilapia originated from Africa, the Middle East, Central and South America, and Southern India, and nowadays, they are produced as more prolific and fast-growing tropical fish species in the world [1]. Different types of tilapia species are farmed, of which Nile tilapia (*Oreochromis niloticus*) production represents the largest percentage in total annual production [2]. The use of by-products produced during processing as production of food ingredients and extraction of bioactive compound in different technologies has a great role in biotechnological and pharmaceutical applications [3]. Marine capture fisheries generate more than half of the total world fish production, and 70% of processed fish is brought for sale [4]. If there is no further valorization process, the fish demand increases, as well as by-products that cause environmental pollution as fishes are gutted and 50%–60% of these fishes contain offal

that are discarded to the environment. Most of the time, this offal are useless or are sometimes used in animal meal and fertilizer [5]. The generated Nile tilapia processing residues are usually in the form of heads, viscera, skin, and bones; this represents a large volume of the fillet, indicating high amount of essential protein that had nutritional value for both human and animal [6,7]. Fishing industries make better uses of the potential of fish wastes as food ingredients [8]. Omega-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, can be obtained from the different body parts of fish. Eicosapentaenoic acid and docosahexaenoic acid compounds have great role in human health; both compounds help in the prevention of atherosclerosis, reduction of blood pressure, and protection against arrhythmias [9,10]. Polyunsaturated fatty acids (PUFAs) omega-3 (n-3) and omega-6 (n-6) are essential nutrients with a multiplicity of similar and often complementary physiological roles mediated by their functions as structural components of membranes, substrates

for eicosanoids, and modulators of gene transcription, to name a few [11]. Fish by-product composed nearly of 6% skin, 7% viscera, 10% offcuts, 18% head, and 10% backbone frames [12]. Nowadays, people are more health-conscious and demand for processed foods without loss of the nutritive value and organoleptic properties. Processing food products while preserving the color, flavor, texture, and nutritive and functional qualities at acceptable levels are those minimally required, including easily handled, good quality and safe [13]. Green extraction technology, like supercritical carbon dioxide CO₂ (SC-CO₂) extraction technique, which represents a new, mild, and selective food processing and is environmentally friendly, is becoming more acceptable. At present, with the use of this technology, high-quality fish oil and potentially useful bioactive compounds from food by-products have been acquired [14,15].

In addition, this supercritical fluid extraction technology, which commonly uses CO₂ as a solvent, also has many advantages, such as low critical pressure and temperature. Furthermore, the final extracts are typically free of contaminants and impurities, and the extracts can easily eliminate the CO₂ used [16]. On the other hand, subcritical water hydrolysis is a flexible technology with several advantages in the processing of useful biomass materials. It uses effective clean solvent for the extraction of bioactive compounds and by-products from the food industry [17]. By acting as a catalyst or reactant, CO₂ is also useful for enhancing chemical reactions such as hydrolysis and conversion reactions of organic compounds. Subcritical water hydrolysis uses water near its critical state, which is below the water's boiling point and at a sufficiently high pressure to retain water in the liquid state [18]. This study aims to valorize tilapia by-product from processing industry using green technology. In this work, fish oil from tilapia head, viscera, and skin has been extracted using SC-CO₂ and conventional solvents. The oil obtained from both techniques was characterized (oil stability, oil quality, fatty acid composition, and antioxidant activity). In the SC-CO₂ extraction technique using subcritical water, the de-oiled residue of tilapia waste resulted in hydrolysate; the protein

content, total sugar content, Maillard reaction, pH, color, and antioxidant activity of the hydrolysate were evaluated. In this study, it is assumed that consecutive utilization of these combined methods will help in obtaining PUFA-enriched fish oil and protein-enriched hydrolysate. This study also targets to achieve a zero-waste production from fishery by-products, which is very important for future food security and furthermore getting food with rich nutritional value.

2. Materials and Methods

2.1 Sample Preparation

About 10 kg of Nile tilapia was bought from the Philippines Company. The average total body weight of the sample was 0.38 kg/piece. The inedible portion (head, skin, and viscera) of Nile tilapia was obtained by manually separating it from the other portion. Samples were then washed and freeze-dried for 72 h at -80 °C using Hyper COOL Freeze Dryer (HC8080, Gyrozen Co., Ltd., Daejeon, Republic of Korea). After drying, samples were crushed using electric blender (PN SMKA-4000 mixer, PN Co., Ltd., Republic of Korea) and finally kept at a refrigerator at -20 °C for oil extraction.

2.2 Experimental Chemical and Reagents

The CO₂ gas (99 % purity) was obtained from KOSEM (Yongsan, Korea). HPLC- grade ethanol, water, acetic acid (99.5 %) glacial, phenolphthalein indicator and other chemicals used in this experiment were purchased from Sigma-Aldrich, Mo., USA, and were of either analytical or HPLC grade.

2.3 Oil Extraction Methods

2.3.1 Hexane Extraction

n-hexane oil extraction was carried out by placing 40 g of freeze-dried powdered viscera, skin, and head into different beaker with 300 mL of n-hexane in each sample followed by stirring at 250 rpm for 16 h at 45 °C. To prevent solvent evaporation, aluminum foil paper was used as lid. Then, the extracted oil was filtered to separate the residue. Finally, it was evaporated at 40 °C to separate the solvent from the oil. The extracted oil was stored at -20 °C for further investigation. The total yield was calculated as follows.

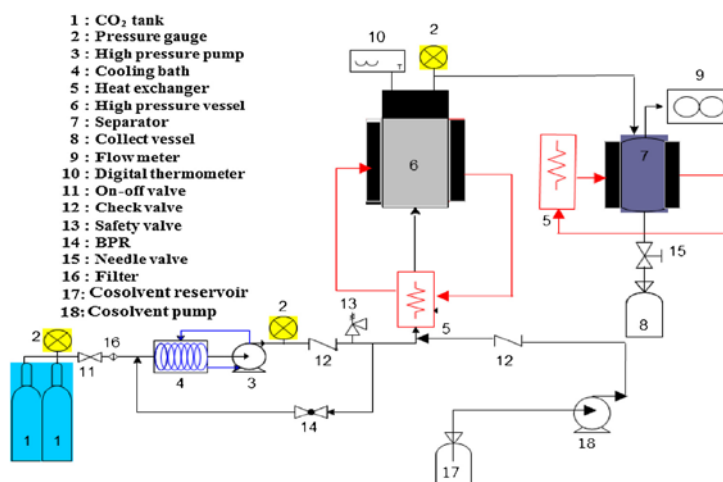
$$\text{Yield (\%)} = \frac{\text{Oil extracted (g)}}{\text{Weight of sample (g)}} \times 100 \quad (1)$$

2.4 Supercritical Carbon Dioxide Extraction

SC-CO₂ extraction mechanism (Fig.1) was used for extraction of oil from the head, skin and viscera of tilapia. Different sample sizes were used in this method. In 200 mL stainless steel extraction vessel first of all the light piece of cotton was put down at the lower, then 50 g head, skin or viscera of freeze-dried ground sample were filled and the light piece of cotton was put at the upper side before closing the vessel to protect the entering of the sample to the gas line. A high-pressure pump (USA) and back pressure regulator (BPR) are used to pump liquid CO₂ into the extraction vessel for the

required pressure and managed CO₂ pressure respectively. To achieve the extraction temperature in the extraction vessel and the separator, preheated water baths connected to the system were used as heat exchangers, and the consumed CO₂ was measured by using gas flow meter (Shinagawa, DC-1, Tokyo, Japan). The extraction temperature and pressure were set at 45 °C and 25MPa, and the CO₂ flow rate was maintained at 27 g/min for the 3 h duration of the extraction. The recovered oil was stored at -20 °C until further analysis. The total yield was calculated as follows.

$$\text{Yield (\%)} = \frac{\text{Oil extracted (g)}}{\text{Weight of sample (g)}} \times 100 \quad (2)$$



1: . Schematic Diagram Oof The Sc-Co2 Extraction System Used Ffor Extraction Tilapia By-Product

2.5 Quality Evaluation

2.5.1 Color

Color measurement of extracted oil or hydrolyzate was carried out using a Lovibond RT Series, Portable Reflectance spectrophotometers, Solstice Park, Amesbury, UK. The instrument was standardized as black and white. The result was showed through L^* , a^* and b^* . Where indicating L^* lightness, a^* redness/greenness and b^* yellowness/blueness.

2.5.2 Viscosity

The viscosity of extracted oil was evaluated by apparatus (BOOKFIELD DV-II+Viscometer model). With small sample adapter, spindle 40 at, 60 RPM 3 mL of sample used in all analysis. The temperature was managed using water bath at 25 °C with a precision of ± 2 °C.

2.6 Oil Stability Evaluation

The acid value (AV), peroxide value (PV), and free fatty acid (FFA) value were analyzed following the previous methods from our laboratory [15].

2.7 Fatty Acid Composition Analysis

The fatty acid composition was determined by using an Agilent 6890 Gas chromatography (Agilent Technologies,

Wilmington, USA) equipped with a silica capillary column (100 m length, 90.25 mm diameter, and 0.2 μ m film) (Supelco, Bellefonte, USA). The AOCS Ce 2-62 official method for the preparation of fatty acid methyl esters was employed for the experiment. Briefly, the oven temperature was set at 240 °C, and programmed to start at 130 °C with an increment of 4 °C/min until reaching the oven temperature setting. Both injector and detector temperatures were set at 250 °C. The identification of fatty acid methyl esters was achieved by comparing the retention time with the system's internal standard.

2.8 Radical Scavenging Activity

2.8.1 DPPH+ Radical Scavenging Activity

The antioxidant activity of tilapia by-products recovered materials by DPPH assay was conducted according to the method by with slight adjustment. Briefly, 50 μ L of extracted sample were dissolved in DPPH+ ethanol solution, and the mixture was vortexed thoroughly, and kept in dark for 30 min. After the reaction, the absorbance of the mixture was read at 517 nm against the blank [15]. A standard control made of 300 μ g/mL of Trolox in ethanol was performed for comparison. The percentage radical scavenging activities for the samples were calculated following the equation below.

$$\% \text{ DPPH radical scavenging activity} = [1 - (\frac{A_s - A_b}{A_c})] \times 100 \quad (3)$$

Where, A_s denote the absorbance of the sample mixed with DPPH solution, A_c is the absorbance of the control containing DPPH solution only, and A_b is absorbance of sample mixed with ethanol. All samples were read at 517 nm.

2.9 ABTS+ Radical Scavenging Activity

The antioxidant activity of recovered materials by 2, 2-azino-di (3-ethylbenzthianzoline-6-sulfonate (ABTS+) assay were performed according to [15]. Both extracted oils and hydrolysates were evaluated for their free radical scavenging ability. Briefly, ABTS was dissolved in distilled water to make 7 mM ABTS solution. After, 2.45 mM potassium persulfate

and ABTS solution were mixed in equal volumes, and the solution was allowed to stand in dark for 16 h to prepare the ABTS+ radicals. The solution containing ABTS+ radicals were constantly diluted with ethanol until the absorbance was adjusted at 0.70 ± 0.02 at 734 nm. Finally, the absorbance of all samples (oil and hydrolysates) was evaluated by mixing 3.9 mL of ABTS+ radical solution with 50 μ L of the recovered materials, and the solution was held in dark at room temperature for 6 min followed by reading the absorbance at 734 nm. A standard control analysis made out of 300 μ g/mL Trolox in ethanol was performed for comparison.

$$\text{ABTS}^+ \text{ radical scavenging activity (\%)} = \left[1 - \left(\frac{A_s - A_b}{A_c} \right) \right] \times 100 \quad (4)$$

Where, A_s is the absorbance of the sample, A_c is the absorbance of the control containing only ABTS solution, and A_b indicates the absorbance of the sample mixed with ethanol at 734nm.

2.10 Subcritical Water Hydrolysis

The tilapia de-oiled residue hydrolysis was performed in the subcritical water experimental apparatus used methods [18]. Briefly, 10 g of de-oiled sample was loaded in to 800 mL volume reactor and filled with 600 mL distilled water. After placed the reactor properly the targeted temperature was seated and the heater was on for pre heated until reach the targeted temperature. The extraction condition kept constant for each sample was a time (30 min), pressure (60 bars) and solid-liquid ratio (10 g/600 mL). The temperature of hydrolysis was (160 °C) and (190 °C). Finally, the hydrolysis sample was taken out of the reactor after cooling it. Then the centrifuge was done at 40 °C temperature, 8000 RPM and 15 min. The F1093 grade filter paper was used to filter the sample and kept at 4 °C until the use of analysis. The hydrolysis yield was determined by the lyophilized extracted sample.

2.11 Protein Content

The total protein content of the hydrolyzed de-oiled sample was performed according to the method (Pomory,

2008). Briefly, a fresh solution was prepared by mixing 1% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 2% tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6$) and 2 % Na_2CO_3 in 1:1:100 ratio. After a fresh solution prepared 0.5 mL of the sample was mixed with 5 mL solution and vortex for vigorously then kept for 10 min. Then 0.5 mL of 1N Folin-Ciocalteu reagent was added to a mixed sample and fresh solution. Finally, vortex and allow the mixture to stand for 2 h before being measured at 660 nm. Bovine serum albumin (Sigma-Aldrich) was used as a standard compound and the total content was expressed as mg BSA per gram dried sample (mg BSA/g).

2.12 Total Sugar

The total sugar of hydrolysate de-oiled sample was determined according to reported previously [19]. The absorbance of the sample measured at 490 nm and the sugar mixed (glucose:fructose:galactose, 1:1:1) used as standard compounds and the total sugar content is expressed as milligram glucose equivalent per gram dried (mg glucose/g).

2.13 Maillard Reaction Product Measurement

Maillard reaction of hydrolysates was evaluated following the method [20,21]. Hydrolysate was diluted 5 times using distilled water and absorbance was measured at 294 nm and 420 nm.

$$\text{Formation of UV – absorbing compound} = \frac{\text{Abs } 294\text{nm}}{\text{Abs } 420\text{nm}} \quad (5)$$

2.14 Statistical Analysis

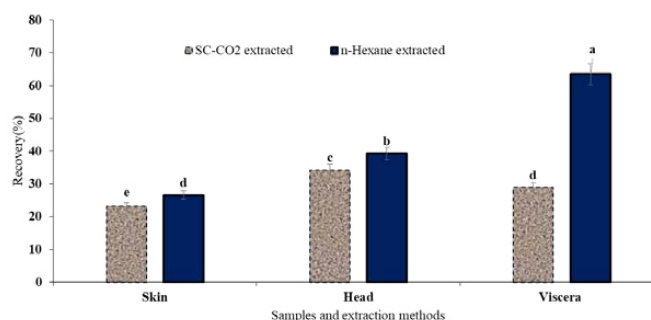
The results are presented using SPSS software (version 25 for windows, Chicago, IL, USA) with calculation of mean $\pm$ standard deviation in triplicate using one-way analysis of variance.

3. Results

3.1 Percentage Yield of The Extracted Oil

For n-hexane extraction, percentage yield of oil was $63.5\% \pm 0.69\%$, $39.3\% \pm 0.64$ and $26.6\% \pm 0.41$ from viscera, head and skin of tilapia, respectively (Fig.2). Comparable percentage yield of oil was obtained from tilapia viscera in previous study using Soxhlet technique [22]. For SC-CO₂ extraction, percentage yield of oil was $34.3\% \pm 1.12\%$, ----- and 23.1%

$\pm 0.24\%$ from viscera, head and skin, respectively (Fig.2) [23]. Obtained comparable percentage yield of oil from squid viscera with the amount extracted from head in SC-CO₂ extraction of the present study. Statistically significant difference was observed in the percentage yield of oil from different parts of tilapia, and between n-hexane and SC-CO₂ extraction methods. The variation in the yield of parts extracted by n-hexane and SC-CO₂ may be due to variations in the chemical composition or solubility of the components in different solvents. While some parts of tilapia by-products may contain active components that are extracted more with n-hexane, others may contain active components that are extracted more with SC-CO₂.



2: . Yield Oof Oil Ffrom Different Parts Oof Tilapia.

3.2 Color of Tilapia By-Product Oil

Color is a significant sensory property of the food substance, pigment content, consistency, oxidation compound, and manufacturing process [23,24]. In the present study, oils extracted from skin and viscera through n-hexane extraction had larger L^* values which were 40.08 ± 0.35 and 39.23 ± 0.24 , respectively. Oils extracted by n-hexane have greater lightness, leading to the formation of dark compounds due to the lower oxidation process that occurs during extraction.. The total color differences of the organic solvent extracted oils showed a lower value relative to the extracted oils of SC-CO₂, which could be attributed to the lightness value of the samples [17]. The b^* values of oil extracted from tilapia skin by-product via n-hexane and viscera by SC-CO₂ were observed to be lower ($b^* = 4.17 \pm 0.11$ and 7.22 ± 0.31 , respectively). Impurity, pigment content, oxidation compound, and processing conditions contributed to the color of tilapia by-product oil. Consumers favor oils having lower b^* values having bright and yellow characteristics as such oils have high content of purity [23].

3.2.1 Viscosity

Tilapia oil extracted via SC-CO₂ from the skin, head, and viscera showed lower viscosity than all oil extracted via n-hexane, where there was a significant difference in values (centipoises, cP) between the extraction process and extracts ($p < 0.05$) (Table 1). The lowest value of viscosity found in the result reflects the high quality and purity. The highest value of viscosity, which was reported in n-hexane extraction, is due to the capacity of hexane to extract all groups of poorly selective nonpolar and amphiphilic lipids such as unsaponifiable gum compounds, e.g., phospholipids [23]. Oil extracted via SC-CO₂ from the skin was observed to have the lowest cP value (34.43 ± 0.05), and the highest was from the viscera (56.7 ± 0.45) obtained via n-hexane (Table 1). This suggests that more purification is needed for higher viscosity values, and adsorbent treatment can be used to minimize the viscosity, which will result in increased costs [25].

	SC-CO ₂ extraction oil			n-hexane extracted oil		
	Skin	Head	Viscera	Skin	Head	Viscera
Color						
L^*	35.63 ± 1.39 b	34.153 ± 0.13 c	34.14 ± 0.30 c	40.08 ± 0.35 a	36.41 ± 0.61 b	39.23 ± 0.24 a
a^*	5.75 ± 0.65 a	2.443 ± 0.09 c	1.173 ± 0.01 e	1.55 ± 0.01 e	0.93 ± 0.10 d	4.48 ± 0.06 b
b^*	11.41 ± 1.61 b	10.49 ± 0.43 b	7.22 ± 0.31 c	4.17 ± 0.11 d	9.59 ± 0.54 b	12.85 ± 0.08 a
Viscosity (cP)	34.43 ± 0.05 d	37.7 ± 0.36 c	37.7 ± 2.62 c	35.97 ± 0.04 d	49.53 ± 1.40 b	56.7 ± 0.45 a

Table 1: Color and Viscosity Analysis of Nile Tilapia By-Product Oil

3.3 Acid and Peroxide Value

The presence of FFAs and other non-lipid acid compounds in fish oil determines the quality of oil. The AVs of oils collected through SC-CO₂ and n-hexane extraction for the skin, head, and viscera were 5.46 ± 0.25 , 3.35 ± 0.28 , and 7.19 ± 1.06 mg KOH/g, and 5.58 ± 0.55 , 4.12 ± 0.42 , and 7.65 ± 1.74 mg KOH/g, respectively (Table 2). Furthermore, The PVs of oils collected through SC-CO₂ and n-hexane extraction for the skin, head, and viscera were 1.77 ± 0.43 , 1.34 ± 0.46 , and 4.48 ± 0.17 meq/kg, and 4.34 ± 0.43 , 4.70 ± 0.52 , and 3.97 ± 0.34 meq/kg, respectively (Table 2). During the hydrolysis reaction of triacyl glycerides, FFA and peroxide (PO) are generated due to the spoilage of raw material that has been formed as non-

lipid acid compounds; the assessment of these formations was used to evaluate the amount of hydroperoxides formed by thermo-oxidative or lipid oxidation phase in primary oxidation [23,25]. According to (Vegneshwaran VR), in the current study findings, the AVs of oil extracted from the viscera parts of tilapia by-product via SC-CO₂ and n-hexane were confirmed to be within the limit range (7–8 mg/kg); however, that from the skin and head by means of both extraction methods were found to be below the limit range [26]. On the other hand, PVs of oil extracted from the skin, head, and viscera parts of tilapia by-product via SC-CO₂ and n-hexane were within the limit range (<5 meq/kg).

Parameters	SC-CO ₂ extracted oil			n-hexane extracted oil		
	Skin	Head	Viscera	Skin	Head	Viscera
Acid value (mgKOH/g)	5.46 ± 0.25 b	3.35 ± 0.28 c	7.19 ± 1.06 a	5.58 ± 0.55 b	4.12 ± 0.42 bc	7.65 ± 1.74 a
Peroxide value (meq/kg)	1.77 ± 0.43 b	1.34 ± 0.46 b	4.48 ± 0.17 a	4.34 ± 0.43 a	4.70 ± 0.52 a	3.97 ± 0.34 a
Free fatty acid value (%)	3.62 ± 0.10 b	1.79 ± 0.09 c	4.29 ± 0.41 a	3.19 ± 0.35 b	1.69 ± 0.02 c	4.44 ± 0.55 a

Table 2: Oil Stability Analysis of Nile Tilapia By-Product Extracted By Sc-Co2 Oil and N-Hexane Extracted Oil

3.4 Free Fatty Acid Values

The generated FFAs through hydrolysis of ester bonds of triacylglycerol acid content in tilapia by-product extracted oil were determined through FFA value analysis. In this study, the FFA values from oil extracted via SC-CO₂ and n-hexane were determined, and the values showed insignificant difference between the different parts of tilapia by-product and extraction methods. The FFA values in SC-CO₂ extraction were as follows: skin, 3.62% ± 0.10%; head, 1.79% ± 0.09%; and viscera, 4.29% ± 0.41%. The FFA values via n-hexane extraction were as follows: skin, 3.19% ± 0.35%; head, 1.69% ± 0.02%; and viscera, 4.44% ± 0.55%. According to, all recorded FFA were in the range of acceptable limit (1%–7%) [27–36].

3.5 Radical Scavenging Activity

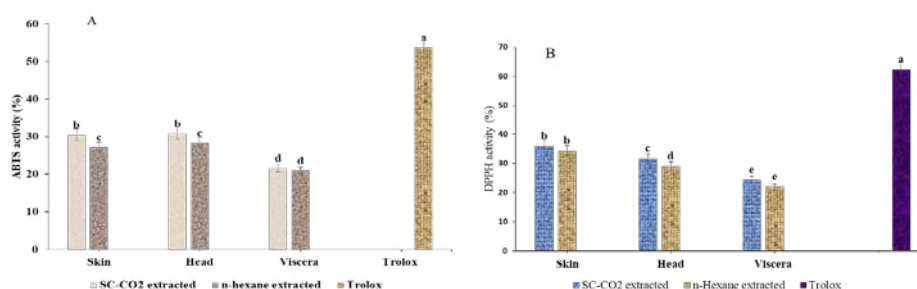
3.5.1 ABTS Free Radical Scavenging Activity

The scavenging ability of ABTS was observed in oils extracted from tilapia by-products (skin, head, and viscera) via SC-CO₂ and n-hexane is presented in Fig. 3A. The occurrence in the viscera component of oil obtained both through SC-CO₂ and n-hexane was slightly lower at 21.68% ± 1.33 % and 20.94% ± 1.11%, respectively, relative to the other parts (skin and head) extracted via both techniques. The highest ABTS scavenging activity and insignificant difference were observed in SC-CO₂ extraction for both skin and head

samples at 30.56% ± 1.05% and 30% ± 1.83%, respectively. In previous studies, the maximum scavenging activities of Atlantic salmon oils extracted via SC-CO₂ from the abdominal segment were recorded at 85.18% ± 0.95% [23]. The highest scavenging activity was found in all samples (skin, head, and viscera) from SC-CO₂ extraction. The maximum scavenging ability (53% ± 0.28%) was detected as compared with the standard Trolox.

3.6 DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activity of tilapia by-product oils (skin, head, and viscera) is presented in Fig. 3B. The maximum scavenging activity was found to be 35% ± 0.51%, which was from the oil extracted from the skin through SC-CO₂, followed by skin component oils extracted through n-hexane at 34% ± 1.44%. In a previous study, the maximum DPPH scavenging activity of oil from the skin of Atlantic salmon extracted through SC-CO₂ was more than two times this result at 83.2% ± 2.24% [23]. The oil from the viscera extracted through SC-CO₂ and n-hexane showed lower scavenging behavior, relative to skin and head samples, at 24.37% ± 1.20% and 21.89% ± 2.20%, respectively. The maximum scavenging activity (62.20% ± 0.40%) of the standard Trolox was substantially reported relative to all samples of oil collected in both techniques.



3. : Antioxidant Activities ; ABTS activity (A), DPPH activity (B).

3.7 Fatty Acid Compositions

The components of fatty acids in fish oil derived from tilapia by-products using SC-CO₂ and n-hexane extraction methods are presented in Table 3. There were 36 components of fatty acids determined in oils from tilapia by-product (skin, head, and viscera) derived via both techniques. The percentages of the total saturated fatty acids (SFAs) in all parts of tilapia by-products were found to be higher than that of monounsaturated fatty acids (MUFAs) and PUFAs. The viscera parts from extraction methods SC-CO₂ and n-hexane showed the highest value of SFAs (46.40% ± 0.36% and 48.67% ± 0.11%, respectively). However, the SFA components of palmitic acid (C16:0) from the skin, head, and viscera by SC-CO₂ and n-hexane extraction were the highest (24.07% ± 0.01%, 23.99% ± 0.01%, and 23.97% ± 0.01%, and 24.78% ± 0.78%, 25.97% ± 0.01%, and 26.14% ± 0.01%, respectively). This was followed by myristic acid (C14:0; from 7.29% ± 0.01% to 8.22% ± 0.37%) and stearic acid (C18:0; from 5.21% ± 0.01% to 6.52% ± 0.01%). Tricosanoic acid was also observed in oil from the skin via SC-CO₂ and n-hexane extraction (0.23% ± 0.01% and 0.20% ± 0.02%,

respectively). Significant variations were found in the overall SFA from tilapia by-product oils derived from the skin, head, and viscera. This result resembles that of the report of which showed the significant variations found between the total SFAs among tuna by-product oil [27]. The largest volume of overall PUFAs was found in head oil extracted via SC-CO₂ (19.19% ± 0.38%), followed by skin (16.56% ± 0.09%) and viscera (14.18% ± 2.31%) oils. The PUFAs of oil extracted through SC-CO₂ were slightly higher than that extracted through n-hexane, whereas there was no substantial difference between the oils extracted via n-hexane and SC-CO₂ from the skin, head, and viscera. The percentage of docosahexaenoic acid (C22:6n3) content was higher in the oil extracted by SC-CO₂ from the head (1.12% ± 0.01%), followed by the skin (1.06% ± 0.01%). Among all MUFAs, oleic acid (C18:1, cis9) showed maximum quality (29.41% ± 0.01% to 32.57% ± 0.01%), and significant variation of the value was observed between SC-CO₂ and n-hexane extraction techniques on all parts of tilapia by-products used in this study.

Fatty acids (%)	SC-CO ₂ extracted oils			n-hexane extracted oils		
	Skin	Head	Viscera	Skin	Head	Viscera
Saturated fatty acids (SFA)						
Butyric acid	0.11±0.02 ^d	0.29±0.01 ^a	0.21±0.01 ^b	0.16±0.02 ^c	0.00±0.00 ^e	0.12±0.00 ^d
Caproic acid	0.10±0.00 ^b	0.20±0.04 ^a	0.10±0.00 ^b	0.12±0.01 ^b	0.11±0.11 ^b	0.18±0.01 ^a
Caprylic acid	0.15±0.01 ^b	0.21±0.04 ^a	0.14±0.01 ^b	0.09±0.01 ^c	0.10±0.0 ^c	0.15±0.01 ^b
Capric acid	0.31±0.06 ^{a,b}	0.29±0.06 ^{a,b}	0.32±0.07 ^a	0.00±0.00 ^d	0.20±0.0 ^c	0.23±0.01 ^{bc}
Undecanoic acid	0.18±0.01 ^a	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b
Lauric acid	6.68±0.01 ^{a,b}	6.13±0.01 ^{bc}	7.22±0.01 ^a	5.75±0.81 ^c	5.58±0.02 ^c	6.55±0.01 ^{a,b}
Tridecanoic acid	0.00±0.00 ^c	0.06±0.01 ^b	0.00±0.00 ^c	0.00±0.00 ^c	0.97±0.02 ^a	0.00±0.00 ^c
Pentadecanoic acid	0.17±0.06 ^b	0.28±0.05 ^a	0.19±0.01 ^b	0.18±0.03 ^b	0.23±0.01 ^{a,b}	0.23±0.06 ^{a,b}
Palmitic acid	24.07±0.01 ^c	23.99±0.01 ^c	23.97±0.01 ^c	24.78±0.78 ^b	25.97±0.01 ^a	26.14±0.01 ^a
Stearic acid	5.33±0.01 ^c	5.21±0.01 ^c	5.71±0.26 ^b	5.58±0.17 ^b	5.87±0.1 ^b	6.52±0.01 ^a
Behenic acid	0.22±0.01 ^b	0.25±0.01 ^a	0.13±0.01 ^d	0.15±0.02 ^d	0.18±0.01 ^c	0.18±0.01 ^c
Lignoceric acid	0.18±0.01 ^a	0.18±0.01 ^a	0.12±0.01 ^c	0.11±0.01 ^c	0.16±0.01 ^b	0.16±0.01 ^b
Heneicosanoic acid	0.11±0.01 ^{ab}	0.08±0.02 ^{cd}	0.06±0.01 ^d	0.09±0.02 ^{bc}	0.12±0.01 ^a	0.06±0.01 ^d
Tricosanoic acid	0.23±0.01 ^a	0.00±0.00 ^c	0.00±0.00 ^c	0.20±0.02 ^b	0.00±0.00 ^c	0.00±0.00 ^c
Euric acid	0.13±0.01 ^a	0.08±0.01 ^b	0.08±0.01 ^b	0.09±0.02 ^{b,c}	0.08±0.01 ^b	0.09±0.01 ^b
Myristic acid	7.73±0.01 ^b	7.29±0.01 ^c	8.13±0.01 ^a	8.22±0.37 ^a	7.39±0.1 ^c	8.06±0.01 ^a
Monounsaturated fatty acids (MUFA)						
Oleic acid	30.93±0.01 ^c	30.76±0.02 ^c	29.41±0.01 ^d	31.44±0.99 ^{b,c}	32.57±0.01 ^a	31.79±0.01 ^b
Myristoleic acid	0.22±0.01 ^{a,b}	0.24±0.05 ^a	0.24±0.02 ^a	0.19±0.03 ^{b,c}	0.17±0.01 ^c	0.21±0.01 ^{b,c}
Cis-10-pentadecanoic acid	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.23±0.06 ^a
Palmitoleic acid	4.33±0.01 ^a	3.88±0.01 ^b	4.07±0.01 ^a	4.21±0.13 ^a	3.87±0.06 ^b	4.11±0.01 ^{a,b}
Cis-10-heptadecanoic acid	0.21±0.02 ^{a,b}	0.09±0.02 ^c	0.19±0.01 ^b	0.22±0.01 ^a	0.22±0.01 ^a	0.20±0.01 ^{a,b}
Elaidic acid	0.39±0.01 ^b	0.41±0.01 ^b	0.52±0.07 ^a	0.35±0.05 ^b	0.38±0.03 ^b	0.49±0.01 ^a
Nervonic acid	0.17±0.01 ^b	0.09±0.02 ^c	0.15±0.01 ^b	0.28±0.04 ^a	0.29±0.01 ^a	0.16±0.01 ^b
Eicosenoic acid	1.56±0.01 ^a	1.24±0.01 ^c	1.28±0.01 ^c	0.00±0.00 ^d	1.37±0.01 ^b	1.41±0.01 ^b
Polyunsaturated fatty acids (PUFA)						
Linolelaidic acid	0.11±0.01 ^c	0.11±0.00 ^c	0.18±0.01 ^a	0.10±0.00 ^c	0.15±0.01 ^b	0.15±0.01 ^b
Linoleic acid	11.54±0.06 ^{ab}	13.09±0.01 ^a		7.88±1.11 ^d		9.15±0.01 ^{c,d}
r-Linoleic acid	0.56±0.01 ^b	0.77±0.01 ^a	0.28±0.01 ^d	0.25±0.04 ^d	0.37±0.01 ^c	0.38±0.01 ^c
Linolenic acid	0.75±0.01 ^b	1.04±0.01 ^a	0.43±0.03 ^{d,e}	0.39±0.06 ^e	0.57±0.01 ^c	0.50±0.10 ^{c,d}
Eicosadienoic acid	0.81±0.01 ^{b,c}	0.90±0.01 ^b	0.77±0.01 ^c	0.75±0.11 ^c	1.09±0.01 ^a	0.87±0.06 ^b
Elcosatrienoic acid	0.47±0.01 ^b	0.56±0.01 ^a	0.27±0.01 ^e	0.24±0.03 ^f	0.32±0.01 ^d	0.35±0.01 ^c
Eicosatrienoic acid	0.16±0.01 ^a	0.17±0.01 ^a	0.10±0.00 ^c	0.09±0.02 ^c	0.11±0.00 ^{b,c}	0.12±0.01 ^b
Arachidic acid	0.29±0.01 ^a	0.23±0.01 ^{c,d}	0.22±0.01 ^d	0.24±0.03 ^{b,c,d}		0.28±0.01 ^{a,b}
Arachidonic acid	0.58±0.01 ^b	0.71±0.01 ^a	0.28±0.01 ^d	0.10±0.02 ^f	0.24±0.01 ^e	0.40±0.01 ^c
Eicosapentanoic acid	0.11±0.01 ^d	0.22±0.01 ^b	0.16±0.01 ^c	0.26±0.05 ^a	0.30±0.00 ^a	0.16±0.01 ^c
Docosahexanoic acid	1.06±0.01 ^b	1.12±0.01 ^a	0.16±0.01 ^d	0.29±0.04 ^c	0.26±0.01 ^c	0.28±0.01 ^c

Docosadienoic acid	0.13±0.01b	0.22±0.01a	0.09±0.01d	0.11±0.02c	0.14±0.01b	0.10±0.00c,d
ΣSFA		44.54±0.24c		45.64±1.98b,c		48.67±0.11a
ΣMUFA		36.72±0.10b		37.20±2.11a,b	38.90±0.07a	38.60±0.08a,b
ΣPUFA	16.56±0.09b	19.19±0.38a	14.18±2.31c	12.79±0.57c	13.86±0.03c	12.74±0.10c

Table 3: Total Fatty Acid Composition

3.8 Total Yield, Protein, and Sugar Content of Hydrolysate

Hydrolysis of de-oiled tilapia waste (DTW; de-oiled waste of the skin, viscera, and head) was conducted at temperatures between 160 °C and 190 °C using the subcritical water hydrolysis method. This resulted in different percentage yield (protein and total sugar content) between different parts in hydrolysis at both 160 °C and 190 °C (Table 4). The skin, head, and viscera are among the main components of fish body parts, which were the origins of fish protein hydrolysates [28]. In the human body, proteins have roles in the development, control of biochemical reactions, and transport of nutrients across cell membranes. In the present analysis, the maximum percentage yield was obtained from de-oiled tilapia skin waste (S2) hydrolyzed at 190 °C (7.44%) and the minimum percentage yield was obtained from de-oiled tilapia visceral waste (V1) hydrolyzed at 160 °C (2.04%). The DTW protein content increased in subcritical water hydrolysis as extraction temperatures rise; this result revealed that higher hydrolysis activity caused by rising constant water ionization at high temperature increased protein yield. The hydrolysate indicated that the total protein content in all samples varied, and the values recorded were from 31.33 ± 1.23 to 40.42 ± 0.79 (BSA mg/g) in V1 hydrolyzed at 160 °C and V2 hydrolyzed at 190 °C. This finding is similar to the previous report, which reported that the highest protein yields in raw and de-oiled squid viscera hydrolysates were 340.55 ± 6.25 and 660.58 ± 2.94 mg g⁻¹ at 180 °C and 280 °C, respectively [23]. The maximum total sugar observed in V1 and V2 was 30.06 ± 4.07 mg/g and 28.51 ± 0.83 mg/g, respectively, whereas the minimum amounts reported in head waste (H2) and S2 were 10.20 ± 0.68 mg/g and 10.27 ± 1.68 mg/g, respectively. The overall sugar content of DTW hydrolysate decreased when temperature was increased to 190 °C. These findings were not similar to the previous research conducted by, in which the reported total sugar content of *Penaeus japonicus* hydrolysates depends on the hydrolysis temperature and was seen to rise with increasing temperature with a minimum and maximum total sugar content of 19.10 ± 0.90 and 49.30 ± 1.10 mg/g dried mass and 7.48 ± 1.05 and 14.18 ± 1.23 mg/g dried mass at 100 °C and 200 °C, respectively [19,20].

3.9 pH of De-Oiled Tilapia Waste Hydrolysate

pH is a crucial parameter for the extraction of useful materials from their natural origins [26]. Table 4 presents the pH level of DTW hydrolysate with Maillard reaction

and color formation; the data indicated that the DTW pH value ranged from 6.70 ± 0.01 to of 8.14 ± 0.01 . The pH level of hydrolysates is one of the key factors influencing the functional properties of hydrolyzed proteins, including their foaming and emulsifying ability and water holding and fat binding capacity (Taheri et al., 2013). In the DTW of skin sample where the highest Maillard reaction products were registered, the pH values increased as Maillard reaction products increased (S1 = 17.43 ± 0.90); Maillard reaction product with pH = 8.14 ± 0.01 and (S2 = 13.75 ± 1.75); Maillard reaction product with pH = 8.07 ± 0.01 . The pH values were increased in all DTW samples (skin, head, and viscera) as Maillard reaction products increased [27]. According to the oxidation of organic acids and acidic compounds that have reduced pH in the past, as well as the formation of salts and the production of alkaline compounds such as methylamine and other amines due to the decomposition of glycine, results in the increase of pH with temperature.

3.10 Color and Maillard Reaction Products

Table 4 reveals the results of the color and Maillard reaction products of DTW in the current study, most of which used instrumental color measurements (L*, a*, b* systems), with a key parameters of lightness (L*), redness (a*), and yellowness (b*) [19,20,30]. Suggested that color and taste are produced during the heating of foods due to Maillard reaction agent produced by the reduction of sugar reactive carbonyl group reacting with a nucleophilic amino acid. In the current study, the highest and insignificant difference of L* values (40.65 ± 1.55 and 39.94 ± 1.75) was observed in the S1 and H1 in the same hydrolysis temperature (160 °C), respectively. The L* values in all samples were observed to decrease when temperature increases (Table 3); this might be due to the formation of Maillard reaction. a* showed less value in S1, S2, and H1, whereas b* in H2 was the highest. In the research work of Ames (1990), it was noted that Maillard reactions influence several parameters of food quality, including organoleptic properties, color, and protein functionality [31]. Based on temperature time profiles used during food processing, specific aroma profiles were created. In the DTW product, the Maillard reaction increased at the highest pH of S1 (17.43 ± 0.90) and lowest pH of V2 (10.15 ± 0.22). The parameters influencing Maillard reaction are temperature, time, pH, water behavior, and reactant source and concentration [37].

Sample	Color				Maillard reaction product	pH	Hydrolysis yield (%)	Total protein (BSA mg/g)	Total sugar (Glu mg/g)
	<i>L*</i>	<i>a*</i>	<i>b*</i>	ΔE					
S1	40.65±1.55a	0.67±0.55c	7.01±0.03d	31.84±0.98d	17.43±0.90a	8.14±0.01a	6.58±0.03b	36.40±0.33b	14.58±0.44b
S2	37.94±0.74b	0.40±0.09c	8.48±0.35b	33.57±0.80c	13.75±1.75bc	8.07±0.01c	7.44±0.01a	39.86±0.59a	10.20±0.68b
H1	39.94±1.75a,b	0.35±0.07c	7.41±0.145c	33.03±0.94c,d	14.83±0.16b	8.10±0.02b	4.56±0.02d	31.28±0.92c	11.65±3.62b
H2	37.72±0.29b	1.32±0.02b	9.06±0.02a	33.32±0.04c,d	13.10±0.33c	7.93±0.01d	5.18±0.02c	37.39±0.95b	10.27±1.68b
V1	35.56±0.66c	1.49±0.03b	7.68±0.13c	35.97±0.55b	10.61±0.04d	6.82±0.01e	2.04±0.01f	31.33±1.23c	30.06±4.07a
V2	31.35±1.39d	3.62±0.20a	5.54±0.15e	40.64±1.17a	10.15±0.22d	6.70±0.01f	2.28±0.01e	40.42±0.79a	28.51±0.83a

Table 4: Total Yield, Protein, and Sugar Content Of Hydrolysates

3.11 Antioxidant activity of DTW

3.11.1 ABTS Radical Scavenging Activity

ABTS radical scavenging activity was used to assess antioxidant activity in DTW, and the result was shown in Fig. 4. The ABTS radical scavenging assay considered the popular, simple, sensitive, hydrophobic and hydrophilic, as well as lipophilic, antioxidant [9,19,20,35]. DTW hydrolysates, based on hydrolysis temperature, showed variable ABTS radical scavenging behaviors. With rising hydrolysis temperature in all hydrolysate samples, ABTS radical scavenging activity gradually increased with increasing hydrolysis temperature (Fig. 4). The maximum radical scavenging activity of ABTS was $99.15\% \pm 0.14\%$ (V2), followed by $98.81\% \pm 0.30\%$ (S2) at the same temperature (190 °C). According to the behavior of ABTS radical scavenging differed between hydrolysates because of variations in the constituents of amino acids and peptide length. In general, de-oiled hydrolyzed extracts rich in protein contain amino acids/peptides that serve as donors of hydrogen and neutralize radicals by converting them to the most stable material, thus ending radical chain reactions [38-41].

4. Discussions

4.1 Conclusion

The study outcomes show that green technologies such as SC-CO₂ and subcritical water hydrolysis techniques are favored for oil extraction from by-products of tilapia and subcritical water hydrolysis of by-products of DTWs. Valorization of DTW (skin, head, and viscera) plays a significant role in the manufacture of bioactive compounds used in food manufacturing, medicinal applications, and the protection from environmental pollution. Tilapia by-product is rich in PUFAs, which have crucial beneficial effect in health. The extracted hydrolysates are also rich in peptides and have good antioxidant activity. SC-CO₂, n-hexane, and subcritical water hydrolysis techniques could be applied for the valorization of tilapia waste for the extraction of bioactive material and for making better uses of the potential fish by-products in fishery industries.

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Conflict of Interest

The authors have no conflict of interest to declare.

Reference

- Yue, G. H., Lin, H. R., & Li, J. L. (2016). Tilapia is the fish for next-generation aquaculture. *International Journal of Marine Science and Ocean Technology*, 3(1), 11-13.
- Abdel-Ghany, H. M., El-Sayed, A. F. M., Ezzat, A. A., Essa, M. A., & Helal, A. M. (2019). Dietary lipid sources affect cold tolerance of Nile tilapia (*Oreochromis niloticus*). *Journal of thermal biology*, 79, 50-55.
- Lordan, S., Ross, R. P., & Stanton, C. (2011). Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Marine drugs*, 9(6), 1056-1100.
- Ghaly, A. E., Ramakrishnan, V. V., Brooks, M. S., Budge, S. M., & Dave, D. (2013). Fish processing wastes as a potential source of proteins. Amino acids and oils: A critical review. *J. Microb. Biochem. Technol*, 5(4), 107-129.
- Leira, M. H., NASCIMENTO, A. F. D., Alves, F. R., Orfao, L., Lacerda, Y. G., Botelho, H. A., ... & Lago, A. D. A. (2019). Characterization of different techniques for obtaining minced fish from tilapia waste. *Food Science and Technology*, 39(Suppl. 1), 63-67.
- de Medeiros, E. F., da Silva Afonso, M., dos Santos, M. A. Z., Bento, F. M., Quadro, M. S., & Andreazza, R. (2019). Physicochemical characterization of oil extraction from fishing waste for biofuel production. *Renewable Energy*, 143, 471-477.
- Sary, C., Paris, L. D. D., Bernardi, D. M., Lewandowski, V., Signor, A., & Boscolo, W. R. (2017). Tilapia by-product hydrolysate powder in diets for Nile tilapia larvae. *Acta Scientiarum. Animal Sciences*, 39(1), 1-6.
- Roslan, J., Kamal, S. M. M., Yunos, K. F. M., & Abdullah, N. (2017). Assessment on multilayer ultrafiltration membrane for fractionation of tilapia by-product protein hydrolysate with angiotensin I-converting enzyme (ACE) inhibitory activity. *Separation and Purification Technology*, 173, 250-257.
- Blanco Comesaña, M., González Sotelo, C., Chapela, M. J., & Pérez Martín, R. I. (2007). Towards sustainable and efficient use of fishery resources: present and future trends.

10. Byun, H. G., Eom, T. K., Jung, W. K., & Kim, S. K. (2008). Characterization of fish oil extracted from fish processing by-products. *Journal of Food Science and Nutrition*, 13(1), 7-11.
11. Howe, P. R., Evans, H. M., Kuszewski, J. C., & Wong, R. H. (2018). Effects of long chain omega-3 polyunsaturated fatty acids on brain function in mildly hypertensive older adults. *Nutrients*, 10(10), 1413.
12. Fouda, T. (2018). Waste management for smoking salmon by-products to extract omega-3 fish oil.
13. Singh, S. K., Pavan, M. S., SaiPrasanna, N., & Kant, R. (2018). Applications of super critical fluid extraction in milk and dairy industry: A review. *J. Food Process. Technol*, 9(10.4172), 2157-7110.
14. Franklin, E. C., Haq, M., Roy, V. C., Park, J. S., & Chun, B. S. (2020). Supercritical CO₂ extraction and quality comparison of lipids from Yellowtail fish (*Seriola quinqueradiata*) waste in different conditions. *Journal of Food Processing and Preservation*, 44(11), e14892.
15. Roy, V. C., Getachew, A. T., Cho, Y. J., Park, J. S., & Chun, B. S. (2020). Recovery and bio-potentialities of astaxanthin-rich oil from shrimp (*Penaeus monodon*) waste and mackerel (*Scomberomorus niphonius*) skin using concurrent supercritical CO₂ extraction. *The Journal of Supercritical Fluids*, 159, 104773.
16. Ivanovs, K., & Blumberga, D. (2017). Extraction of fish oil using green extraction methods: A short review. *Energy Procedia*, 128, 477-483.
17. Franklin, E. C., Haq, M., Roy, V. C., Park, J. S., & Chun, B. S. (2020). Supercritical CO₂ extraction and quality comparison of lipids from Yellowtail fish (*Seriola quinqueradiata*) waste in different conditions. *Journal of Food Processing and Preservation*, 44(11), e14892.
18. Ho, T. C., & Chun, B. S. (2019). Extraction of bioactive compounds from *Pseuderanthemum palatiferum* (Nees) Radlk. using subcritical water and conventional solvents: A comparison study. *Journal of food science*, 84(5), 1201-1207.
19. Chow, P. S., & Landhäusser, S. M. (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree physiology*, 24(10), 1129-1136.
20. Cho, Y. J., Getachew, A. T., Park, J. S., Lim, C. T., Lee, H. J., & Chun, B. S. (2020). Influence of temperature on decomposition reaction of compressed hot water to valorize *Achatina fulica* as a functional material. *Food and Bioproducts Processing*, 122, 89-97.
21. Cho, Y. J., Haq, M., Park, J. S., Lee, H. J., & Chun, B. S. (2019). Physicochemical and biofunctional properties of shrimp (*Penaeus japonicus*) hydrolysates obtained from hot-compressed water treatment. *The Journal of Supercritical Fluids*, 147, 322-328.
22. Seboka, A. D. D., & Deressa, S. G. (2026). Properties of Oils and Hydrolysates Obtained from Tilapia (*Oreochromis niloticus*) By-Products Using Pressurized Fluid Technologies.
23. Uddin, M. S., Ahn, H. M., Kishimura, H., & Chun, B. S. (2010). Production of valued materials from squid viscera by subcritical water hydrolysis. *Journal of Environmental Biology*, 31(5), 675-679.
24. 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 supercritical carbon dioxide and conventional methods. *Waste and Biomass Valorization*, 8(6), 1953-1967.
25. Farag, R. S., & Basuny, A. M. (2009). Improvement in the quality of used sunflower oil by organic and inorganic adsorbents. *International journal of food science and technology*, 44(9), 1802-1808.
26. Luther, M., Parry, J., Moore, J., Meng, J., Zhang, Y., Cheng, Z., & Yu, L. L. (2007). Inhibitory effect of Chardonnay and black raspberry seed extracts on lipid oxidation in fish oil and their radical scavenging and antimicrobial properties. *Food Chemistry*, 104(3), 1065-1073.
27. Lee, H. J., Chae, S. J., Saravana, P. S., & Chun, B. S. (2017). Physical and functional properties of tunicate (*Styela clava*) hydrolysate obtained from pressurized hydrothermal process. *Fisheries and Aquatic Sciences*, 20(1), 14.
28. Pourali, O., Asghari, F. S., & Yoshida, H. (2010). Production of phenolic compounds from rice bran biomass under subcritical water conditions. *Chemical Engineering Journal*, 160(1), 259-266.
29. Henle, T. (2005). Protein-bound advanced glycation endproducts (AGEs) as bioactive amino acid derivatives in foods. *Amino acids*, 29(4), 313-322.
30. Rubio-Rodríguez, N., De Diego, S. M., Beltrán, S., Jaime, I., Sanz, M. T., & Rovira, J. (2012). Supercritical fluid extraction of fish oil from fish by-products: A comparison with other extraction methods. *Journal of food engineering*, 109(2), 238-248.
31. Lund, M. N., & Ray, C. A. (2017). Control of Maillard reactions in foods: Strategies and chemical mechanisms. *Journal of agricultural and food chemistry*, 65(23), 4537-4552.
32. Deepika, D., Vegneshwaran, V. R., Julia, P., Sukhinder, K. C., Sheila, T., Heather, M., & Wade, M. (2014). Investigation on oil extraction methods and its influence on omega-3 content from cultured salmon.
33. Hao, G., Cao, W., Li, T., Chen, J., Zhang, J., Weng, W., ... & Ren, H. (2019). Effect of temperature on chemical properties and antioxidant activities of abalone viscera subcritical water extract. *The Journal of Supercritical Fluids*, 147, 17-23.
34. Halim, N. R. A., Yusof, H. M., & Sarbon, N. M. (2016). Functional and bioactive properties of fish protein hydrolysates and peptides: A comprehensive review. *Trends in Food Science & Technology*, 51, 24-33.
35. Asaduzzaman, A. K. M., Haq, M., & Chun, B. S. (2018). Reduction of histamine and heavy metals in mackerel hydrolysates produced by catalysts associated-subcritical water hydrolysis. *Journal of Industrial and Engineering Chemistry*, 68, 301-310.
36. Ferdosh, S., Sarker, Z. I., Norulaini, N., Oliveira, A., Yunus, K., Chowdury, A. J., ... & Omar, M. (2015). Quality of tuna fish oils extracted from processing the by-products of three species of neritic tuna using supercritical carbon dioxide. *Journal of food processing and preservation*,

- 39(4), 432-441.
37. Jing, H., & Kitts, D. D. (2002). Chemical and biochemical properties of casein-sugar Maillard reaction products. *Food and Chemical Toxicology*, 40(7), 1007-1015.
38. Mæhre, H. K., Dalheim, L., Edvinsen, G. K., Elvevoll, E. O., & Jensen, I. J. (2018). Protein determination—method matters. *Foods*, 7(1), 5.
39. Pomory, C. M. (2008). Color development time of the Lowry protein assay. *Analytical biochemistry*, 378(2), 216-217.
40. Taheri, A., Anvar, S. A. A., Ahari, H., & Fogliano, V. (2013). Comparison the functional properties of protein Hydrolysates from poultry byproducts and rainbow trout. *Iranian journal of fisheries sciences*, 12(1), 154-169.
41. Watchararужи, K., Goto, M., Sasaki, M., & Shotipruk, A. (2008). Value-added subcritical water hydrolysate from rice bran and soybean meal. *Bioresource technology*, 99(14), 6207-6213.