BIOREFINERY FOR YELLOWFIN TUNA (*THUNNUS ALBACARES*) BY-PRODUCTS FOR CHARACTERIZATION AND IDENTIFICATION OF VALUE-ADDED MATERIALS

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ABSTRACT

Valorization of fish by-products is gaining interest because a huge amount of biomass is discarded as by-products from fish processing industries. Seafood companies in Sri Lanka use only 50% of the total catch for actual human consumption. This work aims to find out the appropriate extraction methods to maximize the recovery of high-value-added compounds from juvenile yellowfin tuna by-products and extract those compounds using a novel biorefinery approach. The chemical composition of yellowfin tuna by-products was examined. Oil, collagen, and protein isolates were recovered using the pH shift method combined with the acid-soluble collagen extraction from the head, dorsal and caudal samples of juvenile yellowfin tuna (n=7, mean weight = 0.875 ± 0. 107 kg). In terms of protein content, the highest protein content was obtained in the caudal sample (23.80 ± 0.14%) followed by dorsal (22.75 ± 0.64%) and head (18.75 ± 0.21%). Omega-3 PUFA was higher in the dorsal (40.02%) than in the caudal (44.56%) and head (32.78%). The alkaline pH shift method resulted in high yield (Dorsal:10.9%, caudal (Post H_2O_2): 10.85%, caudal (Pre H_2O_2): 10.77%, head: 8.81% on a wet weight basis) and high purity (<90%) protein isolates from heads, caudal and dorsal samples. Collagen extracted with 0.6 M hydrochloric acid could increase the collagen yield from the head (21.16% on a dry weight basis) and caudal sample (39.44% -Pre H_2O_2, 23.17% -post H_2O_2; on a dry weight basis) than the dorsal sample (9.51% on a dry weight basis) which was extracted with 0.5M acetic acid. The oil was extracted through the pH shift method at room temperature and the conventional heat base method. Yellowfin tuna oil was rich in omega-3 polyunsaturated fatty acids, particularly DHA (26.84%). Freeze-thawing and repeated centrifugation at 4^0C during the pH shift method recovered a higher oil yield (1.52%) than the conventional heat base method (0.75%). A higher yield could be expected from adult yellowfin tuna. The outcomes of this study depicted the maximum recovery of high-added value compounds with high purity of proteins from the raw materials through this biorefinery approach.

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1 INTRODUCTION

1.1 Background

Sri Lanka is one of the oldest and most important tuna-producing countries in the Indian Ocean (Jayasooriya & Bandara, 2013). Tuna fisheries in Sri Lanka are developing rapidly with the expansion of offshore and high-sea fishing activities. The major tuna species are Yellowfin tuna (*Thunnus albacares*), Bigeye tuna (*Thunnus obesus*), Skipjack tuna (*Katsuwonus pelamis*), Frigate tuna (*Auxis thazard*) and Bullet tuna (*Auxis rochei*). Among the major tuna fisheries, Yellowfin tuna is the dominant commercial catch, and it is the backbone of the fish export industry in Sri Lanka (Jayasooriya & Bandara, 2013). The contribution of tuna to total fish export represented 42.2% of total fish export and it generated approximately 113 million USD income per year (Ministry of Fisheries, 2021). Among the world's major yellowfin tuna exporters, Sri Lanka is the fourth largest exporter while Indonesia is the first and Maldives is the world's second largest market. (Lecomte, Rochette, Laurans, & Lapeyre, 2017).

The tuna supply chain contributes to increased demand for processed tuna products in Sri Lanka. Yellowfin tuna are generally processed and exported as fresh or frozen products. The principal products of yellowfin tuna are fresh loins and steaks, frozen, and canned. These processing operations require bleeding, gutting, de-heading, filleting, skinning, and trimming. Seafood companies in Sri Lanka use only 50% of the catch for actual human consumption (Ampitiya, Gonapinuwala, Fernando, & De Croos, 2022). During the processing, the large biomass of non-processed parts is generally used as low-value products as fertilizers or fish feed. Fish waste is produced in solid and liquid forms. The major amounts of by-products are represented by the head, tail, and offal collected through eviscerating, cutting, and filleting processes. Skin, blood, bones, and frames are the second major by-products produced during the skinning and cutting process (Ferraro, et al., 2010). Tuna waste obtained during processing is mainly used as a raw material to produce low value fish feed in Sri Lanka. But these by-products consist of valuable compounds such as collagen, peptides, poly-unsaturated fatty acids, chitin, enzymes, and minerals (Ferraro, et al., 2010).

1.2 Justification

The yellowfin tuna processing companies in Sri Lanka are using only 20% - 50% of the fish as edible portions consisting mainly of white muscles. According to the annual yellowfin tuna export volume, approximately, 4500Mt of by-products are annually produced by seafood companies. Most of these off-cuts are used for low value products such as fish meal, compost, or direct fertilizer while the rest is dumped without getting any use but creating environmental pollution. Furthermore, modern quality control and hygienic standards enforced on seafood companies have caused a significant increase in the amount of solid and liquid waste (Ferraro, et al., 2010).

In recent years, researchers have paid attention to the valorisation of by-products for human consumption. Fish heads, dorsal skins, dark muscles, and caudal fin with caudal peduncle

represent around 35% of total off-cuts. These off-cuts may contain collagen, lipids with omega-3 fatty acids, and high-quality proteins. In addition, they are possible resources for vitamins (A, D, and niacin), and minerals (zinc, iodine, iron) (Olsen, Toppe, & Karunasagar, 2014). More conventionally, fish skin and fins, as well as bones are employed by the global seafood industry as a source of collagen. Fish heads are a known source of protein and oil (Boronat, et al., 2022). Even though a substantial amount of an offcut of yellowfin tuna is generated at seafood companies in Sri Lanka, inadequate knowledge and techniques hinder its potential utilization to produce value-added materials for human consumption. Seafood companies in Sri Lanka are keen to develop value-added products to increase operational efficiency and improve the income gotten from the by-product transformation.

A number of studies have been conducted globally to produce value-added materials from tuna by-products for human consumption including extraction of polyunsaturated fatty acids, collagen, and protein (Ampitiya, Gonapinuwala, Fernando, & De Croos, 2022; Ferraro, et al., 2010). The different methodologies (pH shift method, wet rendering method, cold press method) have been developed worldwide and applied alone to get a high yield of a certain product while breaking down remaining compounds for other uses. However, there are very few studies conducted on the potential of extraction of collagen, oil, and protein from the dorsal skin, head, and caudal fin as a value-addition method to tuna by-products in Sri Lanka (Ampitiya, Gonapinuwala, Fernando, & De Croos, 2022; Kumara, Rajapakshe, & Jayamanne, 2011). Extraction of those high-added value compounds which can be profitable owing to their beneficial role in human health joined to the development of new technologies. Hence, the complete use of this fish waste could open new valorisation pathways for new industries in Sri Lanka. Therefore, it is necessary to develop a comprehensive strategy to maximize the utility of by-products.

1.3 Goals

Limited knowledge and technology still exist for the integration of different extraction methods from a biorefinery perspective in Sri Lanka. Therefore, the main goals of this study are to focus on the integral valorisation strategy for maximizing the utility of the yellowfin tuna by-products in Sri Lanka, dealing efficiently with the by-products, and increasing knowledge of extraction methods for high value compounds. The transfer of this knowledge and technology to the university of Jaffna and relevant seafood industries can be used to promote the production of value-added materials and higher value end-products made out of the fish by-products that are currently under-utilized (as fish meal and fertilizers). Furthermore, this study will be important to focus on how to deal with the aforementioned by-products in efficient ways including useful applications for direct human consumption. The knowledge behind the extraction of high value compounds for food formulation and pharmaceutical application would have concomitant economic benefits for tuna processing industries in Sri Lanka.

1.4 Specific objectives

1. Characterize the chemical composition of the yellowfin tuna by-products of heads, dorsal skin, and caudal fin with muscles (offcuts).

2. Find the appropriate extraction methods to extract valuable materials such as crude oil, collagen, and protein.

2 LITERATURE REVIEW

2.1 Yellowfin tuna production in Sri Lanka

Figure 1. Yellowfintuna (*Thunnus albacares*) *(Syndey Fish Market, 2023)*.

Yellowfin tuna (Thunnus albacares) is a pelagic shoaling species that can grow from 30 to 170 cm and close to an age of 8 years (Besada, et al., 2006). Typically, they are distributed in the tropical and subtropical water including the Indian Ocean. Yellowfin tuna is commercially the second most important species of tuna worldwide. More than 70 countries are fishing and processing tuna globally (Garofalo & Tommasi, 2023).

The major tuna processing countries in the Indian Ocean are Sri Lanka, Indonesia, and Maldives, contributing to more than 50% of the total catches of tuna species in the Indian Ocean (Indian Ocean Tuna Commission, 2020). Purse seining and longlining are the major fishing methods used to catch tuna in coastal water and high seas in Sri Lanka.

Multiday fishing fleets are mainly targeting tuna and tuna-like species. Long-line fishing is promoted by the Sri Lankan government to ensure the quality of tuna production to cater to the rapidly developing export market. Annual yellowfin tuna production in 2020 was 37930 metric tonnes (Mt) which represents 11.6% of total marine catch (Ministry of Fisheries, 2021). Yellowfin tuna are generally processed and exported as fresh and frozen products. The main international trade markets for Sri Lankan tuna products are the European Union, the United States of America, Japan, and the United Kingdom, (Silva & De Mashiro, 2006). The estimated total export quantity in 2020 was 8992 Mt consisting of 3576 Mt (39.8%) for the European Union followed by 1317 (14.6%) Mt for other European countries, 864 Mt (9.6%) for the U.S.A., and 164 Mt (1.8%) for Japan (Ministry of Fisheries, 2021). The rest of the tuna production is targeted at the local market and made a marginal contribution to per capita fish consumption.
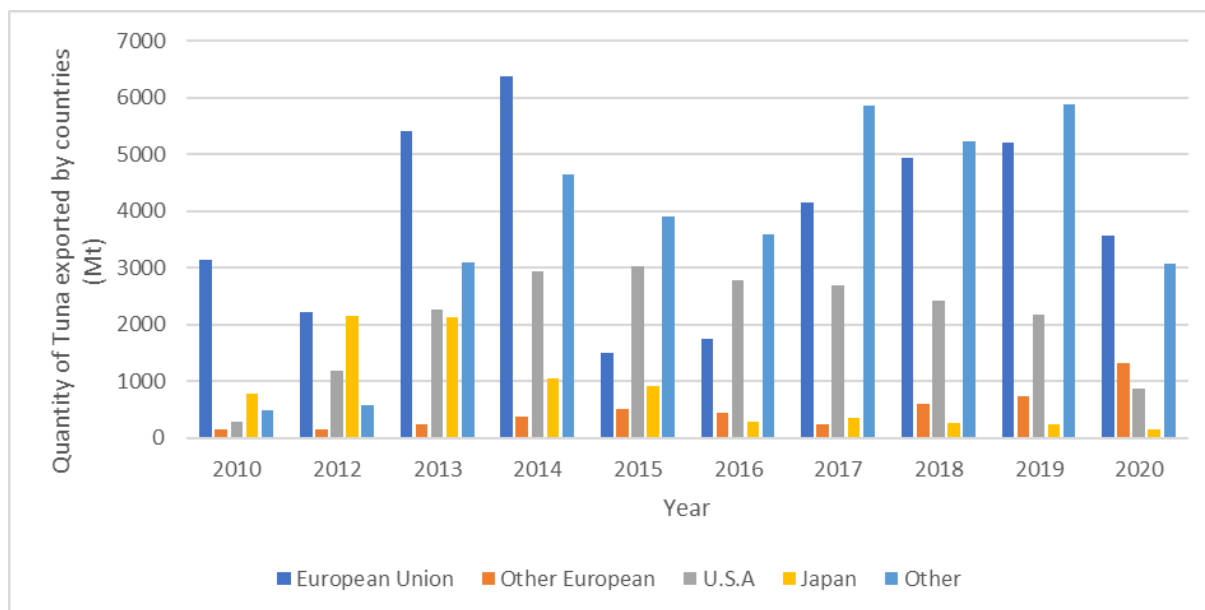
Figure 2. Export volume of yellowfin tuna in Sri Lanka (Ministry of Fisheries, 2021).

The contribution of tuna to the export market has increased in recent times. During the last decades, approximately 75 new seafood processing plants have been established to expand the export quantity, and it is becoming an important sector of the country's economy (Ampitiya, Gonapinuwala, Fernando, & De Croos, 2022).

2.2 Yellowfin tuna processing industry in Sri Lanka

The tuna processing industry in Sri Lanka is completely operated by the private sector. The government provides technical and legal assistance for export by giving tax relief and low-interest-rate loans (De Silva & Yamao, 2006).

After capture the tuna are transported to processing plants in frozen condition. The process of fresh frozen tuna involves several steps. The frozen tunas are brushed and washed in chilled running water. Fishes are chilled with a layer of flake ice at a 1:1 ratio. Histamine and mercury levels are detected before the primary processing stage. If the concentrations are at acceptable levels, chilled fish are subjected to gill and gut-removing processes. Fins and heads are completely removed and washed thoroughly. Then de-gutted fish are filleted except whole fish shipment and undergo a deboning process. The skin of each loin is removed during the skinning process. The skinned loins are tested to find out any internal damage, cuts, bite marks, and parasites. The loins are processed as blocks, chunks, brochettes, steaks, and saku in the trimming and portioning process (Figure 3). The rest of the raw materials after filleting are considered as by-products (NJ Marine (PVT) LTD, 2022).

The processing units are targeted at two markets consisting of the tuna steak market and the sashimi market. The type of market is closely related to the catch quality. The highest quality fresh tuna is destined for the Japanese and American sashimi markets while the second quality catches are targeted at European and American tuna steak consumers (Lecomte, Rochette, Laurans, & Lapeyre, 2017). Tuna catches which do not meet the export quality standards are sent to the local markets. The main products are fresh and frozen whole tuna, fresh and frozen tuna loins, and fresh and frozen fillets (Figure 3). The main export market for processed and

semi-processed tuna are the United Kingdom, USA, France, Germany, Switzerland, and Netherlands (Figure 2). The total tuna export contributes approximately 17003 million rupees for annual export income to the Sri Lankan economy and it accounts for 42.6% of the total fish export value in 2020 (Ministry of Fisheries, 2021).

Figure 3. Yellowfin tuna export products (weight ranged 25 kg-90 kg) in Sri Lanka; Left to right; Tuna H&G, Tuna G&G, Tuna Saku, Tuna steak, Tuna centre cut (Source: http://www.nnsuperseafood.com/category/tuna-fish).

2.3 By-product generation in tuna processing industries in Sri Lanka

Most exported tuna products require processing that generates a large quantity of by-products that are not commonly used in further processing. The by-products include 20 to 35% solid waste and 20 to 35% liquid waste (Sayana & Sirajudheen, 2017). The by-product consists of the head (17%), fins (2%), skin (8%), bones (4%), viscera (5%), scales (5%), and some damaged muscles (Figure 4) (Sayana & Sirajudheen, 2017).
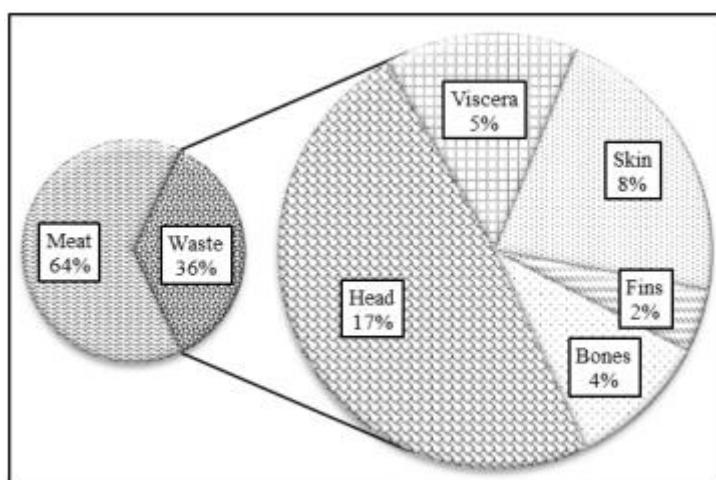
Figure 4. Quantification of different components of tuna processing wastes (Sayana & Sirajudheen, 2017).

In recent decades many studies have been focused on transforming by-products into functional or bioactive compounds due to environmental and economic benefits. The transformation of by-products into commercial value-added products is emphasized as a way of producing maximum food from limited resources.

2.4 High value-added materials extraction from by-products

The discards of by-products by fish processing companies are currently rising, driven by the increase in fish consumption. Seafood is an excellent source of nutrients such as lipids, proteins, and minerals which are important for human health. The main high value compounds extracted from fish by-products worldwide are summarized in Table 1.

Table 1. High-value compounds extracted from fish by-products worldwide (Ferraro, et al., 2010).

High-added value compounds	Marine by-products	Content (% w/w)
Polyunsaturated fatty acids (omega-3 and omega-6)	Cod liver, mackerel flesh residues	58-80% in cod liver
Free amino acids	Muscles, white fish flesh residues	0.8-2% of taurine
Collagen and gelatin	Pelagic fish skin, scales, and bones	Up to 80% in the skin, up to 50% in scales
Hydroxyapatite	Pelagic fish Scales and bones	60-70% in bones, up to 50% in scales

2.5 Collagen extraction from by-products

The extraction of collagen from tuna by-products remains a topic of interest for the pharmaceutical, cosmetics, and food industries. Collagen is one of the most abundant animal-derived proteins which consists of 30% of the total protein in humans (Ampitiya, Gonapinuwala, Fernando, & De Croos, 2022). Collagen is used for pharmaceutical, medical, and technical applications such as improving the stability of foods, and encapsulation formation. Collagen is mainly used to produce gelatine, due to its unique gel-forming capacity. Despite its low biological value, gelatine is commonly used in medical and pharmaceutical applications due to its biodegradability. However, only 1% of collagen is derived from marine sources while others are obtained from mammalian sources. There are a few threats to human health identified from mammalian collagen due to diseases such as bovine spongiform encephalopathy and hoof-and-mouth disease. Marine-derived collagen is the best alternative to overcome those threats (Ferraro, et al., 2010).

More intensive studies have been carried out on collagen extraction from tuna-by products. Collagen can be recovered from acid, basic or enzymatic treatments. Di and colleagues (2014) isolated acid-soluble collagen and pepsin-soluble collagen from the spine and skull of skipjack

tuna. The results revealed that extracted collagen was mainly composed of type I collagen and contained Glycine (330.2−339.1 residues/1 000 residues) as the major amino acid. Woo and colleagues (2008) extracted and optimized collagen extraction from the dorsal skin of yellowfin tuna using basic extraction (NaOH treatment). According to their findings, the yellowfin tuna dorsal skin collagen had 20.5% amino acid content. Ahamed and his group extracted and characterized collagen from the skin, scales, and bone of bigeye tuna using acid and pepsin extraction methods. The yield of acid-soluble collagen and pepsin-soluble collagen in the skin were recorded as the highest yield of $13.5 \pm 0.6\%$ and $16.7 \pm 0.7\%$, respectively (dry weight). All the extracted collagens consisted of type I collagen and had a high level of amino acids (227–232/1000 residues). Therefore, this study proved that bigeye tuna skin can be used as a potential source of collagen and used as an alternative to mammalian collagen (Ahmed, Haq, & Chun, 2019). Fish-derived collagen has a few drawbacks such as low stability, fishy odour, and dark colour, but this collagen is still well-suited for many industrial applications (Ferraro, et al., 2010).

2.6   Extraction of fish oil from by-products

Fish by-products are used to extract essential fatty acids that cannot be synthesized by mammalian cells. Polyunsaturated fatty acids (PUFA) are important derivatives from fish by-products that have gained interest from the pharmaceutical and food industries. PUFA can be either omega-3 or omega-6 PUFA (depending on their first double bond position). Omega-3 fatty acids have been found to be important in regulating biological and physiological functions in the human body and preventing some diseases (Ferraro, et al., 2010).

Some fish oils (i.e., cod liver oil) are important sources of omega-3 eicosapentaenoic (C20:5n-3; EPA) and docosahexaenoic (C22:6 n-3; DHA) fatty acids. The most important natural sources of omega-3 PUFA are indeed fish oils which are extracted from common species such as sardine, tuna, mackerel, cod, and shark. The PUFA level is 30%, which makes them commercially interesting raw materials to prepare omega-3 PUFA (Ferraro, et al., 2010).

In particular, the cod liver has been widely used to extract omega-3 PUFA. Cod liver oil contains vitamins A, D, and E, and lipids with 50-80% (w/w). Salmon head and Anchovy are good sources of PUFA due to a significant content of lipids, ca. 15–18% (w/w). Among fish flesh, Mackerel flesh PUFA content is around 1810mg per 100g, followed by salmon with 1800mg per 100g, tuna with 1500mg per 100g, herring with 1200mg per 100g, trout with 1060mg per 100g and cod with 240mg per 100g (Ferraro, et al., 2010).

Fats are deposited beneath the skin, head, and dark muscles in tuna. Not only is the lipid content highly diversified, the fatty acid composition of lipids also varies within different body parts (Ferraro, et al., 2010). Tuna heads are known to be a rich source of omega-3 fatty acids. WHO/FAO standard for tuna fish oil is summarized in Table 2.

Many technologies have been developed to extract fish oil from by-products. Wet pressing is one of the common traditional methods to obtain fish oil at an industrial scale. The process involves cooking the raw material, pressing the cooked material, and centrifugation to extract the oil. Ferraro and colleagues (2010) described that the first stage of this production includes mincing, cooking, and pressing of solid fish by-products, from which wastewater and a solid

cake are generated. In the second stage, those wastewaters are dislodged to remove any remaining solid particles, which are then added to the solid cake, and then centrifuged to séparate the oil fraction. Fish oil can have edible and non-edible applications depending on its composition (Ferraro, et al., 2010).

Table 2. Fatty acid composition of tuna oil as determined by gas-liquid chromatography from the authentic sample, expressed as a percentage of total fatty acids

Fatty acid formula	Fatty acid	Fatty acid composition in tuna oil (%)
C14:0	Myristic acid	ND -0.5
C15:0	Pentadecanoic acid	ND-1.5
C16:0	Palmitic acid	14.0-24.0
C16:1(n-7)	Palmitoleic acid	ND-12.5
C17:0	Heptadecanoic acid	ND-3.0
C18:0	Stearic acid	ND-7.5
C18:1 (n-7)	Vaccenic acid	ND-7.0
C18:1 (n-9)	Oleic acid	10.0-25.0
C18:2 (n-6)	Linoleic acid	ND-3.0
C18:3 (n-3)	Linoleic acid	ND-2.0
C18:3 (n-6)	y-linoleic acid	ND-4.0
C18:4 (n-3)	Stearidonic acid	ND-2.0
C20:0	Arachidic acid	ND-2.5
C20:1 (n-9)	Eicosenoic acid	ND-2.5
C20:1 (n-11)	Eicosenoic acid	ND-3.0
C20:4 (n-6)	Arachidonic acid	ND-3.0
C20:4 (n-3)	Eicosatetraenoic acid	ND-1.0
C20:5 (n-3)	Eicosapentaenoic acid	2.5-9.0
C21:5 (n-3)	Heneicosapentaenoic acid	ND-1.0
C22:1(n-9)	Erucic acid	ND-2.0
C22:1 (n-11)	Cetoleic acid	ND-1.0
C22:5 (n-3)	Docosapentaenoic acid	ND-3.0
C22:6 (n-3)	Decosahexaenoic acid	21.0-42.5

2.7 Extraction of protein from tuna by-products

Proteins are extensively used in food to improve the nutritional quality of products such as milk replacers, protein supplements, stabilizers in beverages, and flavour enhancers. Proteins are responsible for several of the functional and bioactive properties of foods. They play an important role in food product development industries. The consumption of animal-derived proteins have been paid attention to the use of alternative protein sources such as fish, for human consumption due to the availability, cost, and risk associated with some animal-derived protein (i.e., pig protein isolates) (Hayes, Mora, Hussey, & Aluko, 2016).

Different extraction methods can be used to isolate fish protein including the pH shift method, solvent extraction method, repeated water washing and refining, enzyme hydrolysis, and a combination of several methods (Shaviklo A. R., 2015). Of these, the pH-shift process is particularly high-performing and inexpensive. Developed in the late 1990s, this method consists of modifying the pH to high (over 11) or low (under 5) to solubilize muscle proteins and separate them from bones, scales, skin, and separate lipids (which can then be separated by centrifugation). The solubilized isolated proteins are then precipitated by adjusting the pH of the protein (i.e., to the other end, high if it was low and low if it was high initially). The pH-shift processing method has been used previously for the isolation of proteins from herring and other small pelagic fish species (Hayes, Mora, Hussey, & Aluko, 2016). Fang and colleagues (2020) described pH shift as the most suitable method for obtaining high-quality protein powder from complex raw materials.

3 MATERIAL AND METHODOLOGY

3.1 Raw material and sample pre-treatment

Juvenile yellowfin tuna (n=7, mean weight = 0.875 ± 0.107 kg) were purchased from Malabe, Colombo fish market in Sri Lanka. Whole frozen tuna were transported at 4^0C to the Tropical Fish Export company's laboratory in a styrofoam box with ice gel packets. Samples were stored at -18^0C in a freezer until further utilized. Frozen samples were thawed with running tap water followed by washing cold water until the temperature reached 5^0C. Thawed yellowfin tuna were dissected longitudinally, and the head, dorsal skin with some muscles, and caudal fin with caudal muscles were manually removed with a filleting knife. Then each sample was washed three times with cold distilled water to remove the surface dirt. Each sample was minced using a laboratory grinder.

Each by-product was vacuumed packed separately and stored at -18^0C until further transport. Then samples were packed in a styrofoam box with ice gel packets and transported to France by air. Then samples were stored at -18^0C C in a freezer until further transport. Finally, samples were transported to Matis, Iceland, by air. The samples were then thawed and placed in polythene bags separately and stored at -20^0C until further use.

3.2 Chemicals

All chemicals used were an analytical grade from Sigma-Aldrich Corporation MO, USA.

3.3 Determination of chemical composition

Characterization of the chemical composition of yellowfin tuna by-products was obtained by measuring moisture content, total lipids, fatty acid composition, ash percentage, and total protein.

3.3.1 Determination of protein content

Protein content was measured by the Dumas method (International Organization of Standardization, 2008). All samples were analysed in duplicate. The crude protein content was calculated as:

Crude protein = Nitrogen content * 6,25

3.3.2 Determination of moisture content

Moisture content was determined by ISO 6496:1999 method. All samples were analysed in duplicate. An empty porcelain bowl was weighed using an electronic balance (GR 200 semi-micro analytical scale, AANDD, Germany). Approximately 5g of minced sample were placed in a bowl and weighed again. Then samples were oven dried at 102 -104 ^{0}C for 24 hours. The bowls were removed from the oven and allowed to cool to ambient temperature in a desiccator for about 30 minutes. Then the dried weight of each sample in the bawl was measured. The results were calculated as the weight loss during drying as a percentage of the wet muscle (% m/m) (International Organization of Standardization, 1999).

$$Water\ content\ (W) = 1 - \frac{m3 - m1}{m2 - m1} * 100(\%)$$

Where:
m_1 is the weight of the bowl (g)
m_2 is the weight of the bowl with wet sample (g)
m_3 is the weight of the bowl with dried sample (g)

3.3.3 Determination of ash content

Ash content was determined by ISO 5984:2022 method. All samples were analysed in duplicate. Approximately 3-5g of the sample was heated at 550^{0}C for 12 -18 hours. Ash content was weighed, and the total ash content was calculated as a percentage of the sample mass (ISO, 2022).

3.3.4 Extraction and determination of total lipid content

Total lipid content was determined using the method described by Bligh & Dyer (1959), with some modifications. All samples were analysed in duplicate. 25g of sample (adapted to the quantity of water in the sample) were added into a 250/500 mL centrifuge bottle. 25mL chloroform and 50mL of methanol were added and homogenized for 2 minutes using a homogenizer (T 25 digital ULTRA-TURRAX, IKA, Germany coupled with S 25 N -25G dispersing tool, IKA, Germany). Then 25mL of chloroform was added and continued mixing for 1 minute. 25mL of 0.88% of KCl was added and mixed for 1 minute. Then the lower chloroform phase was extracted using pipettes. The chloroform phase was filtrated on a glass

microfiber under suction. Then the suction flask content was poured into a 50mL volumetric flask. The aqueous phase was removed using a pipette. The solution was diluted to 50mL using chloroform.

A screw cap glass tube without a cap was weighed using the electronic balance (GR 200 semi-micro analytical scale, AANDD, Germany). 2mL of lipid extraction was added to a screw cap culture tube. The solvent contained in the lipid extract was removed at 55^0C using a nitrogen jet. The sample was allowed to cool and weighed. The weight difference in 2mL was calculated and multiplied by the total volume of chloroform (50 mL) solution and divided by the initial weight of the sample used for lipid extraction.

$$Total\ lipid\ content\ (\%) = \frac{(W2 - W1) * 50 * 100}{2 * W3}$$

Where:
W1: Initial weight of screw cap glass tube (g)
W2: Final weight of screw cap glass tube with lipid extract (g)
W3: Initial weight of raw sample used to extract lipids (g)

3.3.5 *Determination of Fatty acid composition*

Between 60-90 mg of extracted lipid was taken (the chloroform phase from the B&D extract was removed with a nitrogen jet). 1.5 mL of 0.5 NaOH in methanol was mixed with extracted lipid and heated in the oven for 7 minutes at 100 ^{0}C. Samples were allowed to cool. 2mL of BCl3 12% in methanol was added into each sample and heated in an oven for 30 minutes at 100^0C. Then the samples were allowed to cool and 1mL of standard solution (C23:0 in isooctane) and 5mL of concentrated NaCl was added. Solution was vortexed for ½ minute. Then Isooctane layer was transferred into a small test tube with a small amount of natrium sulphate.1mL of clean isooctane was added to the former screw cap glass tube and vortexed again for ½ minute. Remaining isooctane layer was transferred into a small test tube. Then 1.5 mL of solution was transferred to small glass vials for gas chromatography. All samples were analysed in duplicate.

Fatty acid methyl esters (FAME) were separated on a Varian 3900 GC equipped with a fused silica capillary column (Omegawax 250, 30 m x 0.25 mm x 0.20 µm film), split injector and flame ionization detector fitted with Galaxie Chromatography Data System, Version 1.9.3.2 software. The injection volume was 1µL with a 15:1 split at an inlet temperature of 100^0C. Data for each fatty acid were expressed as g/100g of extracted fish oil. Peak areas were determined using 1.9.3.2. software.

3.4 Extraction of possible value-added materials

Based on the chemical composition of residuals from preliminary extraction I, it was decided to follow the valorisation strategy to recover oil, protein, and collagen from by-products (Figure 5).
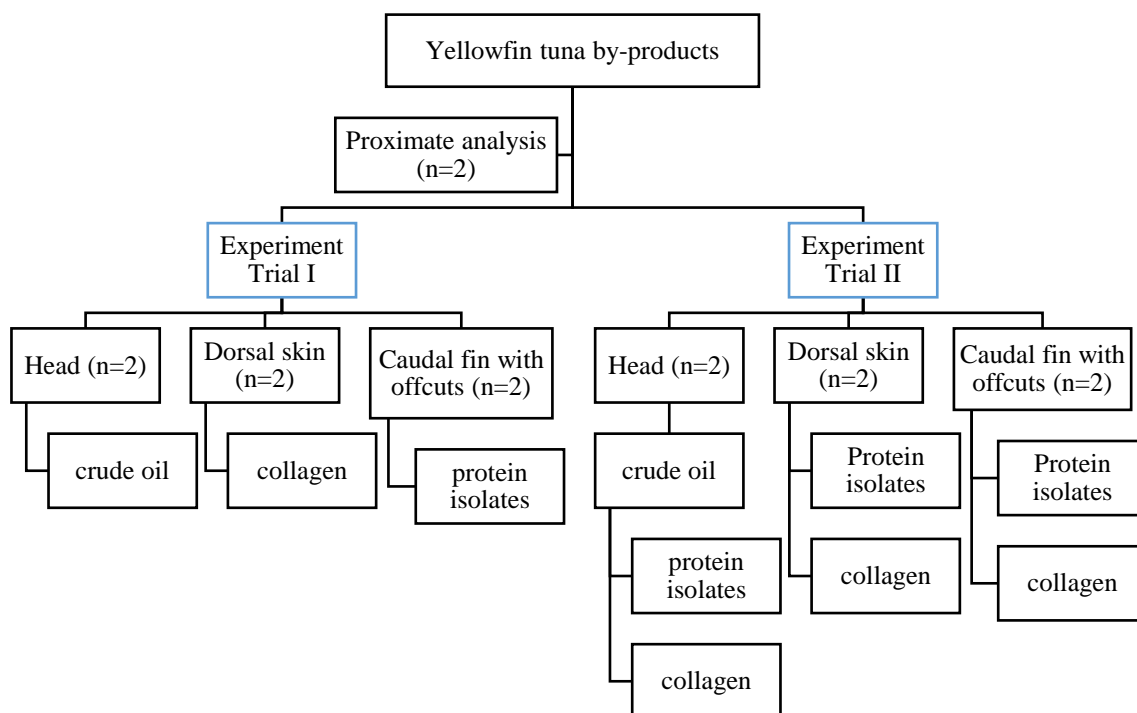
Figure 5. Experimental plan based on chemical composition of raw materials and residuals.

3.5 Experiment Trial I

The suitable extraction methods to extract collagen, crude oil, and proteins from each by-product were done based on the chemical composition of the yellowfin tuna by-products.

3.5.1 Isolation of Fish protein from yellowfin tuna caudal fin with offcuts

The protein powder was isolated from the yellowfin tuna caudal fin with offcuts using the pH shift method. The sample was taken with cold water of a ratio of 1:3 and mixed for 30 minutes. The solution pH was adjusted up to pH11 by adding 13.00ml of 2M NaOH slowly. Then the solution was filtered using cheesecloth. All the bones, fins, skin, and other residuals were taken out and weighed separately. Precipitation of soluble protein is accomplished by adjusting the solution from pH5.3 to 5.5 to reach the isoelectric point of the myofibrillar proteins using 3M HCl. The protein isolate (PI) was drained through the 50 µm pore size cheesecloth to remove soluble impurities. The fish protein isolate was weighed. The protein isolate was frozen at -18^{0}C and after that, the water contained in the fish protein isolate was removed through freeze-drying.

The yield of protein isolate was calculated as the percentage ratio of the weight of the initial caudal fin sample in gram to the wet weight of the protein isolate obtained in gram.

$$\text{Yield (\%) (wet weight basis)} = \frac{weight\ of\ protein\ isolate\ (g)}{\text{Initial wet weight of the raw material (g)}} X100$$
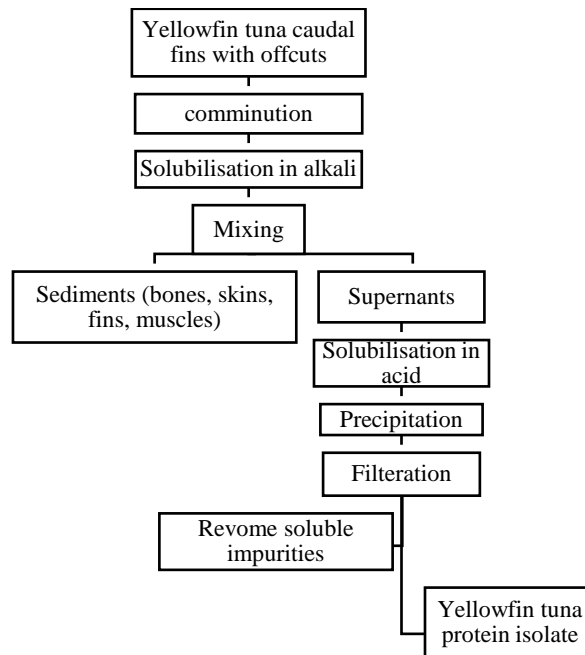
Figure 6. Chart of the pH shift method of extracting yellowfin tuna protein isolates

The functional properties, including foaming capacity and foaming stability, emulsion stability and gelation properties of extracted dry protein powder were calculated.

To analyse foaming capacity, 20mL of 1% protein sample was homogenized with a homogenizer (Ultra Turrax T25) for 2 minutes at 10000 rpm. Then immediately transferred to a 25 mL measuring cylinder. The total volume was written down at time zero, 15, 30, and 60 minutes after homogenization. Foaming capacity and stability were calculated by following formula.

$$\text{Foaming Capacity (\%):} \qquad FC = \frac{(V2-V1)}{V1} * 100$$

$$\text{Foaming Stability (\%):} \qquad FS = \frac{V3-V1}{V2-V1} * 100$$

Where:
V1: volume before homogenization
V2: volume after homogenization
V3: volume after standing.

To analyse emulsion stability, 0.5g of protein powder was added into 500 mL plastic beaker. Then, 50mL of 0.1M NaCl and 50mL of oil were added and homogenized with Ultra Turrax at 13500 rpm for 2 minutes. The solution was poured into a 50mL plastic graduated cylinder and let stand for 15 minutes. The total volume water phase volume were recorded.

Emulsion stability was calculated according to the method described by Kristinsson & Rasco, (2000):

$$Emulsion\ stability\ (ES) = \frac{(total\ volume\ [mL] - waterphase\ volume\ [ml]) * 100}{total\ volume\ [mL]}$$

To analyse gelation properties, 0.09g and 0.21g of protein powder were mixed with 3mL of deionized water in test tubes. The solution was mixed on vortex for 10 seconds. Then the test tubes were placed in boiling water bath at 90^0C for one hour. Test tubes were cooled under running tap water and placed in the cooler at 4^0C for 3 hours. Gelation properties (sample slip from inverted test tube, slight turbidity and sample fall down from the inverted test tube) were observed and recorded.

3.5.2 Extraction of collagen from yellowfin tuna dorsal skin

Acid-soluble collagen was extracted by the following protocol:

Pre-treatment of dorsal skin
To remove the non-collagenous proteins, the samples were soaked in 0.1M NaOH (sodium hydroxide 98%, Sigma-Aldrich) with a sample and solution ratio of 1:10 w/v at room temperature for one and a half hours. The alkali solution was changed every 30 minutes. Then the solution was strained from the sample and washed with cold tap water continuously until a neutral or faintly basic pH of wash water was obtained.

Extraction of acid-soluble collagen
Pre-treated samples were then soaked in 0.05M acetic acid (Acetic acid 99%, Sigma-Aldrich) solution with a sample/solution ratio of 1:10 w/v with stirring. The solution was changed twice every 45 minutes to remove fat and other impurities. The solution was strained from the sample. The skins were again soaked in 0.05M acetic acid with a skin/solution ratio of 1:10 w/v for 2 hours. Then the solution was strained and washed with tap water until pH reached 6. Thereafter, the collagen was extracted with tap water with a sample/water ratio of 1:3 w/v at 45^0C for 16 hours with continuous stirring (150 rpm) in an incubator shaker. Then the mixture was filtered using cheesecloth with a pore size of 50μm and weighed. Finally, extracted collagen was freeze-dried.

The yield of extracted collagen was calculated as percentage ratio of weight of the initial dorsal skin sample (wet or dry) in gram to the weight of the dry collagen obtained in gram.

$$\text{Yield (\%) (wet weight basis)} = \frac{Acid-soluble\ collagen\ weight\ (g)}{\text{Initial wet weight of the raw material (g)}}\ X100$$

$$\text{Yield (\%) (dry weight basis)} = \frac{Acid-soluble\ collagen\ weight\ (g)}{\text{Initial dry weight of the raw material (g)}}\ X100$$

3.5.3 Extraction of crude oil from yellowfin tuna heads

Crude oil was directly extracted from yellowfin tuna heads using a conventional heat-based method. Samples were extracted in duplicate. In brief, 100g of minced head was manually mixed with 51 mL of water. The slurry of one sample was heated at 85 ^{0}C for 20 minutes and other sample was heated for 40 minutes. The slurry was manually mixed every 10 minutes. Then the slurry was strained from residuals and centrifuged at 5100 x g, 22^0C for 10 minutes. The supernatant was collected and stored in the refrigerator for 2 hours. Finally, the supernatant was separated and stored until further analysis.

Oil recovery yield, the relative amount of extracted crude oil in relation to the initial by-product weight was expressed as a percentage. The fatty acid composition of crude oil was determined according to the method described in section 3.2.5.

$$\text{Oil weight (g)} = \text{Extract oil from by-products}$$

$$\text{Oil percentage recovery} = \text{the weight of oil recovered (g) / weight of the initial sample (g)}$$

3.6   Experiment Trial II

Based on chemical composition of the raw materials, basic extraction methods were applied to extract the crude oil, fish protein isolates, and collagen from yellowfin tuna head, caudal fin with offcuts, and dorsal skin respectively. The ash content of extracted compounds and residual were measured according to the methods described in section 3.3.3. Based on the results, the residual contained a high protein fraction which is referred to biorefinery approach to recover fish protein and/or collagen from tuna head, caudal fin with offcuts, and dorsal skin in Experiment Trial II.

3.6.1   Biorefinery approach for yellowfin tuna heads

The yellowfin tuna heads were defrosted under running tap water. The sample was mixed with cold water of a ratio of 1:3 and homogenized at 15,000 rpm for 90 seconds. The solution pH was adjusted up to pH11 by slowly adding 13.00ml of 2M NaOH. Then the solution was incubated in ice for 10 minutes followed by centrifugation at 5000 x g, 4^0C for 20 minutes to facilitate soluble protein, oil, and collagenous components. After the centrifugation, three layers were generated consisting top emulsion layer, mid layer, and bottom layer (Figure 7).

To separate the fish oil, the top emulsion layer was scooped out using a spatula and frozen at -20^0C for two hours. Then the sample was defrosted with running tap water and centrifuged at 5000 x g, 4^0C for 10 minutes. The supernatant was separated and weighed. The remaining solution was added to mid layer from primary fractionation containing soluble proteins.

To extract the protein isolates, the mid layer was collected from primary fractionation and the soluble proteins were precipitated by adjusting solution pH to 5.3 using 3M HCl. Protein isolate was drained through the 50µm pore size cheesecloth to remove soluble impurities. Then the fish protein isolate was weighed. The protein isolate was frozen at -18^0C and freeze dried.
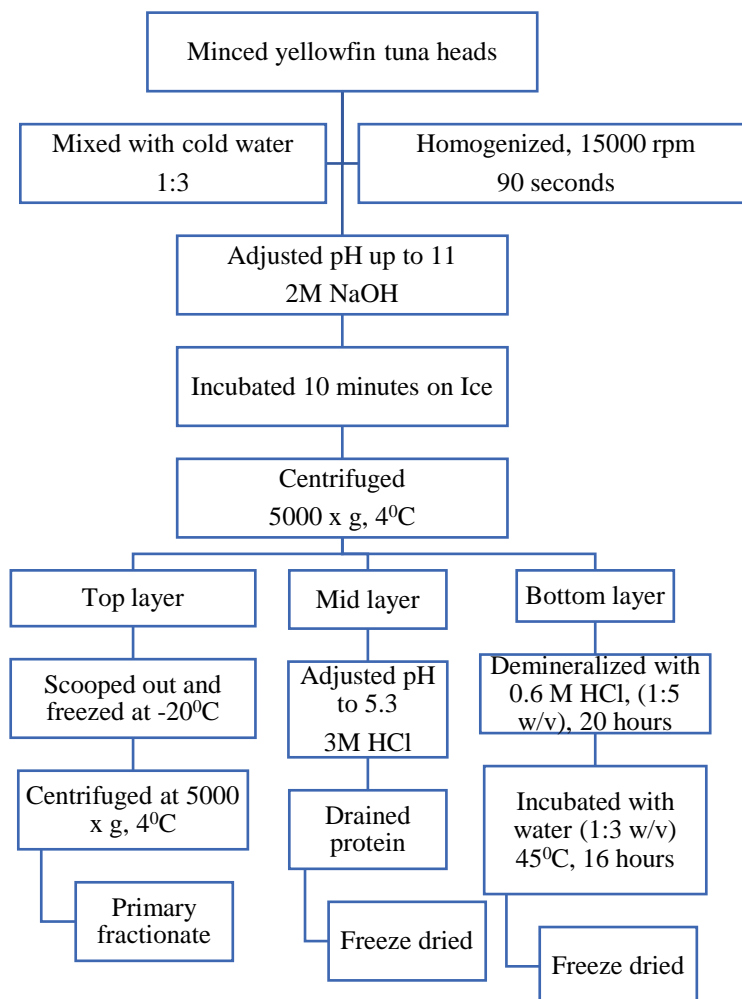
Figure 7. Schematic representation of biorefinery approach for juvenile yellowfin tuna heads (n=2)

To extract acid-soluble collagen, bone demineralization was performed according to the method described by Arnesen & Gildberg (2006), with some modifications. The solid remaining in bottom layer was soaked in 0.6M HCl with a ratio of 1:5 (w/v) for 20 hours at room temperature. The demineralized bones and other residuals were separated from acid solution which contained minerals. Then the sample was washed with cold water until pH reached 3.6. Thereafter, the collagen was extracted with tap water with a sample/water ratio of 1:3 w/v at 45^0C for 16 hours with continuous stirring (150 rpm) in an incubator shaker. Then the mixture was filtered using cheesecloth with a pore size of 50µm and weighed. Finally, extracted collagen was freeze-dried.

3.6.2 Biorefinery approach for yellowfin tuna dorsal skin

The defrosted dorsal skin sample was taken with cold water of a ratio of 1:3 and mixed for 30 minutes. The solution pH was adjusted up to pH11 by adding 13ml of 2M NaOH slowly. Then the solution was filtered using cheesecloth. All the bones, fins, skin and other residuals were taken out and weighed separately. Precipitation of soluble protein was done by adjusting the solution pH into 5.3 to 5.5 using 3M HCl for reaching the isoelectric point of the myofibrillar proteins. The protein isolate (PI) was drained through the 50µm pore size cheesecloth to remove soluble impurities. The fish protein isolate was weighed. The protein isolate was frozen at -18^0C and after that the water contained in fish protein isolate was removed through the freeze-drying.

For extraction of acid-soluble collagen, all fins, skin and other residuals were taken out and weighed separately. The bone sample was soaked in 0.6M HCl with a ratio of 1:5 (w/v) for 20 hours at room temperature. Then the sample was washed with cold water until pH reached 3.6. Thereafter, the collagen was extracted with tap water with a sample/water ratio of 1:3 w/v at 45^0C for 16 hours with continuous stirring (150 rpm) in an incubator shaker. Then the mixture was filtered using cheesecloth with a pore size of 50 µm and weighed. Finally, extracted collagen was freeze-dried.

3.6.3   *Biorefinery approach for yellowfin tuna caudal fin with offcuts*

The defrosted samples were divided into two, Sample A and B. Both samples were taken with cold water in a ratio of 1:3 and mixed for 30 minutes. The solution was adjusted up to pH11 by adding 13ml of 2M NaOH slowly. Then 0.5% $H2O2$ was added in a ratio of 1:2 (w/v) to sample A and allowed to settle for 20 minutes. Then both solutions were filtered using cheesecloth. All the bones, fins, skin and other residuals were taken out and weighed separately. Precipitation of soluble protein is accomplished by adjusting the solution pH from 5.3 to 5.5 using 3M HCl. The protein isolate was drained through the 50µm pore size cheesecloth to remove soluble impurities. Then 0.5% $H2O2$ was added with a ratio 1:2 (w/v) to sample B and allowed to settle for 20 minutes. The fish protein isolate was weighed. The protein isolate was frozen at -18^0C and after that the water contained in fish protein isolate was removed through the freeze-drying.

To extract acid-soluble collagen, all bones, fins, skin and other residuals were taken out and weighed separately. The bone sample was soaked in 0.6M HCl with a ratio of 1:5 (w/v) for 20 hours at room temperature. Then the sample was washed with cold water until pH reached 3.6. Thereafter, the collagen was extracted with tap water with a sample/water ratio of 1:3 w/v at 80^0C for 16 hours with continuous stirring (150 rpm) in an incubator shaker. Then the mixture was filtered using cheesecloth with a pore size of 50µm and weighed. Finally, extracted collagen was freeze-dried.

3.7   Determination of pH and colour

The pH values of extracted protein isolates, collagen and oil were measured using a pH meter (Knick Portavo 902 pH meter with SN E20 a probe).

The colour of yellowfin tuna protein isolates and collagen were measured in colourimeter (CR-400, Minolta). The colourimeter was standardized with a white marble plate and measurements were taken from the instrument monitor. The degree of lightness (L*), redness (a*) and yellowness (b*) were measured using the tristimulus colour coordinates (CIELAB-system). The parameters were indicated as: L* from black (0) to white (100); a* from green (-120/negative values) to red (+ 120/ positive values); and b* from blue (-120/ negative values) to yellow (+120/ positive values) (Jafarpour, et al., 2020). The whiteness was calculated to assess the colour (Judd and Wyszecki, 1963).

$$Whiteness = (100 - [(100\text{-}L^*)^2 + a^{*2} + b^{*2}]^{1/2})$$

Where:
L: Lightness
a: Redness

b: Yellowness

3.8 Data analysis

The mean and standard deviations (Mean ± SD) of yield of protein, moisture content, lipid content and ash contents were calculated using Microsoft Excel 2016.

4 RESULTS

4.1 Approximate composition of yellowfin tuna by-products

Table 3. Approximate composition (mean ± SD) of yellowfin tuna by-products.

By-products	Replicates (n)	Protein (%)	Moisture (%)	Ash (%)	Lipid (%)
Head	2	18.75 ± 0.21	73.10 ± 0.01	8.65 ± 0.78	4.18 ± 0.33
Dorsal	2	22.75 ± 0.64	75.85 ± 1.09	1.30 ± 0.28	0.81± 0.06
Caudal (with off-cuts)	2	23.80 ± 0.14	77.06 ± 0.31	5.60 ± 0.42	1.43 ± 0.16

Table 3 shows the approximate composition of yellowfin tuna heads, dorsal skin, and caudal fin with offcuts. The protein content varied from 18.75% to 23.80%. The highest protein content was obtained from the caudal fin with offcuts (23.80 ± 0.14) and the lowest was obtained from the yellowfin tuna head (18.75 ± 0.21). The highest moisture content was recorded in the caudal fin with offcuts (77.06 ± 0.31%) and the lowest was in the head (73.10 ± 0.01%). The ash content was comparatively higher (8.65 ± 0.78%) in yellowfin tuna heads than in the dorsal and caudal by-products. In terms of total lipid content, dorsal skin contained the lowest amount of fat (0.81±0.06%) while the highest fat content was found in yellowfin tuna heads (4.18±0.33%) followed by the caudal fin (1.43±0.16%). The data on total fat values (mean ± SD) in yellowfin tuna by-products is given in Figure 8.
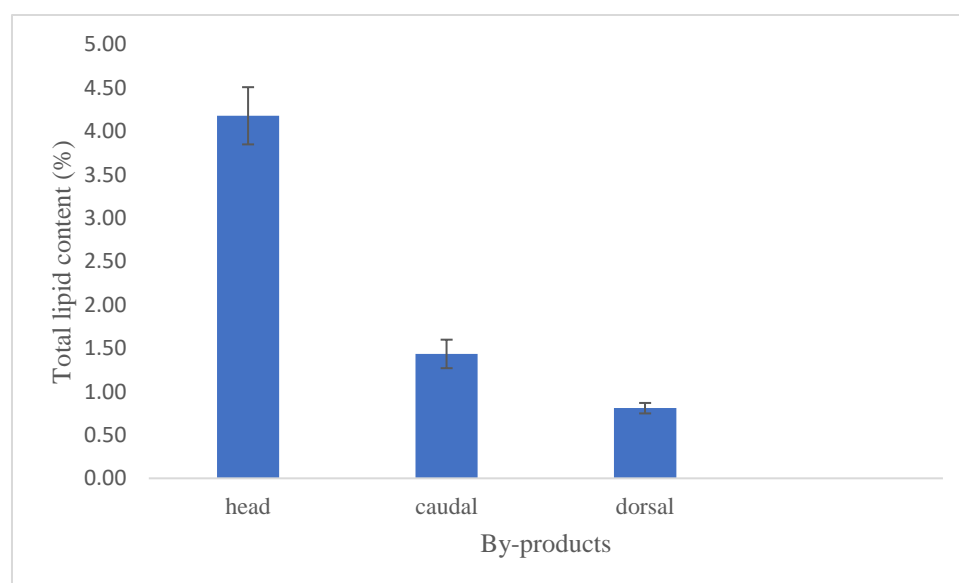
Figure 8. Total lipid content (mean ± SD) in yellowfin tuna by-products (n=2).

4.2 Fatty acid profile in yellowfin tuna by-products
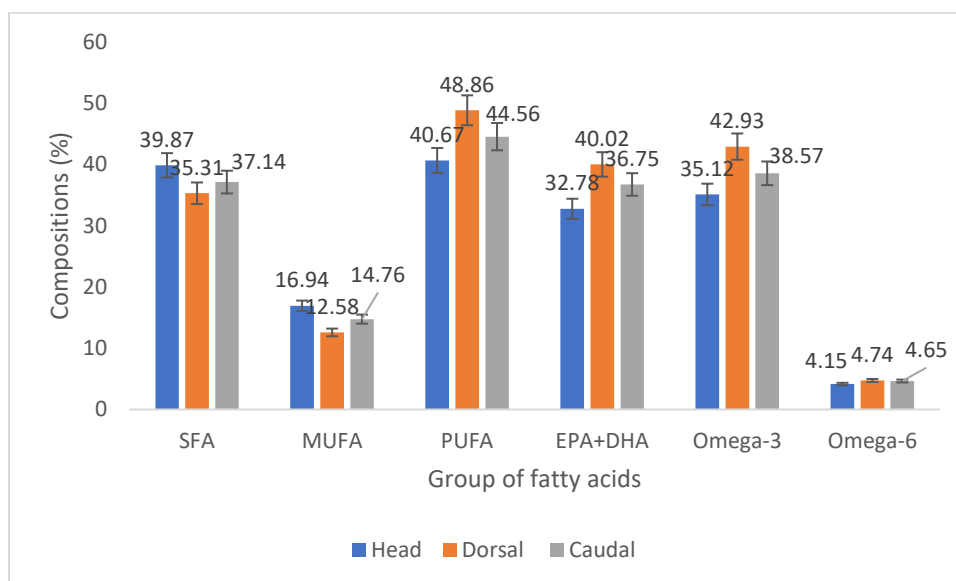
Figure 9. Fatty acid composition of yellowfin tuna by-products.

The fatty acid profile of the main fatty acid groups of yellowfin tuna by-products is presented in Table 4 and the full fatty acid composition is presented in Table A1. The most abundant fatty acids in by-products were identified as docosahexaenoic acid (DHA/PUFA) (Head: 26.63 ±0.19%, Dorsal: 34.71±0.72%, Caudal: 31.27±0.90%), followed by palmitic acid (SFA) (Head: 22.52 ±0.22%, Dorsal: 20.14±0.47%, Caudal: 20.72±0.20%), oleic acid (MUFA) (Head: 10.07 ±0.17%, Dorsal: 7.77±0.12%, Caudal: 8.82±0.28%) and eicosapentaenoic acid (EPA/PUFA) (Head: 6.15 ±0.03%, Dorsal: 5.31±0.04%, Caudal: 5.49±0.03%). Lower frequency fatty acids included Eicosenoic acid, Linoleic acid, Vaccenic acid, Heptadecanoic acid, Palmitoleic acid, and Myristic acid while the rest were ≤ 1.0% at all sampling points. Furthermore, results indicate that Omega-3 PUFAs were ranging from 35.12% to 42.93% and were higher the omega-6 PUFAs ranging from 4.15% to 4.74%.

4.3 pH shift method for recovery of fish protein isolates (FPI) from yellowfin tuna by-products
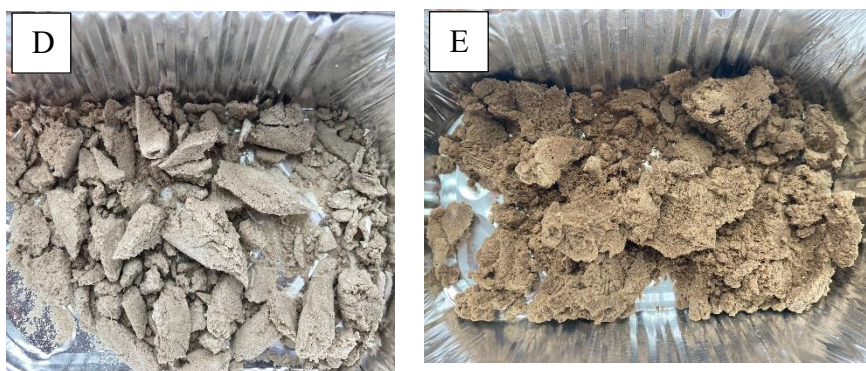
Figure 10. Protein isolates extracted from yellowfin tuna by-products (A: Caudal (Trial I), B: Caudal (Pre-H_2O_2), C: Caudal (post-H_2O_2), D: Dorsal, E: Head)

Table 4. Ash content and yield percentage of FPI recovered from yellowfin tuna by-products.

By-products	Experiment	Ash content (%)	Yield (%) wet weight basis
Caudal	Trial I	4.0	2.65
Caudal (Pre H2O2)	Trial II	2.8	10.77
Caudal (Post H2O2)	Trial II	1.4	10.85
Dorsal	Trial II	2.1	10.90
Head	Trial II	3.2	8.81

*All the data are based on two replicates.

High yield recovery (wet weight basis) was observed from caudal (Pre and post H_2O_2 treatments) and dorsal samples. The lowest yield was obtained from the caudal sample in Trial I. Ash content of protein isolates was higher in caudal (Trial I, 4%) and head (3.2%) than in the other samples. The lowest ash content (1.4%) was reported in the caudal (Post-H_2O_2) sample. Owing to their ash content, similar results of protein content can be expected in caudal (pre-H_2O_2 treatment) samples and dorsal samples. The protein content of protein isolates from Trial I was 88% and ash content was higher than the ash content obtained from the Trial II. Therefore, it is expected that protein content in Trial II will be higher than the Trial I as the ash content was lower than the Trial I.

4.3.1 Colour characteristics of fish protein isolate
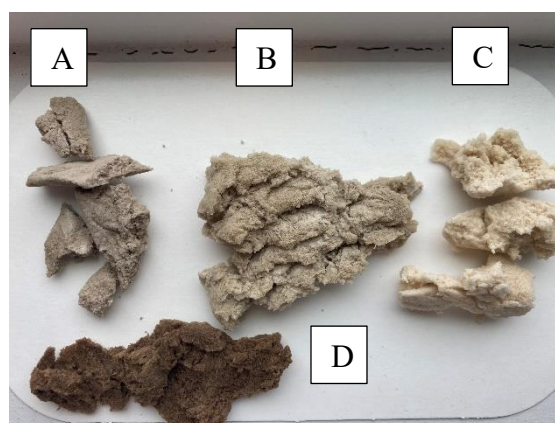
Figure 11. Colour differences of protein isolates extracted from different by-products; A: Dorsal skin, B: caudal fin with pre-H_2O_2 treatment, C: caudal fin with post-H_2O_2 treatment, D: head.

Table 5. Tristimulus colour parameters of fish protein powder recovered from yellowfin tuna by-products.

Sample	Experiment	Lightness (L*)	Redness (a*)	Yellowness (b*)	Whiteness
Caudal	Trial I	56.16 ± 3.23	2.05 ± 0.25	14.01 ± 0.68	53.93 ± 1.35
Caudal (PreH$_2$O$_2$)	Trial II	65.97 ± 4.07	1.46 ± 0.20	16.43 ±1.63	62.09 ± 3.42
Caudal (PostH$_2$O$_2$)	Trial II	73.72 ± 7.56	1.66 ± 0.52	18.7 ± 2.55	67.19 ± 4.92
Head	Trial II	43.67 ± 2.46	5.71 ± 0.46	12.59 ± 1.77	41.95 ± 1.94
Dorsal	Trial II	55.23 ± 5.61	1.95 ± 0.17	10.87 ± 0.91	53.84 ± 5.18

*Values are mean ± standard deviation of triplicate measurements.

Protein isolates with hydrogen peroxide treatments (pre and post) showed the highest lightness (73.72 & 65.97), whiteness (67.19 & 62.09), yellowness (16,43 & 18.7), and lowest redness (1.46 &1.66) compared to other samples.

4.3.2 Functional Properties of Yellowfin tuna protein isolates

Table 6. Functional properties of yellowfin tuna protein isolates

Sample	Emulsifying stability (%)	Foaming capacity (%)	Foaming stability (%)	Gelation
Yellowfin tuna protein isolate	57.95±14.21	26.47±3.05	88.89±0.00	Present in 0.21 concentration

*Values are mean ± standard deviation of duplicate measurements.

The functional properties of emulsifying activity, foaming capacity, foaming stability, and gelation were determined for caudal sample (Trial I) as a key sample and shown in Table 7.

4.4 Extraction of collagen from yellowfin tuna dorsal skin

Figure 12. Acid-soluble collagen extracted from the yellowfin tuna by-products; A: Dorsal skin (Trial I), B: Dorsal skin (Trial II), C: Head, D: Caudal (pre-H_2O_2), E: Caudal (post-H_2O_2).

Table 7. Yield and ash content of extracted collagen from the yellowfin tuna by-products. All the data are based on two replicates.

By-products	Experiment	Ash content (%)	Yield (%) dry weight basis
Head	Trial II	10.4	21.16
Dorsal	Trial I	12.8	3.11
Dorsal	Trial II	4.2	9.51
Caudal (Pre H_2O_2)	Trial II	-	39.44
Caudal (Post H_2O_2)	Trail II	-	23.17

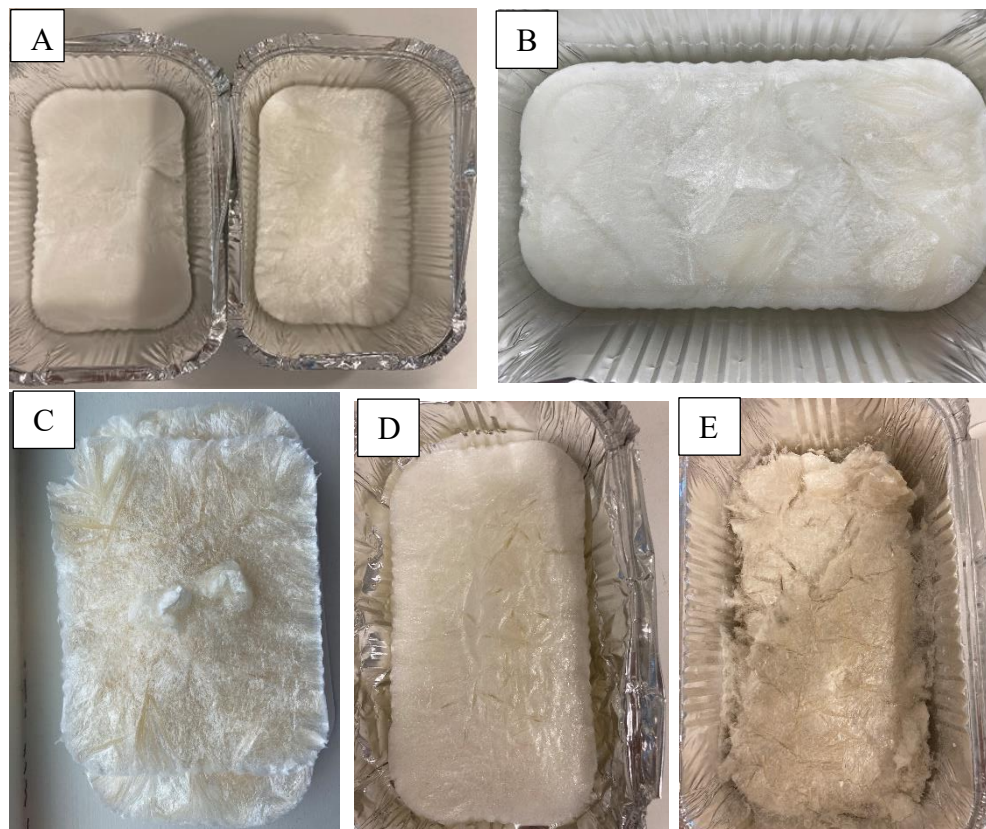The yield acid soluble collagen isolated from yellowfin tuna head, dorsal skin, and caudal fin with offcuts in Trial I and II ranged from 3.119% to 39.44%. The maximum yield (39.44%) was obtained caudal sample treated with pre-H2O2. The lowest yield was 3.11% in the dorsal sample in the first trial. However, the collagen extracted by caudal and dorsal from the residual parts (containing bones) collected after the pH shift treated with HCl for demineralization showed comparatively higher yield than the dorsal skin collagen which was extracted with acetic acid. The highest ash content was recorded in the dorsal sample (Trial I, 12.8%) followed by the head (10.4%) and dorsal (4.2%) in Trial II.

4.4.1 Colour characteristics of acid-soluble collagen

Table 8. Tristimulus colour parameters of acid-soluble collagen recovered from yellowfin tuna by-products.

Sample	Experiment	Lightness (L*)	Redness (a*)	Yellowness (b*)	Whiteness
Head	Trial I	74.64 ±5.79	-1.13 ±0.29	8.58 ±3.93	73.11 ±6.55
Caudal (PostH_2O_2)	Trial II	76.93 ±7.46	-0.89 ±0.32	3.57 ±1.83	76.63 ±7.62
Caudal (PreH_2O_2)	Trial II	68.78 ±7.49	0.78 ±0.31	9.30 ±1.54	73.19 ±9.16
Dorsal	Trial II	92.02 ±1.67	-1.24 ±0.09	5.95 ±1.08	89.95 ±1.84
Dorsal	Trial I	97.12 ±0.78	-0.99 ±0.28	7.11 ±2.13	92.75 ±2.39

*Values are mean ± standard deviation of triplicate measurements.

Acid-soluble collagen extracted from dorsal (Trial I and Trial II) showed the highest lightness (97.12 & 92.02), and whiteness (92.75 & 89.95) compared to other samples. Yellowness of the collagen was highest in caudal (Pre-H_2O_2, 9.30) and head (8.58) samples.

4.5 Extraction of crude oil from yellowfin tuna heads

Table 9.

Total oil recovery yield from yellowfin tuna heads

By-products	Experiments	Yield (%) wet weight basis
Head	Trial I	0.75
Head	Trial II	1.52

*All the data are based on two replicates.

The highest yield of fish oil was recovered in trial II using the pH shift method (1.52% per amount of by-product) while the lowest was in trial I (0.75%) by the conventional heat-based extraction method.
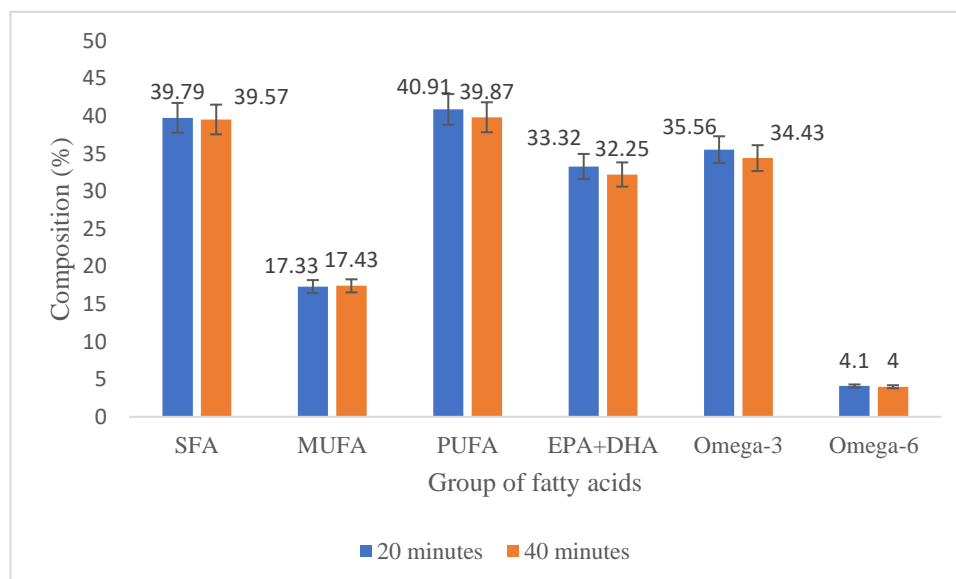
Figure 13. Fatty acid composition (%) of oils recovered from yellowfin tuna heads (n=3) using two different cooking times by conventional heat method.

The fatty acid composition of the extracted oil obtained from the conventional heat-based method was evaluated. The time impact of being heated at 90^0C for 20 to 40 minutes was analysed as heat is known to degrade PUFA. Overall, the two oils had a similar fatty acid composition even if the oil heated for 40 minutes showed slightly lower amounts of omega-3, omega-6, EPA+DHA, and PUFA than the oil heated for 20 minutes.

4.6   Mass balance for yellowfin tuna by-products
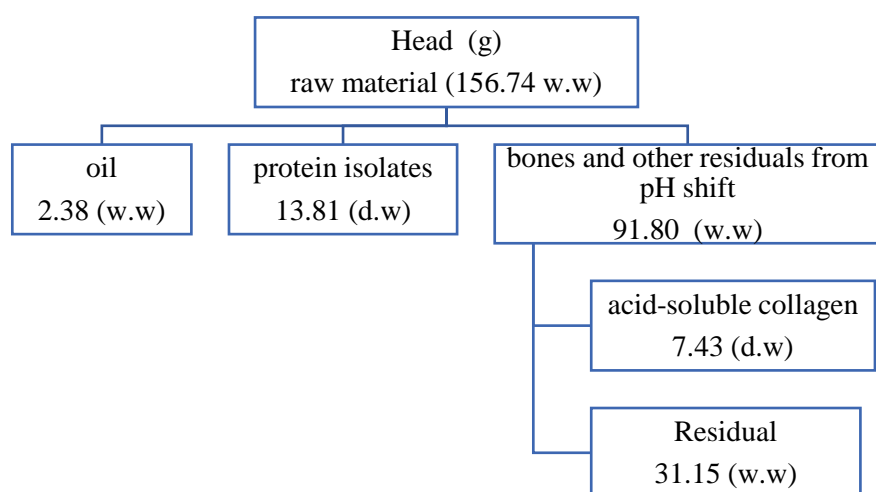
Figure 14. Mass balance of yellowfin tuna head (weight expressed as dry weight (d.w.) or wet weight (w.w)
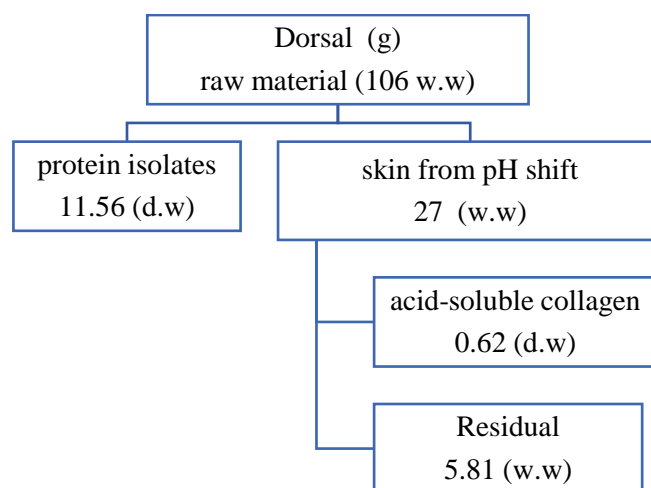
Figure 15. Mass balance of yellowfin tuna dorsal skin (weight expressed as dry weight (d.w.) or wet weight (w.w)
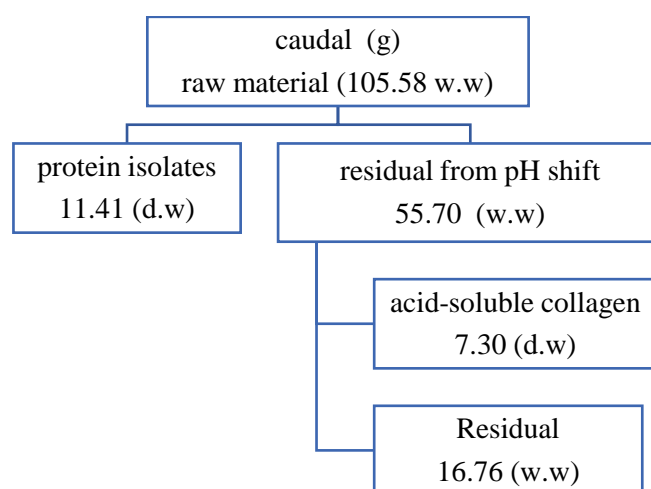
Figure 16. Mass balance of yellowfin tuna caudal with some offcuts (weight expressed as dry weight (d.w.) or wet weight (w.w).

5   DISCUSSION

5.1   Approximate composition of yellowfin tuna by-products

The fish used for this study were juvenile yellowfin tuna which weighed less than 1 kg each. As commercial-size tuna ranged from 20 kg -90kg, their approximate composition could be significantly different from this study. This is especially true of the head, which is smaller and contains more bones when the fish are juveniles than when they are adults.

Table 3 summarizes the approximate composition of yellowfin tuna by-products. The protein content of by-products varied from 18.75% to 23.80%. The highest protein content was obtained from the caudal fin with offcuts. This might be due to the remaining muscle meat attached to the caudal peduncle that have high proteins content. Similar results were reported in a study conducted for yellowfin tuna muscle (23.52%) by Peng and colleagues (2013). Generally, the level of protein content in fish varied from 16% to 25% depending on species, season, sex, and size as shown in the study. According to Karunarathna and Attaygalle (2010), the average protein content of marine and fresh water fish muscles was 18.5 %. In this study,

the protein content in the caudal fin with offcuts muscles was higher than the average value. Moreover, tuna species are known as an excellent source of high-quality protein for humans. Therefore, the caudal fin with offcuts containing the highest amount of protein could be utilized to extract the protein isolate for human consumption.

The moisture content of yellowfin tuna by-products was summarized in Table 2. However, the results were line with other studies done for yellowfin tuna muscle tissue (73.57±0.55%), head (71.93 ± 0.71%), red muscle (70.83 ± 0.70%) (Garofalo & Tommasi, 2023; Karunarathna & Attaygalle, 2010; Peng, Chen, ShiZhaohong, & Wang, 2013).

The ash content in dorsal skin (1.30 ± 0.28%) was similar to the value reported for yellowfin tuna muscle tissue (1.54±0.06%) by Peng and colleagues (2013). However, Karunarathna and Attaygalle (2010) reported that the ash content of yellowfin tuna head (1.00±0.06%) was lower than the value found here. Ash content indicates the mineral concentration and trace elements of yellowfin tuna by-products. Ash content in fish is depends on their body parts, size, feeding behavior, environment, and season. In the present study, ash content in by-products was accumulated more than in previous studies (Karunarathna & Attaygalle, 2010; Peng, Chen, ShiZhaohong, & Wang, 2013). This might be due to the smaller size of yellowfin tuna containing high mineral content and a high bone-to-flesh ratio (Rani, Kumar, Rao, & Shameem, 2016).

Fat content is inversely related to moisture content. In this study, the moisture content of yellowfin tuna was high, therefore the fat content was considerably low. But the value obtained for yellowfin tuna head was higher than the values reported in previous studies done for yellowfin tuna head (0.98±0.13%), skipjack tuna head (0.72±0.23%) and little tuna head (0.67±0.32%) (Karunarathna & Attaygalle, 2010). Therefore, the yellowfin tuna heads are a potential source of valuable fish oil. As described by Mahaliyana and colleagues (2015), lipid content can fluctuate by habitat, growth phase, season, feeding behavior, muscle type, and spawning. Fishes that contain more than 2% fat can be considered fatty fish. In this study, juvenile yellowfin tunas (mean weight: 0.875 ± 0. 107 kg) were used. Although tuna fish are considered fatty fish, the juvenile fish contain less fat than adults. Therefore, we can expect a higher fat content in commercial-size yellowfin tuna by-products (25kg -90kg).

5.2   Biorefinery for yellowfin tuna by-products

The head of yellowfin tuna generally accounts for 15% of total body weight, containing eyes, flesh, skull, bones, and gills. Due to their complex composition of hard waste, it is difficult to achieve maximum utilization. However, oil, protein, and collagen have been recovered from yellowfin tuna head waste.

The caudal fin with offcuts is another principal by-product from yellowfin tuna processing companies. This waste contains a high proportion of protein which is a profitable source for extracted collagen and protein isolates. This study has investigated the recovery of collagen and protein isolates as the first attempt through the biorefinery approach which can be developed as ingredients for food, pharmaceutical and cosmetic industries.

Much research is currently focused on the extraction of collagen from the dorsal skin of fish. Owing to the flesh and skin composition of dorsal skin by-products, collagen, and protein isolates were recovered from the sequential valorization process. Based on the mass balance

(Figure:14, 15, 16), out of 105.58g (wet weight) of caudal sample, 11.41g (dry weight) of protein isolates were recovered. 7.30g (dry weight) of collagen was recovered from 55.70 g (wet weight) of bones while 106 g (wet weight) of dorsal sample produced 11.56 g (dry weight) of protein isolates. 0.62 g (dry weight) of collagen as recovered from 27 g (wet weight) of skin rest materials. Valorization of 156.74 g (wet weight) of yellowfin tuna heads resulted in 2.38 g of oil, 13.81 g (dry weight) of protein isolates, and 7.43 g (dry weight) of collagen from 91.80g (wet weight) rest bones. These yields showed maximum recovery of high-value compounds from the raw materials through this biorefinery approach. Moreover, this biorefinery approach is an excellent candidate to recover environmentally sustainable high-added-value products from yellowfin tuna by-products.

5.3 Extraction of protein isolates from yellowfin tuna by-products

Fish protein isolates were extracted from underutilized fish by-products. In this study, yellowfin tuna fish protein isolates were recovered from the head, dorsal skin, and caudal fish using the pH shift method. These complex raw materials are rich in muscle proteins. Acidic or basic conditions can alter the solubility of the muscle proteins. When the base (OH^-)is added, increase the negative charge on the surface of the protein. Then they start electrostatic interaction with water and become water-soluble (Tahergorabi, Matak, & Jaczynski, 2015). In this study, yellowfin tuna muscle protein was solubilized at basic pH (11.0 ± 0.5) and subsequently precipitated at acidic pH (5.3 ± 0.5). A previous study has shown that solubilization of muscle protein in basic pH allows high recovery protein with high nutritional quality and better colour characteristics (Taskaya, Chen, & Jaczynski, 2010).

The yield achieved by the pH shift method ranged from 2.65% to 10.90% wet weight basis. The results show that the highest protein amount was released from muscles by the dorsal skin sample than the caudal and head samples. Compared to Trial I, the yield obtained from Trial II was higher. This was attributed to reducing the muscle size by homogenizing the sample, increasing the solubility by increasing protein-water interactions, and changes of the muscle composition of by-products (Pezeshk, Rezaei, Hosseini, & Abdollahi, 2021).

The ash content in protein isolates varied from 1.4% to 4%. The highest ash content (4%) was obtained in the caudal sample in Trial I. Lower ash content resulted from the caudal sample with post-H_2O_2 treatment (1.4%), dorsal (2.1%), caudal with pre-H_2O_2 treatment (2.8%) and head (3.2%) respectively. However, these values are much lower than the values of Hayes, Mora, Hussey, & Aluko (2016) who found a higher ash content in boarfish soluble protein isolates (35.4%) and boarfish bone fraction protein powder (45.80%). Raw material type and extraction methods of protein isolates may be responsible for these changes (Shaviklo A. R., 2015).

According to the ash content, high protein content (≥90%) might be expected. Therefore, the protein content of FPI may be approximately similar to protein powder extracted from cape hake (90.0%) (Pires, et al., 2012). The protein content of the FPI varied with different by-products due to the difference in substrate composition of by-products. Moreover, the dorsal sample has the highest yield and protein content as it is comprised of fish muscles rather than bones.

High protein recovery from caudal and dorsal samples in Trial II shows a potential utilization of these isolates in the food industry, although future experiments would be required. Moreover,

the development of these protein isolates as the main ingredient of functional food product may provide a cost-effective, healthy dietary supplement for consumers (Tahergorabi, Matak, & Jaczynski, 2015).

5.3.1 Colour characteristics of fish protein isolates

The colour of protein isolates is the critical quality parameter of the food industry. The colour of extracted protein isolates from yellowfin tuna caudal sample (Trial I & Trial II), dorsal sample, and head sample protein isolate varied from yellow-brownish, light yellow, light grey, and brown colour respectively (Table 6). The L* value of all samples was higher than freeze-dried saithe protein isolate (41.2 ± 0.16) (Shaviklo, Thorkelsson, Arason, & Kolbrun, 2012) and lower than cod (76.37 ± 0.18) and cape hake (76.78 ± 0.098) protein isolates (Abdollahai & Undeland, 2020; Pires, et al., 2012). Protein isolates from the caudal sample (post-H_2O_2) were lighter than salmon protein isolates (L*=72.51 ± 0.15) (Abdollahai & Undeland, 2020). In terms of redness, a* value of all protein isolates in this study was less than salmon and herring protein isolates (Abdollahai & Undeland, 2020). The yellowness values of protein isolates from the caudal sample coincide with the value reported for salmon protein isolates (18.37) (Abdollahai & Undeland, 2020). The yellowness values for cod (14.94) and herring (14.21) protein isolates were lower than head, dorsal, and caudal (Trial I) samples. The results depicted that the whiteness of protein isolates obtained from caudal samples (Trial II) was higher than the herring (54.75) and lower than the cod, salmon, and cape hake protein isolates (71.83, 66.09, 74.34) (Abdollahai & Undeland, 2020) (Park, 2005).

The by-products used to extract the protein were comprised of muscles, blood, bones, and skin with scales. Yellowfin tuna's muscles and blood contain myoglobin and hemoglobin. Scales contain pigments such as melanin. The oxidation of these proteins and pigment during the precipitation of protein (5.3 pH) in the pH shift method causes dark colour protein isolates. According to the obtained results, protein isolates recovered from yellowfin tuna heads were the darkest with L* values of 43.67 and 41.95 for whiteness. This difference might be the presence of a high amount of hemoglobin and myoglobin in yellowfin tuna heads. The colour of fish protein isolates is depending on fish species, by-products, and method of extraction (Shaviklo A. R., 2015).

5.3.2 Color enhancement of fish protein isolates

0.5% of hydrogen peroxide treatment with 1:2 fish weight to the solution was used to enhance the color of protein isolates with two treatments (raw material soaked in hydrogen peroxide solution prior to pH shift and added hydrogen peroxide to protein isolates). The addition of hydrogen peroxide directly to the fish protein isolates (post H2O2 treatment) indicates strong whiting protein from the caudal fin. The whiteness of protein isolates with post-H_2O_2 (67.19 ± 3.42) is higher than pre-H_2O_2 (62.09 ± 3.42).

Hydrogen peroxide can be used as a decolorizing agent under mild alkaline or mildly acidic conditions. According to Carpenter and colleagues (1975), hydrogen peroxide treatment to raw fish with NaOH to adjust pH to 9.5 was preferred without impairing the nutritional value. In this study, the first hydrogen peroxide treatment was done after adjusting pH to 11.0 with NaOH. The second experiment was used by adding hydrogen peroxide to protein isolate under mild acidic conditions at pH 5.3. Both experiments were conducted at room temperature. This experiment illustrate how the reduction of pigments (melanin) and heme protein (hemoglobin and myoglobin) can enhance the color of protein isolates (United kingdom Patent No.

3,879,370, 1975). Therefore, hydrogen peroxide treatment applied on the protein isolates after the pH shift processing could effectively enhance the color characteristic of yellowfin tuna protein isolates more than when it is applied before the pH shift process.

5.3.3 *Functional properties of protein isolates*

The use of protein isolates in the food industry is governed by emulsifying stability, foaming capacity and stability, and gelation. These functional properties were only tested for the caudal sample from Trial I sample and shown in Table 9. In comparison, the functional properties of the yellowfin tuna protein isolates were lower than tuna liver protein powder (Fang, Liu, Li, Chen, & Huang, 2020). These results might suggest that protein molecules were not dispersed evenly in the oil-water interface or low hydrophobicity of protein ((Fang, Liu, Li, Chen, & Huang, 2020; Pezeshk, Rezaei, Hosseini, & Abdollahi, 2021). As those tests were performed only once and on one sample that was not fully homogenized, and the results cannot give a full indication of how the fish protein isolates could react.

5.4 Extraction of collagen from yellowfin tuna by-products

The yield and ash content of collagen obtained from yellowfin tuna dorsal skin, caudal fin, and heads were presented in Table 10. The yield of acid-soluble collagen from the caudal and head, which mainly contained bones, was higher than the dorsal skin. However, the results are contradictory with the results reported for acid-soluble collagen extracted from bigeye tuna skin (13.5% dry matter basis) and bigeye tuna bones (0.1% dry matter basis) (Ahmed, Haq, & Chun, 2019). An earlier study showed that the solubility of collagen depends on the type of acid used. Acids can increase the swelling properties of the raw material and enhances the dissolution of collagen fibers in water and thus result in higher yield (Bhuimbar, Bhagwat, & Dandge, 2019). In this study, 0.5 M acetic acid was used to treat dorsal skin in trials I and II while caudal and head samples were treated with 0.6 M HCl for demineralization prior to collagen extraction. The huge difference in collagen yield might be because of incomplete swelling of dorsal skin with 0.5 M acetic acid. Nevertheless, collagen yield from dorsal skin was less than the yield reported for yellowfin tuna (19.4% dry weight basis) by Woo, Yu, Cho, Lee, & Kim (2008). A lower yield of collagen from skipjack tuna skull, about 2.47% (dry weight) was obtained by Di, Feng, Bin, Fang, & Rui (2014). Only slight differences were observed by Sousa, Vazquez, Martin, Carvalho, & Gomes (2017) for collagen extracted from yellowfin tuna skin (12.51% at 45^0C and 1.26 % at 80^0C, dry weight). Whereas the collagen yield from mackerel head (3.3% dry weight) was much lower than the yellowfin tuna head (Khiari, Rico, Diana, & Rayan, 2011). These differences could be observed due to the variation of species, type of by-products, age, the structure of tissues, starvation condition, and method of extraction (Ahmed, Haq, & Chun, 2019; Elvarasan, et al., 2016).

5.4.1 *Colour characteristics of extracted collagen*

Colour of collagen is an important property in the food industry. The values for lightness and whiteness were higher in dorsal samples (Trial I & II) than in the head and caudal samples. Results obtained from this study clearly indicate that collagen extracted from yellowfin tuna dorsal skin was clear compared to the caudal (Pre-H_2O_2) collagen. Collagen from dorsal skin was extracted with an acetic acid treatment while HCl was used for caudal and head samples. According to the Khiari, Rico, Diana, & Rayan (2011) the color of collagen is affected by the type of acid used for pre-treatment. Therefore these changes might be due to the difference in

acids used for collagen extraction. However, all the collagen samples extracted from yellowfin tuna by-products were lighter and whiter than the collagen extracted from mackerel head (L*=41.5, a*= -1.3 and b*1.1) (Khiari, Rico, Diana, & Rayan, 2011). According to Khiari, Rico, Diana, & Rayan (2011), the darkness of acid-soluble collagen can be decreased by reducing the incubation time.

5.5 Extraction of fish oil from yellowfin tuna by-products

5.5.1 Oil recovery yield

The yield of fish oil extracted from yellowfin tuna is presented in Table 9. Fish oil was recovered using the conventional heat-based method (Trial I) and pH shift method (Trial II). The fish oil could not be recovered efficiently by the conventional heat-based method or manually squeezed from the slurry from the heated sample. The yield was lower than the yield recovered (3.046 ± 0.509) in the study by Kumara, Rajapakshe, & Jayamanne (2011) from yellowfin tuna heads using the wet rendering method which is similar to the conventional heat-based method. This could also partially be due to the fact that juvenile fish were used in this study. Therefore, the fat content available was lower than in adult yellowfin tuna heads. According to their results, the optimum way to recover tuna oil was to heat slurry at 85^0C for 20 minutes (Kumara, Rajapakshe, & Jayamanne, 2011). Using the pH shift method, the oil fraction was recovered from the emulsion layer at 4^0C. The top emulsion layer resulted from high-speed homogenization and extreme pH variation of the sample. Abdollahai & Undeland (2020) described that during the pH shift process, partially denatured proteins can be entrapped into lipids. Therefore, the top emulsion layer consists of protein fraction and oil fraction when the centrifugation force is not enough to precipitate all the protein. In this study, repeated freeze-thawing and centrifugation processes were used to break the protein fraction from the emulsion layer and facilitated recovering 1.52% of yield from the emulsion layer. The yield obtained from this study was lower than the yield recovered from salmon (9%) and higher than the oil recovered from herring (0.75%) by Abdollahai & Undeland (2020) using the pH shift method with freeze-thawing of the emulsion layer. It has been previously shown that this freeze-thawing and centrifugation method is an efficient method to coagulate and precipitate protein from the emulsion layer in order to recover a high yield of oil. However, several factors can affect the efficiency of recovering oil through this process including centrifugation force, emulsification stability of the protein, lipid content of raw material, and solubilization pH (Abdollahai & Undeland, 2020). Although the yield achieved from this study was not sufficient from an economical viewpoint, higher yield can be expected from a commercial size adult yellowfin tuna i.e., 20kg-90kg. Testing this method on a commercial size fish will give a better understanding of the oil yield that could be expected and will make the economic feasibility of this endeavor clear.

5.5.2 Fatty acid profile of the fish oil

Fatty acid composition related to SFA, PUFA, DHA+EPA, total omega-3, and total omega-6 was summarized in Figure 11. The conventional heat-based method was carried out at 85^0C with two different heating treatments (for 20 minutes and 40 minutes) to find the impact of heating duration on their fatty acid composition. In general, prolonged cooking time at high temperatures may accelerate the decomposition of fatty acids (Bako, Umogbai, & Awulu, 2017). But this result revealed that increases in heating time did not significantly affect the fatty acid composition of extracted oil.

The major fatty acids in both treatments (20 minutes and 40 minutes) were docosahexaenoic acid (DHA) (26.83%, 26.11%) followed by palmitic acid (23.13 %, 23.32%), and oleic acid (10.0%, 9.85%). This result is in agreement with the study reported for neritic tuna species (*Thunnus tonggo* and *Euthynnus affinis*) with slight changes in composition (Ferdosh, et al., 2015). Similarly, Chantachum and colleagues (2000) reported that oil extracted from tuna heads at 85^0C for 30 minutes had a high amount of palmitic acid and oleic acid.

In terms of fatty acid classes, the most prominent fatty acid class was PUFA followed by MUFA and SFA in this study. Among the PUFA, DHA was dominant and attributed to the highest percentage than the others in all samples. Yellowfin tuna oil has a higher amount of DHA than the oil extracted from the head of *T. tonggo* (19.9%) and *E.affinis* (18.0%) (Ferdosh, et al., 2015). Abdollahai & Undeland, 2020 reported that salmon (1.47 %) and herring (3.37%) contain comparatively lower DHA than yellowfin tuna oil. Although salmon and herring are known as an important source of PUFA, these results revealed that yellowfin tuna had higher PUFA levels (35.56%) than salmon (25.31%), herring (11.94%), or mackerel (18.10%) (Abdollahai & Undeland, 2020; Ferraro, et al., 2010). According to Ferdosh and colleagues (2015), tuna family fish oils extracted from different by-products are still rich in DHA.

From an economical viewpoint, most food and pharmaceutical companies specify the EPA and DHA percentages for the production of dietary supplements using fish oil. The results showed that the Percentage of DHA and EPA (DHA+EPA%) is slightly higher (33.32%) than the values reported for cod liver oil (30%), salmon (15%), and refined anchovy oils (30%) (Ciriminna, Meneguzzo, Delisi, & Pagliaro, 2017).

Total omega-3 PUFA (35.56% & 34.43%) is higher than the total omega-6 (4.1% and 4%) PUFAs. In contrast, Abdollahai & Undeland (2020) found a lower amount of omega-3 PUFA (17.71% & 10.33%) and a higher amount of omega-6 PUFA (7.40% & 1.25%) in oil extracted from salmon and herrings using the same conventional heat-based extraction method (Abdollahai & Undeland, 2020).

Saturated fatty acids (SFA) were the second most abundant fatty acid group in extracted oil which was higher than the SFA in salmon (13.46%) and herring oil (17.95%) extracted by using the same method (Abdollahai & Undeland, 2020).

These changes of fatty acids between different species were observed due to the species variation, habitat, feeding habits, nature of migration, and differences in extraction methods (Ferdosh, et al., 2015).

6   CONCLUSION

Developing an appropriate valorization strategy to recover value-added compounds could be of paramount importance for the sustainable utilization of tuna fishery by-products. This study tests a novel technology to recover the maximum high-added-value compounds including fish oil, protein isolates, and collagen through the single biorefinery approach. To understand the possible marketability of extracted materials, the approximate composition of by-products was evaluated. The high protein content of yellowfin tuna by-products including caudal and dorsal indicates an excellent source of extracted high-quality protein. The yellowfin tuna head had a higher lipid content than the other by-products. The most abundant fatty acids in by-products

were identified as docosahexaenoic acid (DHA/PUFA) (Head: 26.63%, Dorsal: 34.71%, Caudal: 31.27%) which is an important nutrient in human health products.

The biorefinery approach combined with the pH shift method successfully converted the sequential production of oil, protein isolates, and collagen from yellowfin tuna juvenile heads, protein isolates, and collagen from yellowfin tuna caudal and dorsal samples. In this study, muscle protein of by-products subsequently dissolved in alkaline pH during the pH shift method resulted in high yield and high purity protein isolates from heads, caudal and dorsal samples. In addition, 0.5% H_2O_2 improved the whiteness of protein isolates from caudal samples. According to the tristimulus colour parameters of extracted protein isolates with H_2O_2, post-H_2O_2 treatment resulted in whiter protein isolates than the pre-H_2O_2 treatment. It would be recommended for whiteness and lightness improvement of protein isolates with inherently poor colour characteristics such as protein extracted from the head of the yellowfin tuna.

This present study has shown that it is possible to recover good quality yellowfin tuna oil rich in omega-3 polyunsaturated fatty acid, particularly DHA (26.84%) through the pH shift method at room temperature. The oil recovery yield was higher when using the pH shift method (1.52% wet-weight) than the conventional heat-based method (0.75% wet-weight). The pH shift on other hand efficiently released oil entrapped with protein in the top emulsion layer when subjecting it to freeze-thawing and repeated centrifugation at 4^0C. The result obtained from the conventional heat base method with two different heating times (20 minutes and 40 minutes) revealed that increases in heating time were not affected to the fatty acid composition of extracted oil.

Acid-soluble collagen from the head, caudal and dorsal samples from juvenile yellowfin tuna were isolated using 0.5 M acetic acid and 0.6M HCl. Bone demineralization with 0.6 M hydrochloric acid could increase the collagen yield from the head (21.16% on a dry weight basis) and caudal sample (39.44% -Pre $H2O_2$, 23.17% -post $H2O_2$; on a dry weight basis) than the dorsal sample (9.51% on a dry weight basis). The results indicate that 0.6M HCl showed superior performance in the collagen extraction process on the bones part than the acetic acid did on the dorsal skin sample. The values for lightness and whiteness were higher in dorsal samples (Trial I & II) than in the head and caudal samples. The colour of extracted collagen from the dorsal skin was more white compared to the caudal and head collagen.

Based on the mass balance, 105.58g (wet weight) of the caudal sample was recovered, 11.41g (dry weight) of protein isolates, and 7.30g (dry weight) of collagen from 55.70g (wet weight) of bones while 106g (wet weight) of dorsal sample recovered 11.56g (dry weight) of protein isolates and 0.62g (dry weight) of collagen from 27g (wet weight) of skin materials. Valorization of 156.74 g (wet weight) of yellowfin tuna heads resulted in 2.38g of oil, 13.81 g (dry weight) of protein isolates, and 7.43g (dry weight) of collagen from 91.80g (wet weight) bones. These yields showed maximum recovery of high-added value compounds from the raw materials through this biorefinery approach.

This present study is an important contribution to dealing with low-value by-products in efficient ways to recover the high-added value compounds including oil, collagen, and protein which can be developed for direct human consumption. To the best of this author's knowledge, this is the first study regarding the biorefinery for caudal fin in yellowfin tuna and head valorization with three value-added materials. This improvement will make sustainable utilization of marine resources with limited waste generation and provide a pillar for the

bioeconomy. Future studies will be essential to analyze the functional properties and nutritional quality of extracted compounds in depth to evaluate the economical and technological feasibility of this biorefinery approach in the food and pharmaceutical industry.

6.1   Recommendations

Future research should be carried out to completely characterize the chemical and functional properties and nutritional quality of extracted oil, collagen, and protein isolates extracted from tuna by-products. The acid soluble collagen extraction method with hydrochloric acid seems to be the best method to recover the maximum yield of collagen. This approach should be applied to commercial-size yellowfin tuna in Sri Lanka to better assess potential yield, especially of crude oil.

Further fine-tuning of the pH-shift method combined with freeze-thawing emulsion breaking steps could improve the subsequent oil recovery from the top emulsion layer. Enhancing and improving this extraction method together with purification, stabilization, and quality improvement methods will be important to develop this industry, which could have health, environmental and economic benefits for Sri Lanka.

It is assumed that a biorefinery approach to the valorisation of yellowfin tuna in Sri Lanka at the industrial level would produce concomitant economic benefits for relevant seafood industries. The potential profitability of this venture should be further studied to produce a clear cost-benefit analysis.

6.2   Limitations

Juvenile yellowfin tuna (> 1kg) were used for this study due to practical issues related to sample collection. A low number of replicates was used for these trials due to the limited time and sample availability. Therefore, it was not possible to conduct any statistical analysis to find significant differences in yield or chemical composition between different by-products.

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7   APPENDIX

Table A1. Fatty acid composition (%) of yellowfin tuna by-products

Fatty Acid formular	Name	Head	Dorsal	Caudal
14:0	Myristic acid	2.87 ± 0.09	1.21± 0.16	1.79±0.11
15:0	Pentadecanoic acid	0.95 ±0.02	0.56±0.04	0.71±0.03
16:0	Palmitic acid	22.52 ±0.22	20.14±0.47	20.72±0.20
16:1n7	Palmitoleic acid	2.92 ±0.07	1.59±0.12	2.03±0.16
16:2n4		0.60 ±0.10	0.55±0.06	0.59±0.01
17:0	Heptadecanoic acid	1.42 ±0.04	1.02±0.05	1.27±0.02
16:3n4		0.43 ±0.01	0.32±0.01	0.37±0.02
18:0	Stearic acid	7.75 ±0.18	7.66±0.04	8.51±0.22
18:1n9	Oleic acid	10.07 ±0.17	7.77±0.12	8.82±0.28
18:1n7	Vaccenic acid	1.68 ±0.02	1.43±0.02	1.58±0.02
18:2n6	Linoleic acid	1.22 ±0.08	0.84±0.01	0.92±0.04
18:3n6	y-linolenic acid	0.31 ±0.01	0.26±0.00	0.32±0.00
18:3n3	Linolenic acid	0.41 ±0.04	0.22±0.01	0.27±0.02
18:4n3	Stearidonic acid	0.43 ±0.01	0.21±0.00	0.27±0.03
20:0	Arachidic acid	0.37 ±0.003	0.23±0.01	0.31±0.01
20:1(n11+n9)	Eicosenoic acid	1.47 ±0.06	0.79±0.11	1.26±0.01
20:2		0.38 ±0.007	0.33±0.01	0.37±0.01
21:0		2.65 ±0.04	4.08±0.18	3.37±0.02
20:3n3		0.39 ±0.01		
20:4n6	Arachidonic acid	0.35 ±0.03	0.25±0.02	0.34±0.02
20:4n3	Eicosatetraenoic acid	0.34 ±0.003	1.54±2.61	0.26±0.02
20:5n3	Eicosapentaenoic acid (EPA)	6.15 ±0.03	5.31±0.04	5.49±0.03
22:00		0.28 ±0.01	0.20±0.00	0.25±0.01
22:4n6		2.26 ±0.04	3.39±0.13	3.08±0.08
22:5n3	Docosapentaenoic acid	0.76 ±0.49	0.94±0.02	1.02±0.01
24:0		1.06 ±0.01	0.22±0.01	0.21±0.01
22:6n3	Docosahexaenoic acid (DHA)	26.63 ±0.19	34.71±0.72	31.27±0.90
24:1n9		0.79 ±0.09	1.00±0.32	1.06±0.02
SFA		39.87	35.31	37.14
MUFA		16.94	12.58	14.76
PUFA		40.67	48.86	44.55
EPA+DHA		32.78	40.02	36.75
Omega 3		35.12	42.93	38.57
Omega 6		4.15	4.74	4.65

*Values are mean ± standard deviation from four measurements.

Table A2. Fatty acid composition (%) of oils recovered from yellowfin tuna heads (n=3) using two different cooking times by conventional heat method

Fatty Acid formular	Name	20 minutes	40 minutes
14:0	Myristic acid	3.14	2.96
15:0	Pentadecanoic acid	1.00	0.98
16:0	Palmitic acid	23.31	23.32
16:1n7	Palmitoleic acid	3.06	3.01
16:2n4		0.24	0.44
17:0	Heptadecanoic acid	1.59	1.58
16:3n4		0.44	0.44
18:0	Stearic acid	7.72	7.75
18:1n9	Oleic acid	9.86	10.00
18:1n7	Vaccenic acid	1.74	1.68
18:2n6	Linoleic acid	1.05	1.04
18:3n6	y-linolenic acid	0.34	0.34
18:3n3	Linolenic acid	0.36	0.40
18:4n3	Stearidonic acid	0.43	0.4
20:0	Arachidic acid	0.43	0.44
20:1(n11+n9)	Eicosenoic acid	1.87	1.8
20:2		0.43	0.42
21:0		2.33	2.25
20:4n6	Arachidonic acid	0.47	0.44
20:4n3	Eicosatetraenoic acid	0.34	0.34
20:5n3	Eicosapentaenoic acid (EPA)	6.48	6.13
22:00		0.27	0.29
22:4n6		2.25	2.17
22:5n3	Docosapentaenoic acid	1.10	1.02
22:6n3	Docosahexaenoic acid (DHA)	26.84	26.11
24:1n9		0.60	0.70
SFA		39.79	39.57
MUFA		17.33	17.43
PUFA		40.91	39.87
EPA+DHA		33.32	32.43
Omega 3		35.56	34.43
Omega 6		4.10	4.00

*Values are mean ± standard deviation from four measurements.