Food Research 8 (2) : 64 - 77 (April 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Effects of different preparation methods on the physical, chemical and functional properties of protein powders from Skipjack tuna (*Katsuwonus pelamis*) liver

[1]Jeerakul, C., [1]Kitsanayanyong, L., [1]Pansawat, N., [2]Boonbumrung, S., [1]Klaypradit, W. and [1,*]Tepwong, P.

[1]*Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand*
[2]*Institute of Food Research and Product Development, Kasetsart University, Bangkok 10900, Thailand*

Article history:
Received: 7 September 2022
Received in revised form: 27 October 2022
Accepted: 17 February 2023
Available Online: 17 March 2024

Keywords:
Tuna liver protein powder,
Byproducts utilization,
Heat treatment,
Ultrasound-assisted extraction,
Alkaline pH shift process,
Supercritical carbon dioxide fluid extraction

DOI:
https://doi.org/10.26656/fr.2017.8(2).465

Abstract

Tuna livers (TL), which are often discarded as waste, are a valuable source of protein for human consumption. However, the preparation method used affects the nutritional and functional characteristics of protein powders. This work aimed to investigate the effects of different preparation methods on the physical, chemical, and functional properties of tuna liver protein powders (TLPPs) following heat (H) treatment, heat and ultrasound-assisted (HU) extraction, alkaline pH shift (APS) process, and supercritical carbon dioxide fluid (SC-CO_2) extraction. H at 85°C (H85), HU at 80 kHz and 100 W (HU-80-100), APS at pH 11.5 (APS 11.5), and SC-CO_2 at 350 bars (SC-CO_2-350) resulted in the remarkably highest total protein content among the different preparation conditions. All TLPPs, except for APS 11.5, showed lighter color characteristics. The most abundant amino acids in all TLPPs were glutamic acid, aspartic acid and alanine. The protein solubility and foaming capacity were efficiently improved by SC-CO_2-350. Nevertheless, the emulsion properties and oil holding capacity were greatly enhanced by H85 and HU-80-100, and a significant foaming stability and water holding capacity were found in APS 11.5. Therefore, the TLPPs obtained following different preparation methods are unique and could be potentially utilized as a source of protein ingredients in several food systems.

1. Introduction

Skipjack tuna (*Katsuwonus pelamis*) liver (TL) is considered an underutilized solid byproduct of the canned tuna industry. It is generally used in the production of low-value products, such as animal feed, or the worst-case scenario, it is discarded as waste. This creates huge economic and environmental concerns (Shen *et al*., 2021). Recently, research has been focused on the utilization of TL for fish oil production due to its high n-3 polyunsaturated fatty acids (PUFAs) and fat-soluble vitamin contents (Fang *et al.,* 2019). Besides lipids, TL is also a promising source of proteins, consisting of up to 18.1 g per 100 g (Kang *et al.*, 2007). The latter finding leads to the assumption that TL can be utilized as an alternative protein source for human consumption, particularly in the form of powdered protein as a product with economic value that can be included as a food ingredient (Shen *et al.*, 2021). Finding a suitable preparation method, which is necessary for the enrichment of the valuable protein presented in TL is a key challenge for both the industry and food scientists (Shaviklo, 2015).

However, removing the lipids and water is the most difficult and important aspect of protein powder preparation (Fang *et al*., 2020). Several methods, i.e., wet reduction, pH shift process, enzymatic extraction, microwave-assisted extraction, ultrasound-assisted extraction, and supercritical fluid extraction, have been demonstrated to be effective at removing oil from TL, leaving underutilized residues containing protein. These methods have succeeded in enriching the protein residues derived from the fish processing byproducts (Shaviklo, 2015; Kang *et al.,* 2017). Each of the preparation methods has its advantages and uniqueness. Wet reduction, also known as heat (H) treatment, is a method that relies on cell disruption and coagulates fish protein at high temperatures as part of the process of separating liquids and solids. Also, the fat cells are disrupted, and oil is released into the liquid, resulting in a higher protein content in the protein residues (Chantachum *et al.,* 2000). A combination of heat and ultrasound-assisted (HU) extraction could trigger cell disruption and the rapid release of compounds and macro- and micromolecular components, as well as enhance the functional and structural properties of the

*Corresponding author.
Email: *ffispdt@ku.ac.th*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

protein by cavitation (Nazari *et al.,* 2018; Wang *et al.,* 2021). The pH shift process, especially an alkaline pH shift (APS), leads to the production of a high-yielded water-soluble protein with a low-fat content (Fang *et al.,* 2020; Shen *et al*., 2021). Additionally, supercritical carbon dioxide fluid (SC-CO_2) extraction, a newly developed extraction method that has been dawning research attention recently, could lead to the production of high-purity protein products due to the remarkable removal of lipids (Kang *et al.,* 2017). To the best of our knowledge, limited studies have examined the effects of various preparation methods on the efficiency as well as the nutritional and functional properties of the resulting protein powder prepared from TL.

The above-mentioned byproduct of the canned tuna industry, namely TL, could cause environmental issues if there is no proper management in terms of disposal. However, according to food scientists, due to its high protein content, TL could markedly be used as a protein source. Consequently, this study aimed to develop a high-quality TL protein powder (TLPP) prepared by different methods, including H treatment, HU extraction, APS process, and SC-CO_2 extraction. The comparative physical, chemical, and functional properties of the TLPPs were evaluated.

2. Materials and methods

2.1 Raw materials

The frozen liver of Skipjack tuna (*Katsuwonus pelamis*) was obtained from a canned tuna processing plant in Samut Sakhon Province, Thailand, and transferred to the laboratory in Bangkok within 2 hrs in an icebox at approximately 4°C. After arrival, the tuna liver was immediately kept at -20°C until used.

2.2 Preparation of the tuna liver powder

The frozen TL was partially thawed for 24 hrs at 4°C, then cut into small pieces and ground using a blender (Waring Commercial, Torrington, CT, USA). The ground TL samples were subjected to proximate composition analyses (wet weight basis) following the methods described by the Association of Official Agricultural Chemists (AOAC) No. 950.46, 920.153, 960.39, and 928.08 for moisture, protein, lipid, and ash contents, respectively (AOAC, 2000). The remaining portion of the ground TL was divided into 2 groups: 1) for the different preparation methods of tuna liver protein powders (TLPPs) including heat (H) treatment, heat and ultrasound-assisted (HU) extraction, and alkaline pH shift (APS) process and 2) for freeze-drying. The freeze-dried TL powder was subjected to physical and chemical analyses (control) and for preparation of TLPP by supercritical carbon dioxide fluid (SC-CO_2) extraction.

2.3 Preparation of the tuna liver protein powders

The following four preparation methods were performed: 1) H treatment, 2) HU extraction, 3) APS process, and 4) SC-CO_2 extraction. A comprehensive flowchart of the TLPP preparation and analysis following the different preparation techniques is depicted in Figure 1.

2.3.1 Preparation by heat treatment

The heat (H) treatment for the TLPPs was prepared by modifying the method of Fang *et al.* (2019). In brief, the ground TL was thawed at 4°C for 24 hrs, diluted 1:1 ratio (w/v) with distilled water (DW), and homogenized in an ice bath using an Ultra-Turrax T25 homogenizer (IKA®, Staufen, Germany), at 10,000 rpm for 1 min. The liver homogenate was then heated to 75, 85, and 95°C (marked as H75, H85, and H95, respectively) using a continuous horizontal water bath shaker (Memmert, Bavaria, Germany), at 100 rpm. After 30 mins of incubation, the samples were cooled to room temperature and the protein residues were collected after centrifugation at 10,000×*g* for 10 mins at 4°C using a refrigerated centrifuge (Tomy Seiko, Tokyo, Japan). The protein was freeze-dried and the lyophilized H samples were kept at -20°C.

2.3.2 Preparation by heat and ultrasound-assisted extraction

Following the preparation of heat at 85°C for 30 mins (H85) as described in the previous section, ultrasound-assisted (HU) extraction was performed using an ultrasonic bath (DK-3000TS, Guangdong, China) at different frequencies (40 and 80 kHz) and powers (100 and 300 W) (marked as HU-40-100, HU-40-300, HU-80-100, and HU-80-300, respectively). After 30 mins, the samples were centrifuged at 10,000×*g* for 10 mins at 4°C using a refrigerated centrifuge. The protein residual was collected, freeze-dried, and stored at -20°C.

2.3.3 Preparation by alkaline pH shift process

The alkaline pH shift (APS) process was performed according to the method described by Fang *et al.* (2020). Briefly, the ground TL was diluted at a 1:6 ratio (w/v) with DW and homogenized at 10,000 rpm for 1 min in an ice bath using a homogenizer. After homogenization, the protein in the TL homogenate was solubilized at pH 11.5 by adding 1M NaOH under continuous stirring. The sample was centrifuged at 10,000×*g* for 10 mins at 4°C and the soluble protein in the middle layer was collected and precipitated by adjusting the pH to 5.5. The

https://doi.org/10.26656/fr.2017.8(2).465

precipitated protein was neutralized to pH 7.0 with 1M NaOH and finally collected via centrifugation at 10,000×*g* for 10 mins at 4°C. The protein residual (marked as APS 11.5) was freeze-dried and stored at −20°C.

2.3.4 Preparation by supercritical carbon dioxide fluid extraction

The supercritical carbon dioxide fluid (SC-CO_2) extraction was performed as described by Fang *et al.* (2019) with some modifications using a supercritical fluid extractor (Spe-ed SFE Helix, Applied Separations, Allentown, PA, USA). Initially, fifteen grams of TL powder were loaded into the extraction vessel and CO_2 was supplied (3.0 L/min) at a temperature of 55°C under different pressures (250 and 350 bar). The static extraction lasted for 1 hr, followed by 4 hrs of dynamic extraction. The samples (marked as SC-CO_2-250 and SC-CO_2-350) were then collected and stored at −20°C.

2.4 Determination of extraction yield, protein content, and protein recovery yield of tuna liver protein powders

The extraction yield of the TLPPs using the H, HU, APS, and SC-CO_2 methods was determined according to the following equation (1):

$$\text{Extraction yield (\%)} = \frac{\text{Weight of TLPP (g dry basis)}}{\text{Weight of TL (g dry basis)}} \times 100 \quad (1)$$

The protein content of the TLPPs was determined using the Kjeldahl method and the conversion factor of 6.25 (AOAC Method No. 920.153, 2000). The protein recovery yield of the TLPPs was calculated as a percentage according to the following equation (2):

$$\text{Protein recovery yield (\%)} = \frac{\text{Weight of TLPP (g)} \times \text{Protein content of TLPP}}{\text{Weight of TL (g)} \times \text{Protein content of TL}} \times 100 \quad (2)$$

2.5 Determination of the physical and chemical properties of tuna liver powder and selected tuna liver protein powders

The TL powder and selected TLPPs (H85, HU-80-100, APS 11.5, and SC-CO_2-350) were subjected to proximate composition analysis according to the AOAC (2000). The physical and chemical properties were analyzed according to the methods described in the following section.

2.5.1 Water activity

The water activity (a_w) of the TL powder and selected TLPPs was determined using a water activity meter (4TEV, Aqualab, Pullman, WA, USA).

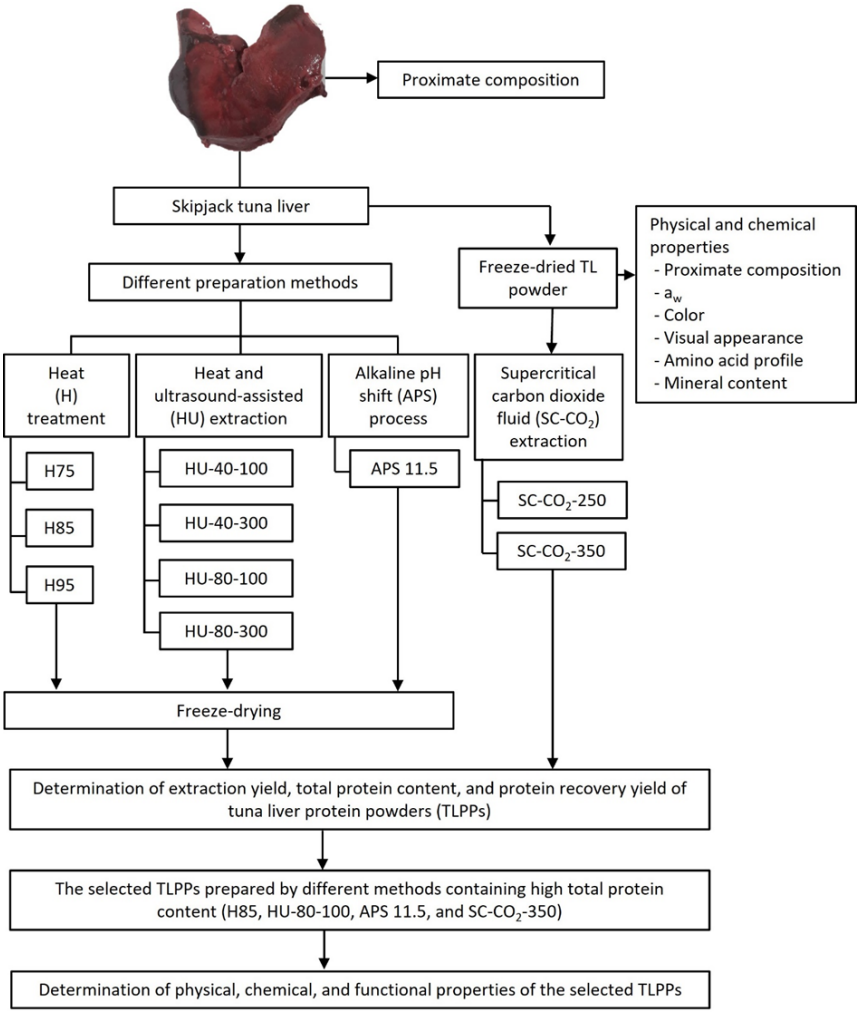


Figure 1. A flowchart of TLPPs preparation by different methods.

https://doi.org/10.26656/fr.2017.8(2).465

2.5.2 Color

The color of the TL powder and selected TLPPs was determined using a Hunter Lab colorimeter (Hunter Associates Laboratory, Reston, VA, USA) and reported using the CIE system. The lightness (L^*), redness (a^*), and yellowness (b^*) values were measured after calibrating the colorimeter with a white standard plate.

2.5.3 Visual appearance

The appearance of the TL powder and selected TLPPs was captured on a white background using a digital camera (Apple iPhone 11 Pro max).

2.5.4 Amino acid composition

The amino acid (AA) profile of the TL powder and selected TLPPs was identified using the methods of Jajic *et al*. (2013) with some modifications. The samples (0.25 g) were hydrolyzed with 6 mol/L HCl or 4.2 mol/L NaOH (for determination of tryptophan) in a B-300 oil bath (Buchi, Flawil, Switzerland) at 110°C for 24 hrs. The hydrolyzed samples were diluted with HPLC-grade water and filtered through a 0.22-µm nylon membrane filter (Filtrex Technologies, Encinitas, CA, USA). The AA profile was then analyzed using high-performance liquid chromatography (HPLC; 1200 Infinity Series, Agilent Technologies, Santa Clara, CA, USA). The chromatographic separation of a 10 µL-portion of the sample was carried out in a Poroshell-120 column (HPH-C18, 4.6 mm × 100 mm, 2.7 µm particle size; CA, USA) maintained in a column oven set at 40°C. Mobile phase A was 10 mM disodium hydrogen phosphate (Na_2HPO_4), 10 mM sodium tetraborate ($Na_2B_4O_7{\bullet}10H_2O$), and 0.5 mM sodium azide (NaN_3), adjusted to pH 8.2, and mobile phase B was a mixture of acetonitrile, methanol, and water (45:45:10 (v/v/v)). The gradient conditions were set as follows: 2% B at 0 - 0.35 min, 57% B at 13.4 min, 100% B at 13.5-15.7 min, and 2% B at 15.8-18.0 min, at a flow rate of 1.0 mL/min. The eluted amino acids were detected by a fluorescence detector with an excitation and emission wavelength of 230 nm and 450 nm, respectively. AAs were identified and quantified based on peak area integration using the peak area determined from a known concentration of mixed AA standard (Agilent Technologies). The data was reported as g/100 g of protein.

2.5.5 Mineral composition

The concentration of selected minerals in the TL powder and selected TLPPs was determined by the Center of Scientific Equipment for Advanced Research (Thammasat University, Pathum Thani, Thailand). The minerals were identified using an inductively coupled plasma-mass spectrometry (ICP-MS; PerkinElmer, Shelton, CT, USA) according to the AOAC standard methods 2011.14 (AOAC, 2013) and quantified based on the known concentration of a multielement standard (PerkinElmer, Shelton, CT, USA).

2.6 Functional properties

The functional properties of the selected TLPPs were evaluated according to the methods described in the following sections. Additionally, two types of commercial protein powders from plant and animal origins, including soy protein concentrate (SP) and egg white powder (EW), were studied and compared to the TLPPs obtained in the present study.

2.6.1 Protein solubility

The protein solubility of the selected TLPPs was examined using the method described by Cha *et al*. (2020) with slight modifications. In brief, a 10-mg portion of TLPPs was dissolved in 10 mL of DW and the pH of the suspension was adjusted in the range of 2 to 13 using 0.1 mol/L HCl or 0.1 mol/L NaOH. After 30 mins of constant stirring, the sample was centrifuged at 10,000×g for 15 mins at 4°C and the supernatant was collected. The protein content in the supernatant (protein solubilization at pH 2 to 12) and total protein content in the sample (protein solubilization at pH 13) were then quantified using the Lowry method (Lowry *et al.*, 1951) against a known concentration of standard bovine serum albumin (BSA). The percentage of the protein solubility was calculated as indicated in the following equation (3) and expressed as the relative protein solubility in relation to the maximum soluble protein sample.

$$\text{Protein solubility (\%)} = \frac{\text{Protein content in the supernatant}}{\text{Total protein content in the sample}} \times 100 \quad (3)$$

2.6.2 Emulsifying properties

The emulsifying activity index (EAI) and emulsion stability index (ESI) of the TLPPs were determined according to the method described by Han *et al.* (2019) with modifications. Briefly, the selected TLPPs solution (10 mg/mL) was mixed with palm oil at a ratio of 3:1 (v/v) and the emulsion was generated by homogenization at 10,000 rpm for 1 min. After 0 and 10 mins of incubation, a 50 µL aliquot of the emulsion was collected and thoroughly mixed with 0.1% (w/v) SDS solution (5 mL) for 30 s. The absorbance of the resulting solution was then determined at 500 nm using an Evolution 300 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). The EAI and ESI of the TLPPs were calculated as indicated in the following equations (4) and (5), respectively.

$$\text{EAI } (m^2/\text{g}) = \frac{2 \times 2.303 \times \text{dil} \times A}{\text{C} \times 1\ \text{cm} \times \phi \times 10000} \quad (4)$$

Where dil is the dilution factor (100), A is the

https://doi.org/10.26656/fr.2017.8(2).465

absorbance analyzed immediately after the emulsion formation, C is the protein concentration (g/mL) before emulsion formation in the aqueous phase, and ϕ is the oil volume fraction of the emulsion (0.25)

$$\text{ESI (min)} = \frac{A_0}{A_0 - A_{10}} \times t \quad (5)$$

Where A_0 is the absorbance at 0 min, A_{10} is the absorbance at 10 min after homogenization, and t is the time between measurements (10 mins).

2.6.3 Foaming properties

The foaming capacity (FC) and foaming stability (FS) of the TLPPs were performed using the method of Cha *et al*. (2020). In brief, the foam was generated by whipping the TLPP solution (10 mg/mL) at 10,000 rpm for 3 min using a homogenizer before being transferred to a 50 mL accredited cylinder. After 0- and 60-min incubation at room temperature, the total foam volume was recorded. The FC and FS of the TLPPs were then calculated as indicated in the following equations (6) and (7), respectively:

$$\text{FC (\%)} = \frac{V_T}{V_0} \times 100 \quad (6)$$

$$\text{FS (\%)} = \frac{(F_t/V_t)}{(F_T/V_T)} \times 100 \quad (7)$$

Where V_0 is the initial volume before whipping, V_T is the total volume after whipping, V_t and F_t are the total volume and total foam of sample solution after 60 mins, respectively, and F_T is the total foam volume after whipping and standing at room temperature for different durations (t = 0 and 60 mins).

2.6.4 Water holding capacity

The water holding capacity (WHC) of the TLPPs was assessed according to the method of Han *et al.* (2019) with minor modifications. In brief, a 0.5 g-portion of TLPPs was dispersed in 10 mL of DDW and vortexed for 60 s using a vortex Genie 2 mixer (Fisher Scientific, Bohemia, NY, USA). After 30 mins of incubation at room temperature, the sample solution was centrifuged at 6,000×*g* for 30 mins, and the volume of the resulting supernatant was determined. Then, the difference between the initial volume of DDW and the volume of the supernatant was determined. The WHC was then calculated as indicated in the following equation (8) and expressed as milliliters of the absorbed water per gram sample.

$$\text{WHC (mL/g)} = \frac{\text{The volume of water absorbed (mL)}}{\text{Weight of sample (g)}} \quad (8)$$

2.6.5 Oil holding capacity

The oil holding capacity (OHC) of the TLPPs was conducted according to the method of Han *et al.* (2019) with a slight modification. In brief, a 10-mL portion of palm oil was added to a tube containing TLPPs (0.25 g) and mixed for 60 s using a vortex mixer. After 30 mins of incubation at room temperature, the sample was centrifuged at 6,000×*g* for 30 mins and the difference between the volume of the supernatant and the amount of oil added to the sample was measured. Then, the OHC was calculated as indicated in the following equation (9) and expressed as milliliters of oil per gram sample.

$$\text{OHC (mL/g)} = \frac{\text{The volume of oil (mL)}}{\text{Weight of sample (g)}} \quad (9)$$

2.7 Statistical analysis

All analyses were performed in triplicate (n = 3), and the values were expressed as mean ± standard deviation (SD). The statistical analysis of the data was conducted using SPSS version 26 (SPSS Inc., Chicago, IL, USA). A t-test or one-way analysis of variance (ANOVA) and Duncan's multiple range test were applied to clarify the differences between the mean values obtained from each of the analyses and preparation methods. A *p*-value of less than 0.05 was a statistical difference.

3. Results and discussion

3.1 Proximate composition of tuna liver

The proximate composition, including moisture, protein, lipid, and ash content of the raw materials was 65.90±0.12%, 18.10±0.04%, 10.64±0.03%, and 2.12±0.08%, (wet weight basis), respectively (data not shown). Similar results were obtained by Kang *et al.* (2007) and Fang *et al.* (2018) who studied the proximate composition of Skipjack tuna liver, wherein the protein, moisture, lipid, and ash contents ranged between 16.44-18.10%, 61.41-66.70%, 6.30-17.60%, and 0.63-1.60%, respectively. The variations in the proximate composition of the Skipjack tuna obtained by different research groups may be caused by various factors such as genetic variation, aging factors, and the extraction methods used (Fang *et al.,* 2018). The results for the proximate composition suggested that TL could be used as a potential protein source.

3.2 Effects of the different preparation methods on the extraction yield, protein recovery yield, and total protein content of tuna liver protein powders

3.2.1 Heat treatment

The extraction yield, protein recovery yield, and total protein content of the TLPPs prepared following the H treatment are shown in Table 1. The results indicated that the extraction yield increased when there was an increase in extraction temperature ($p < 0.05$). However, there were no significant differences between H85 (73.46%) and H95 (75.62%) ($p > 0.05$). The extraction yield of

https://doi.org/10.26656/fr.2017.8(2).465

Table 1. Extraction yield, total protein content, and protein recovery yield of TLPPs from different preparation methods.

Treatment	Content (g/100 g dry weight basis)		
	Extraction yield	Total protein content	Protein recovery yield
TL powder	-	47.95±0.37	-
H treatment			
H75	71.37±1.47^{b}	50.85±0.04^{c}	75.67±1.55^{b}
H85	73.46±0.88^{ab}	53.81±0.32^{a}	82.44±0.98^{a}
H95	75.62±0.97^{a}	51.91±0.55^{b}	81.87±1.05^{a}
HU extraction			
HU-40-100	71.14±0.31^{c}	50.60±0.40^{b}	75.08±0.33^{b}
HU-40-300	72.24±0.24^{b}	49.43±0.71^{c}	74.50±0.25^{b}
HU-80-100	73.75±0.64^{a}	54.39±0.04^{a}	83.66±0.73^{a}
HU-80-300	72.27±0.73^{b}	49.64±0.40^{c}	74.82±0.76^{b}
APS process			
APS 11.5	48.82±1.11	51.41±0.44	52.35±1.19
SC-CO_2 extraction			
SC-CO_2-250	75.19±0.50^{a}	60.97±0.66^{b}	95.61±0.64^{b}
SC-CO_2-350	74.02±0.92^{b}	71.75±1.06^{a}	99.38±1.39^{a}

Values are presented as mean±SD (n = 3), dry weight basis. Values with different superscripts within the same column are statistically significantly different ($p < 0.05$).

TLPPs obtained following the H treatment was comparable to that of poultry meat (71.01%) following the heat treatment (76°C) (Przybylski *et al.,* 2021). Additionally, an increase in extraction temperature increased the protein recovery yield of TLPPs. However, increasing the extraction temperature above 85°C did not further increase the recovery yield of TLPPs, and a significant protein recovery yield was found in H85 and H95 (82.44% and 81.88%, respectively) compared to that of H75 (51.68%). The total protein content of TLPPs was independent of the increasing temperature. The highest protein content was observed in H85 (53.81%), followed by H95 (51.91%) and H75 (50.85%), respectively ($p < 0.05$). This could be explained by the higher amount of lipids removed at 85°C compared to 75°C and 95°C. Fewer lipids were removed at 75°C due to less lipid cell disruption than at 85°C. At 95°C, fewer lipids were removed due to lipid trapping inside the aggregated protein structure caused by the high temperature (Chantachum *et al.*, 2000). This resulted in the highest protein content being found in H85. In this part of the study, H85 (85°C) was selected as the most suitable treatment for TLPP preparation by heat due to having the highest protein content obtained among the treatments.

3.2.2 Heat and ultrasound-assisted extraction

The extraction yield, protein recovery yield, and total protein content of TLPPs obtained following HU extraction are shown in Table 1. The results showed that the ultrasound frequency and power significantly affected ($p < 0.05$) the extraction yield, protein recovery yield, and total protein content of TLPPs. An increase in ultrasound frequency from 40 kHz to 80 kHz at 100 W resulted in a significant increase ($p < 0.05$) in extraction yield, protein recovery yield, and total protein content from 71.14% to 73.75%, 75.08% to 83.66%, and 50.60% to 54.39%, respectively. The increase in total protein content may be explained by the reduction in protein loss with a solvent at a higher frequency as a result of protein nucleation (Golly *et al.,* 2020). On the other hand, an increase in the ultrasound frequency from 40 kHz to 80 kHz at 300 W did not influence the extraction yield, protein recovery yield, and total protein content of TLPPs ($p > 0.05$). The significant and highest ($p < 0.05$) value of all parameters was observed by HU-80-100 (73.75, 83.66, and 54.39% for the extraction yield, protein recovery yield, and total protein content, respectively). However, the increase in ultrasound power from 100 to 300 W at 80 kHz resulted in a decrease in extraction yield, protein recovery yield, and total protein content ($p < 0.05$). This could be explained by the increase in ultrasonic cavitation caused by an excess of ultrasonic power resulting in damage to the cell wall and the release of the protein with solvent (Wang *et al.,* 2021). In this part of the study, HU-80-100 was selected as the most suitable treatment for TLPP preparation by the HU due to having the highest protein content obtained among the treatments.

3.2.3 Alkaline pH shift process

The extraction yield, protein recovery yield, and total protein content of TLPP prepared by the APS process are shown in Table 1. According to the results obtained in our preliminary trial (data not shown), both the extraction and protein recovery yields of the TLPP

https://doi.org/10.26656/fr.2017.8(2).465

obtained by APS at pH 11.5 were significantly higher ($p < 0.05$) than those obtained at pHs 2.5, 3.5, and 10.5. This might be related to the presence of negatively charged proteins under extremely alkaline pH conditions which could readily combine with water and consequently increase the protein solubility and yield (Tain *et al.,* 2017).

Additionally, the total protein content of APS 11.5 (51.41%) was significantly ($p < 0.05$) higher than that of the TL powder (47.95%) (Table 1). This result might be explained by the high recovery of myofibrillar and water-soluble proteins due to the APS process (Tian *et al.,* 2017). Therefore, the optimal pH to produce recovered proteins from TL using the pH shift process was determined to be pH 11.5. However, the protein content of APS 11.5 obtained in this study was lower than that of common carp muscle protein isolates obtained following the APS process (Tian *et al.,* 2017). This could be affected by many factors, such as a variation in raw materials, the amino acid composition, the ratio between the raw materials and water, and the trapping of solubilized proteins following the first centrifugation (Abdollahi and Undeland, 2019).

3.2.4 Supercritical carbon dioxide fluid extraction

The extraction yield of the TLPPs prepared by SC-CO_2 extraction is shown in Table 1. The SC-CO_2-250 (75.19%) showed a significantly higher ($p < 0.05$) extraction yield than the SC-CO_2-350 (74.02%). This result indicated that pressure inversely affected the extraction yield of the protein following SC-CO_2 extraction ($p < 0.05$). With the increase in pressure, however, the protein recovery yield and total protein content of the TLPPs obtained following the SC-CO_2 extraction increased.

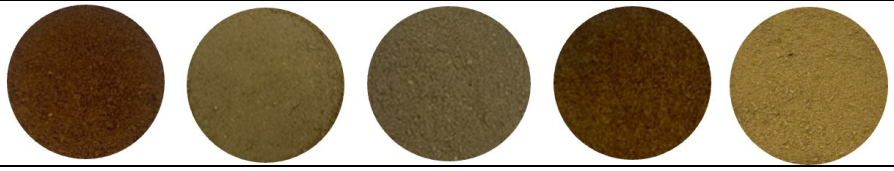
The SC-CO_2-350 showed a significantly higher ($p < 0.05$) protein recovery yield and protein content (99.38 and 71.75%, respectively) than the SC-CO_2-250 (95.61 and 60.97%, respectively). The application of SC-CO_2 in protein preparation is due to the removal of lipids and lipophilic substances from the protein matrices (Fang *et al.,* 2019). Generally, most of the lipid components were effectively removed at higher pressures (Kang *et al.,* 2017), resulting in an increase in the total protein content of SC-CO_2-350 compared to SC-CO_2-250. In this part of the study, SC-CO_2-350 was selected as the most suitable treatment for TLPP preparation following SC-CO_2 extraction due to having the highest protein content obtained between treatments.

3.3 Physical and chemical characterization of tuna liver powder and selected tuna liver protein powders

3.3.1 Proximate composition and a_w of tuna liver powder and selected tuna liver protein powders

The proximate composition of TL powder and the selected TLPPs obtained following the H85, HU-80-100, APS 11.5, and SC-CO_2-350 treatments are shown in Table 2. Different preparation methods had significant effects on the proximate quality of the TLPPs. After treatment, the protein content was significantly increased, whereas the lipid and moisture content were significantly decreased. However, the ash content was significantly increased in SC-CO_2-350 but decreased in H85, HU-80-100, and APS 11.5. The highest protein content was observed in SC-CO_2-350 (71.75%) which was consistent with the findings of Kang *et al.* (2017)

Table 2. Proximate composition, water activity, color, and visual appearance of TL powder and TLPPs from different reparation methods

	TL powder	H85	HU-80-100	APS 11.5	SC-CO_2-350
Proximate composition (g/100 g)					
Protein	47.95±0.37^{d}	53.81±0.32^{b}	54.39±0.04^{b}	51.41±0.44^{c}	71.75±1.06^{a}
Lipid	21.02±0.30^{a}	16.36±0.84^{b}	16.54±0.33^{b}	13.98±0.04^{c}	2.05±0.30^{d}
Moisture	6.65±0.35^{a}	4.02±0.21^{c}	4.28±0.30^{bc}	3.83±0.11^{c}	4.72±0.41^{b}
Ash	4.04±0.02^{b}	1.41±0.01^{d}	1.43±0.02^{d}	2.92±0.09^{c}	5.13±0.03^{a}
Water activity	0.44±0.00^{c}	0.51±0.00^{a}	0.50±0.00^{b}	0.41±0.00^{d}	0.18±0.00^{e}
Color					
*L**	40.05±0.02^{e}	62.15±0.02^{b}	63.74±0.05^{a}	45.39±0.00^{d}	61.35±0.04^{c}
*a**	14.40±0.03^{a}	7.99±0.01^{d}	8.13±0.02^{c}	11.00±0.03^{b}	6.79±0.07^{e}
*b**	19.95±0.08^{d}	24.71±0.01^{b}	25.64±0.16^{a}	19.70±0.03^{d}	20.59±0.38^{c}
Visual appearance					

Values are presented as mean±SD (n = 3), dry weight basis. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$).

https://doi.org/10.26656/fr.2017.8(2).465

who reported the higher protein content of bovine liver powder by SC-CO_2 extraction in comparison to other methods. All preparation methods significantly reduced ($p < 0.05$) the lipid content in the liver, with SC-CO_2-350 having the lowest lipid content ($p < 0.05$). This could be attributed to the greater diffusivity of SC-CO_2 in the matrix caused by its high compressibility, liquid-like density, and low viscosity characteristics compared to other preparation methods (Klettenhammer *et al.,* 2020), leading to the efficient removal of lipid and lower lipid content (Fang *et al.,* 2019). Compared to the lipid content of bovine liver powder (3.08-5.04%) obtained following SC-CO_2 extraction at 200-450 bar (Kang *et al.,* 2017), SC-CO_2-350 had a lower lipid content (2.05%). The moisture content of all TLPPs (3.83-4.72%) was significantly decreased to less than 10% which agreed well with the findings for most fish protein powders (Shaviklo, 2015). The water activity (a_w) of TL powder and the selected TLPPs ranged from 0.18 - 0.51 which met the standard requirement for low-moisture food (< 0.6) (Thai Industrial Standards Institute (TISI), 2014). The ash content of the SC-CO_2-350 (5.12%) was significantly high among the preparation methods ($p < 0.05$). This is consistent with the findings for rice bran powder that was obtained following SC-CO_2 extraction (Sparks *et al.,* 2006).

3.3.2 Colors and visual appearance of tuna liver powder and selected tuna liver protein powders

The color of dried products is important for the latter application of TLPPs. The color parameters of TLPPs were given in Table 2. Of note, L^* and b^* were both increased, while a^* was decreased after the treatments. The H85 (62.15), HU-80-100 (63.74), and SC-CO_2-350 (61.35) showed an extreme increase in L^* than APS 11.5 (45.39) compared to TL powder (40.35). This could be explained by the effect of heat (Pohlman *et al.,* 1997) and high pressure (Carlez *et al*., 1995) on the denaturation of myoglobin and hemoglobin. The remarkable decrease in a^* of the treated groups, particularly SC-CO_2-350, was probably due to the effect of high pressure on the enzyme responsible for the redox reaction of myoglobin in the samples (Jung *et al.,* 2003). Additionally, the b^* of the HU-80-100 significantly increased compared to the other treatments. Overall, the results of the color analysis were in agreement with the visual appearance of the TLPPs which were whiter and more yellow than the TL powder (Table 2).

From the above results, SC-CO_2-350 may potentially serve as a source of protein containing a lower lipid content and improved coloration.

3.3.3 Amino acids of tuna liver powder and selected tuna liver protein powders

The amino acid profiles of the TL powder and selected TLPPs are presented in Table 3. In the TL powder, the most abundant amino acids were glutamic acid (12.37%), aspartic acid (8.86%), cysteine (8.28%), leucine (8.18%), and alanine (6.59%). Overall, the major amino acid compositions of the TL powder were consistent with those reported by Kang *et al.* (2007), wherein aspartic acid, glutamic acid, leucine, and alanine are the four major amino acids in the Skipjack tuna liver. After the preparation of TLPPs, aspartic acid and glutamic acid (12.19–14.51% and 9.48–9.64% of the total amino acids, respectively), which contributed to the umami flavor (Han *et al.,* 2019), remained the most prominent amino acids. A significant increase ($p < 0.05$) in the amino acid content of TLPPs was observed in aspartic acid, phenylalanine, methionine, and tyrosine compared to the TL powder. However, histidine decreased significantly ($p < 0.05$), particularly in APS 11.5 which could be attributed to the oxidation sensitivity of histidine, resulting in the formation of aspartic acid (increased as shown in Table 3) as a major oxidation product (Hrynets *et al.,* 2011). Among the preparation methods, SC-CO_2-350 provided the highest amount of flavor enhancing amino acids, i.e., glutamic acid, aspartic acid, and alanine, suggesting the feasibility of SC-CO_2-350 as a promising flavor enhancer.

The most abundant essential amino acids (EAA) were leucine, valine, and lysine for TL powder, H85, and HU-80-100; leucine, phenylalanine, and threonine for APS 11.5; and lysine, leucine, and threonine for SC-CO_2-350. Our findings were comparable to those of Fang *et al*. (2020) who found that leucine, phenylalanine, lysine, and valine were the most abundant EAAs in the tuna liver and liver protein powder obtained following the different preparation methods. These EAAs are important as they promote brain and immune functions, are associated with muscle metabolism, boost energy, and assist the body in recovering from strenuous physical activity (Sarojnalini and Hei, 2019). In the present study, APS 11.5 and SC-CO_2-350 can help to maintain total essential amino acid (TEAA) content while H85 and HU-80-100 significantly enhanced ($p < 0.05$) the TEAA content of the TLPPs compared to the TL powder. Overall, the amino acid analysis revealed that the EAA content of all TLPPs meets the Food and Agriculture Organization (FAO, 2013) recommendations for adults, indicating the high nutritional value of TLPPs.

3.3.4 Minerals

The macro and micro mineral content of the TL powder and selected TLPPs are presented in Table 4.

https://doi.org/10.26656/fr.2017.8(2).465

Table 3. Amino acid profile of TL powder and TLPPs from different preparation methods.

Amino acid	Content (g/100 g protein)					
	TL powder	H85	HU-80-100	APS 11.5	SC-CO_2-350	Requirements for adults (FAO, 2013)
Aspartic acid	8.86±0.52^{c}	9.48±0.01^{b}	9.64±0.04^{b}	10.67±0.31^{a}	10.19±0.04^{a}	
Glutamic acid	12.37±0.58^{bc}	12.19±0.03^{c}	12.15±0.07^{c}	12.83±0.19^{b}	14.51±0.05^{a}	
Serine	4.36±0.12^{c}	4.34±0.03^{c}	4.38±0.03^{c}	5.20±0.08^{a}	4.99±0.02^{b}	
Histidine*	2.80±0.01^{a}	2.63±0.05^{e}	2.68±0.03^{b}	2.13±0.04^{e}	2.44±0.03^{d}	1.5
Glycine	5.14±0.24^{a}	4.56±0.04^{b}	4.59±0.01^{b}	4.91±0.12^{a}	5.08±0.03^{a}	
Threonine*	4.62±0.17^{c}	4.75±0.02^{c}	4.98±0.02^{b}	5.26±0.06^{a}	5.40±0.02^{a}	2.3
Arginine	5.26±0.26^{c}	6.10±0.04^{a}	5.86±0.03^{b}	5.99±0.02^{ab}	5.03±0.03^{d}	
Alanine	6.59±0.27^{b}	6.17±0.02^{cd}	6.03±0.04^{d}	6.41±0.10^{bc}	6.85±0.02^{a}	
Tyrosine	2.93±0.18^{c}	3.33±0.01^{b}	3.40±0.01^{b}	3.69±0.04^{a}	3.68±0.01^{a}	
Cysteine	8.28±0.67^{a}	7.17±0.06^{a}	7.31±0.21^{a}	7.30±1.15^{a}	3.23±0.05^{b}	
Valine*	5.91±0.16^{b}	6.19±0.02^{a}	6.07±0.02^{a}	5.02±0.03^{c}	5.02±0.01^{d}	3.9
Methionine*	2.77±0.03^{d}	3.06±0.02^{a}	2.94±0.02^{b}	3.06±0.03^{a}	2.85±0.01^{c}	2.2
Phenylalanine*	4.58±0.08^{c}	5.37±0.05^{a}	5.37±0.03^{a}	5.36±0.07^{a}	4.82±0.01^{b}	3.8
Isoleucine*	4.87±0.12^{b}	5.40±0.08^{a}	5.35±0.02^{a}	4.26±0.04^{c}	3.81±0.01^{d}	3.0
Leucine*	8.18±0.13^{c}	8.97±0.03^{a}	8.82±0.05^{b}	8.78±0.06^{b}	8.01±0.01^{d}	5.9
Lysine*	5.72±0.13^{b}	5.54±0.02^{bc}	5.59±0.08^{b}	5.13±0.50^{c}	8.08±0.05^{a}	4.5
Proline	4.16±0.17^{b}	3.89±0.05^{b}	3.89±0.29^{b}	3.27±0.48^{c}	5.37±0.11^{a}	
Tryptophan*	1.03±0.07^{a}	0.86±0.01^{ab}	0.94±0.00^{a}	0.73±0.01^{bc}	0.57±0.25^{c}	0.6
TEAA	40.46±0.60^{bc}	42.76±0.09^{a}	42.75±0.22^{a}	39.72±0.78^{c}	41.00±0.15^{b}	

Values are presented as mean±SD (n = 3), dry weight basis. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). TEAA: Total essential amino acids.

Most of the macro mineral contents in H85, HU-80-100, and APS 11.5 were decreased while SC-CO_2-350 was increased. The predominant mineral contents were P, K, Na, and Mg for TL powder, H85, and HU-80-100; Na, P, K, and Fe for APS 11.5; and P, K, Na, and Fe for SC-CO_2-350. Interestingly, most of the mineral content, except Se and Ni, was increased in the SC-CO_2-350 treatment. This might be related to the significant lipid removal in SC-CO_2-350 compared to the other treatments (Table 2). A similar phenomenon was reported by Shen *et al.* (2021) who found that the increasing content of minerals in the protein powder resulted from the removal of lipids and moisture during preparation. On the other hand, the contents of P, K, Na, and Mg, in both H85 and HU-80-100 were dramatically decreased. These minerals are the major cation in the extracellular fluids which are easily lost in water during the heat denaturation of the protein compared to intracellular ions and minerals-bound proteins (Goluch *et al.,* 2021). Our findings were found to be consistent with those of Goluch *et al.* (2021) who found that K, P, Na, and Mg were decreased in heat-treated goose breast meat (*Pectoralis major*). Of note, most minerals in APS 11.5 were decreased compared to other treatments, except Na, which was probably due to the minerals easily reacting to the alkaline conditions, resulting in the solubilization (loss) of minerals during the pH shift process (Shen *et al.,* 2021).

In terms of micro mineral content, Zn in all treatments was increased, probably due to the higher binding affinity of the metal to the proteins. This is associated with their role as cofactors in enzymes (Abdollahi *et al.,* 2021). In addition, the content of Fe in the APS 11.5 and SC-CO_2-350 treatments was increased which might be due to the co-precipitation of hemoglobin with the recovered proteins during the pH shift process and the effect of pressure on the increase in protein powder concentration due to the removal of lipids and moisture, respectively (Pires *et al.*, 2012; Fang *et al.,* 2022). Overall, the contents of Fe, Zn, Cu, and Se, as micro minerals in TLPPs, meet the requirements set for the recommended dietary allowances (RDA) established for mineral intake for both male and female adults (FDA, 2018). Therefore, it is likely that the TLPPs obtained following the different preparation methods can be useful as mineral supplements in foods.

3.4 Functional properties

The functional properties of the TLPPs were determined and compared to the reference protein powders (egg white powder - EW and soy protein concentrate - SP) as shown in Figure 2 and Table 5.

3.4.1 Relative protein solubility

The solubility of proteins is important in food

https://doi.org/10.26656/fr.2017.8(2).465

products because it has a range of effects on the functional properties of proteins such as foam ability and emulsification (Kristinsson and Rasco, 2000). The relative protein solubility (%) of TLPPs, EW, and SP in the pH range of 2 to 12 is depicted in Figure 2. The minimum solubilities of the TLPPs were observed at pHs 3–8 (0.13–34.96%) which could be attributed to the proximity between the pH of the solution and the isoelectric point of the protein. This consequently enhanced protein precipitation (Cha *et al.*, 2020). These findings agreed well with those from a study of proteins derived from salmon, cod, and herring byproducts which demonstrated the lowest protein solubilities at pHs 4–8 for the same reason (Abdollahi and Undeland, 2018). An increase in pH from 8 to 11 resulted in an increase in the protein solubility of TLPPs; the maximum protein solubility of the TLPPs was found at pH 11 (39.35-92.75%), indicating that the TLPPs could be utilized in an alkaline pH range. These could be attributed to the increasing charge of amino acids under alkaline pH conditions (Kristinsson and Rasco, 2000). According to the results, the protein solubility pattern of the TLPPs was consistent with that of fish myofibrillar proteins which are highly solubilized at an extremely high pH (Abdollahi and Undeland, 2018).

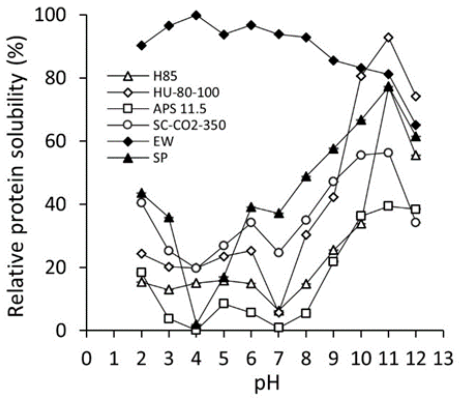


Figure 2. Relative protein solubility (%) at pH 2.0 to 12.0 of TLPPs from different preparation methods. Values are presented as mean±SD (n = 3), dry weight basis

Among the different preparation methods, SC-CO_2-350 (19.67–47.20%) showed higher protein solubility at pHs 2–9. These results indicated that SC-CO_2 extraction remarkably improved protein solubility in this pH range, most likely due to the minimum surface hydrophobicity resulting from the removal of bound lipids (Kang *et al.,* 2017; Fang *et al.,* 2019), as confirmed by the lowest lipid content in SC-CO_2-350 (Table 2).

Notably, the highest protein solubility at pHs 10–11 was found in HU-80-100 (80.55–92.75%). This could be attributed to the partial unfolding and opening of the protein by the mechanical force created by cavitation, leading to the exposure of the hydrophilic groups of amino acids and a reduction in the particle size of the protein which consequently improves protein-water interactions (Nazari *et al.,* 2018). An enhancement of protein solubility has also been observed in millet protein subjected to ultrasonication (Nazari *et al.,* 2018). On the other hand, APS 11.5 showed the lowest protein solubility across all pHs (<40%). This could be explained by the larger amount of salt formation in APS 11.5 compared to the other preparation methods (Table 4). This could lead to salting-out effects and a consequent decrease in protein solubility (Kaur *et al.,* 2021).

In this study, EW and SP were used as the reference controls. Interestingly, TLPPs showed an almost similar pattern of protein solubility to SP, a plant-derived protein, but not to EW of animal origins. This was similar to the findings of Abdollahi and Undeland (2018). Overall, the protein solubility of SC-CO_2-350 and HU-80-100 was found to be comparable to that of SP, indicating the applicability of these TLPPs in food products.

Table 4. Mineral content TL powder and TLPPs from different preparation methods.

Mineral	Content (mg/kg)					RDA for adult (mg/day)	
	TL powder	H85	HU-80-100	APS 11.5	SC-CO_2-350	Male	Female
Macro minerals							
Mg	652.91	486.55	443.88	193.48	1030.30	400	310
Ca	54.31	51.72	45.16	49.30	63.50	1000	1000
Na	3608.84	1699.00	1471.93	22986.00	4477.67	1500	1500
K	6631.22	3430.97	3187.09	1694.48	8695.49	3400	2600
P	7529.99	4317.68	4247.03	2604.42	10821.57	700	700
Micro minerals							
Fe	533.21	446.04	436.00	538.01	1080.29	8	18
Zn	123.02	163.90	161.83	172.97	132.68	11	8
Cu	30.69	36.26	37.51	23.00	64.01	0.9	0.9
Se	21.07	16.49	15.07	12.40	20.85	0.055	0.055
Ni	<0.40	<0.40	<0.40	0.46	<0.40	1	1

Values presented are means of two replicates of each measurement, dry weight basis.

https://doi.org/10.26656/fr.2017.8(2).465

Table 5. Functional properties of TLPPs from different preparation methods.

Functional properties	Protein powders					
	H85	HU-80-100	APS 11.5	SC-CO_2-350	EW	SP
EAI (m^2/g)	4.02±0.08^{e}	4.55±0.07^{e}	13.79±0.43^{c}	6.71±0.09^{d}	17.36±0.15^{b}	23.46±0.03^{a}
ESI (min)	39.97±0.72^{c}	55.88±0.59^{b}	34.76±1.06^{e}	38.33±0.86^{d}	25.01±0.81^{f}	79.70±0.66^{a}
FC (%)	125.83±1.14^{d}	119.17±1.14^{e}	124.17±1.14^{d}	155.83±1.44^{b}	174.17±1.14^{a}	145.83±1.14^{c}
FS (%)	44.35± 1.91^{f}	88.77±1.03^{d}	91.47±0.63^{c}	82.09±1.80^{e}	100.01±1.95^{a}	96.19±0.16^{b}
WHC (mL/g)	4.07±0.12^{c}	4.50±0.10^{c}	18.61±1.27^{b}	3.53±0.12^{cd}	2.50±0.83^{d}	27.22±0.96^{a}
OHC (mL/g)	11.33±0.23^{a}	6.40±0.40^{d}	8.67±0.23^{b}	7.73±0.23^{c}	5.07±0.46^{e}	7.07±0.61^{cd}

Values are presented as mean±SD (n = 3), dry weight basis. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$).

3.4.2 Emulsion properties

The ability of the protein to form an emulsion was determined by EAI and ESI (Cha *et al.,* 2020). The results show that the preparation methods significantly affected ($p < 0.05$) both the EAI and ESI of TLPPs (Table 5). Overall, the highest EAI was found in APS 11.5 (13.79 m^2/g), indicating that APS can highly reduce the interfacial tension of emulsion in comparison to SC-CO_2-350, HU-80-100, and H85, all of which possessed lower EAIs (6.71, 4.55 and 4.02 m^2/g, respectively). This might be related to the denaturation and partial unfolding of proteins by the APS which enhances the adsorption of the hydrophobic globular head of protein with lipids (Panpipat and Chaijan, 2017). However, the EAI of EW and SP was found to be significantly higher ($p < 0.05$) than that of the TLPPs. This might be due to the increased hydrophobicity of EW and SP that enhances their emulsifying capacity (Pires *et al.,* 2012).

HU-80-100 had the significantly highest ($p < 0.05$) ESI (55.88 mins), followed by H85 (39.97 mins), SC-CO_2-350 (38.33 mins), and APS 11.5 (34.76 mins), respectively. This could be explained by the disruption of the protein structure following the ultrasound treatment, resulting in the reduction of the protein size, leading to the enhancement of protein-bound lipids at the oil-water interface (Nazari *et al.,* 2018). This result was found to agree well with the findings of Kang *et al.* (2022) regarding chickpea protein in which the ESI was significantly enhanced following ultrasound treatment. Additionally, the ESI of the TLPPs was significantly higher ($p < 0.05$) than the EW. This might be explained by the exposure of the hydrophobic groups, the proper protein arrangement (Kang *et al.,* 2022), and varying degrees of the proteins breaking down (Kang *et al.,* 2017) caused by the different methods of protein production. This stabilized the network of proteins formed in the emulsion and consequently increased the emulsion stability (Panpipat and Cajian, 2017). However, the ESI of the TLPPs was significantly lower than that of SP ($p < 0.05$) which might be correlated with the lower EAI (Shen *et al.,* 2021).

3.4.3 Foaming properties

Foaming properties (FC and FS) are the major properties of protein products used in food applications (Abdollahi and Undeland, 2018). In this study, the FC and FS of TLPPs are presented in Table 5. The preparation methods had a significant influence ($p < 0.05$) on the foaming properties of TLPPs. It was observed that the SC-CO_2-350 (155.83%) showed a significantly higher ($p < 0.05$) FC compared to H85 (125.83%), HU-80-100 (119.17%) and APS 11.5 (127.17%). Similarly, Kang *et al.* (2017) demonstrated that bovine liver protein obtained following SC-CO_2 extraction had a greater FC than the protein produced by other methods. This was probably due to the effect of the SC-CO_2 extraction on the elimination of non-polar lipids which enhanced foam formation by increasing the affinity of the protein at the air-water interface (Yuen *et al.,* 2019). Compared to EW and SP, the SC-CO_2-350 possessed a significantly higher ($p < 0.05$) FC than SP which might be related to the unfolding of the protein that occurred during the SC-CO_2 extraction (Solana *et al.,* 2016). However, all of the TLPPs had a significantly lower ($p < 0.05$) FC than EW which might be related to the lower protein solubility of the TLPPs compared to EW (Figure 2) (Abdollahi and Undeland, 2018).

Generally, the FS is affected by the stability and gas permeability of the protein film that is formed on the air-liquid surface (Barać *et al.,* 2011). APS 11.5 (91.47%) exhibited the significantly highest FS ($p < 0.05$), followed by HU-80-100 (88.77%), SC-CO_2-350 (82.09%), and H85 (44.35%), respectively. This might be explained by the enhancement of the interactions of the protein due to APS, facilitated by the formation of a cohesive protein film at the air-water interface (Panpipat and Chaijan, 2017). Interestingly, the FS of TLPPs, except for H85, was greater than 80%, which was comparable to that of EW and SP. This indicates the potential of TLPPs as excellent foam stabilizers. According to the above results, the TLPPs possessed a high potency for foam stability, which may be applicable

https://doi.org/10.26656/fr.2017.8(2).465

to foam-based foods.

3.4.4 Water holding capacity

WHC is an important property in food systems because it influences the flavor and texture of food (Cha *et al.,* 2020). The WHC of the TLPPs is shown in Table 5. The WHC of APS 11.5 (18.61 mL/g) was significantly higher ($p < 0.05$) than those of HU-80-100 (4.55 mL/g), H85 (4.05 mL/g), and SC-CO_2-350 (3.53 mL/g). These results agree well with the findings of Han *et al.* (2019), who found that the scallop (*Patinopecten yessoensis*) gonad protein obtained following APS extraction showed improved WHC. The higher WHC of APS 11.5 might be related to the lower solubility of protein (Figure 2), resulting in a higher volume of held water (Cha *et al.,* 2020). Compared to EW and SP, the WHC of TLPPs (4.07-18.62 mL/g), except for SC-CO_2-350 (3.53 mL/g), was significantly higher ($p < 0.05$) than that of EW (2.50 mL/g). This was probably due to the conformational structure change of protein, allowing the myofibrillar protein and hydrophilic amino acids to accommodate water, which increases the WHC of TLPPs (Zou *et al.,* 2017; Cha *et al.,* 2020). However, the WHC of all TLPPs was significantly lower ($p < 0.05$) than that of SP (27.22 mL/g) which was consistent with the findings for scallop gonad protein compared to SP (Han *et al.,* 2019).

3.4.5 Oil holding capacity

OHC is an important property required in the meat and emulsion industries to improve product texture (Kang *et al.,* 2017). In this study, it was found that H85 (11.33 mL/g) had a higher OHC than APS 11.5, HU-80-100, and SC-CO_2-350 (6.40-8.67 mL/g) (Table 5). The higher OHC of H85 could be explained by the effects of thermal degradation on the opening of a globular protein which enhances the binding between hydrophobic amino acids, especially leucine and valine (Table 3), and fat (Haryati *et al.,* 2019). However, TLPPs exhibited significantly higher ($p < 0.05$) OHC to EW and similar or higher ($p < 0.05$) OHC to SP, suggesting the probable application of TLPPs in enhancing the mouthfeel and retention of flavor and improving food palatability (El Nasri and El Tinay, 2007).

According to the results above, the SC-CO_2-350 treatment showed the highest protein solubility and FC which may be applied to foam-based food products. H85 and HU-80-100 exhibited superior emulsion properties and OHC can be used for emulsifying food. Excellent FS and WHC were found in APS 11.5 which can be used to improve the flavor and texture of foods. Additionally, the functional property analyses of the TLPPs obtained following the different preparation methods pointed out that these proteins are equivalent in many aspects to commercial proteins such as EW and SP, especially in terms of solubility, foaming properties, WHC, and OHC. Thus, TLPPs can potentially be applied in various food systems and used as alternative protein ingredients in foods.

4. Conclusion

The effect of the different preparation methods on the physical, chemical and functional properties of TLPPs has been successfully addressed in the present study. Of all the methods, SC-CO_2-350 led to the significant removal of lipids and remarkably increased the protein content of the TLPP. The color of all TLPPs was remarkably improved. However, the order of prominent amino acids (glutamic acid, aspartic acid, and alanine) remained unchanged following the application of the different preparation methods, and the EAA contents of all TLPPs were found to meet the requirements for adults as suggested by the FAO. In terms of their functional properties, SC-CO_2-350 showed the highest protein solubility and FC. H85 and HU-80-100 were high for EAI, ESI, and OHC. APS 11.5 possessed significant FS and WHC. Therefore, the TLPPs obtained in the present study are found to be distinctive based on the preparation methods, leading to the application of TLPPs as a protein material for many purposes.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The research work was funded by Kasetsart University Research and Development Institute (KURDI), FF(KU)7.65 under the research program “Development of functional ingredients from by-products of canned tuna processing”.

References

Abdollahi, M. and Undeland, I. (2018). Structural, functional, and sensorial properties of protein isolate produced from salmon, cod, and herring by-products. *Food and Bioprocess Technology*, 11, 1733-1749. https://doi.org/10.1007/s11947-018-2138-x

Abdollahi, M. and Undeland, I. (2019). Physicochemical and gel-forming properties of protein isolated from salmon, cod and herring by-products using the pH-shift method. *LWT*, 101, 678-684. https://doi.org/10.1016/j.lwt.2018.11.087

Abdollahi, M., Wu, H. and Undeland, I. (2021). Impact of processing technology on macro-and micronutrient profile of protein-enriched products from fish

backbones. *Foods*, 10(5), 950. https://doi.org/10.3390/foods10050950

Association of Official Analytical Chemists (AOAC). (2000). Official Methods of Analysis. 17th ed. Maryland, USA: AOAC.

Association of Official Analytical Chemists (AOAC). (2013). Interlaboratory Study Workbook for Blind (Unpaired) Replicates. Ver. 2.1. Rockville, USA: AOAC.

Barać, M., Cabrilo, S., Pešić, M., Stanojević, S., Pavlićević, M., Maćej, O. and Ristić, N. (2011). Functional properties of pea (*Pisum sativum*, L.) protein isolates modified with chymosin. *International Journal of Molecular Sciences*, 12(12), 8372–8387. https://doi.org/10.3390/ijms12128372

Carlez, A., Veciana-Nogues, T. and Cheftel, J.C. (1995). Changes in colour and myoglobin of minced beef meat due to high pressure processing. *LWT*, 28(5), 528–538. https://doi.org/10.1006/fstl.1995.0088

Cha, J.W., Yoon, I.S., Lee, G.W., Kang, S.I., Park, S.Y., Kim, J.S. and Heu, M.S. (2020). Food functionalities and bioactivities of protein isolates recovered from skipjack tuna roe by isoelectric solubilization and precipitation. *Food Science and Nutrition*, 8(4), 1874-1887. https://doi.org/10.1002/fsn3.1470

Chantachum, S., Benjakul, S. and Sriwirat, N. (2000). Separation and quality of fish oil from precooked and non-precooked tuna heads. *Food Chemistry*, 69(3), 289 -294. https://doi.org/10.1016/S0308-8146(99)00266-6

El Nasri, N.A. and El Tinay, A.H. (2007). Functional properties of fenugreek (*Trigonella foenum graecum*) protein concentrate. *Food Chemistry*, 103(2), 582–589. https://doi.org/10.1016/j.foodchem.2006.09.003

Fang, Y., Gu, S., Liu, S., Zhang, J., Ding, Y. and Liu, J. (2018). Extraction of oil from high-moisture tuna liver by subcritical dimethyl ether: feasibility and optimization by the response surface method. *RSC Advances*, 8(5), 2723-2732. https://doi.org/10.1039/C7RA12948C

Fang, Y., Liu, S., Hu, W., Zhang, J., Ding, Y. and Liu, J. (2019). Extraction of oil from high moisture tuna livers by subcritical dimethyl ether: a comparison with different extraction methods. *European Journal of Lipid Science and Technology*, 121(2), 1800087. https://doi.org/10.1002/ejlt.201800087

Fang, Y., Liu, J., Li, J., Chen, W., Huang, G. and Ding, Y. (2020). Rapid preparation of protein powder from high moisture tuna liver: New insight into subcritical dimethyl ether. *LWT*, 124, 109179. https://doi.org/10.1016/j.lwt.2020.109179

Fang, Y., Huang, Q., Lin, Y., Ge, H., Huang, G. and Jiang, H. (2022). Physicochemical, digestive and rheological properties of protein from tuna by subcritical dimethyl ether: Focus on process-related indexes. *Food Chemistry*, 372, 131337. https://doi.org/10.1016/j.foodchem.2021.131337

FAO. (2013). Dietary protein quality evaluation in human nutrition: report of an FAO Expert Consultation. Food and Nutrition Paper 92, p. 72. Rome, Italy: Food and Agriculture Organization.

Food and Drug Administration (FDA). (2018). Dietary supplement products and ingredients. Retrieved on August 20, 2022 from Food and Drug Administration website: www.fda.gov/Food/DietarySupplements/ProductsIngredients/default.htm.

Golly, M.K., Ma, H., Yuqing, D., Dandan, L., Quaisie, J., Tuli, J.A., Mintah, B.K., Dzah, C.S. and Agordoh, P.D. (2020). Effect of multi-frequency countercurrent ultrasound treatment on extraction optimization, functional and structural properties of protein isolates from Walnut (*Juglans regia* L.) meal. *Journal of Food Biochemistry*, 44(6), e13210. https://doi.org/10.1111/jfbc.13210

Goluch, Z., Barbara, K., Haraf, G., Woloszyn, J., Okruszek, A. and Werenska, M. (2021). Impact of various types of heat processing on the energy and nutritional values of goose breast meat. *Poultry Science*, 100(11), 101473. https://doi.org/10.1016/j.psj.2021.101473

Han, J.R., Tang, Y., Li, Y., Shang, W.H., Yan, J.N., Du, Y.N. Wu, H.T., Zhu, B.W. and Xiong, Y.L. (2019). Physiochemical properties and functional characteristics of protein isolates from the scallop (*Patinopecten yessoensis*) gonad. *Journal of Food Science*, 84(5), 1023-1034. https://doi.org/10.1111/1750-3841.14598

Haryati, S., Sukarno, S., Budijanto, S. and Prangdimurti, E. (2019). Characterization of functional properties catfish protein isolates (*Clarias* sp.). *IOP Conference Series: Earth and Environmental Science*, 404, 012032. https://doi.org/10.1088/1755-1315/404/1/012031

Hrynets, Y., Omana, D.A., Xu, Y. and Betti, M. (2011). Comparative study on the effect of acid- and alkaline-aided extractions on mechanically separated turkey meat (MSTM): Chemical characteristics of recovered proteins. *Process Biochemistry*, 46(1), 335-343. https://doi.org/10.1016/j.procbio.2010.09.006

Jajic, I., Krstović, S., Glamocic, D. and Jakšić, S. (2013). Validation of an HPLC method for the determination of amino acids in feed. *Journal of the Serbian Chemical Society*, 78(6), 839-850. https://doi.org/10.2298/JSC120712144J

Jung, S., Ghoul, M. and de Lamballerie-Anton, M. (2003). Influence of high pressure on the color and microbial quality of beef meat. *LWT,* 36(6), 625–631. https://doi.org/10.1016/S0023-6438(03)00082-3

Kang, T.K., Heu, M.S., Jee, J.J., Lee, J.H., Kim, H.S. and Kim, J.S. (2007). Food component characteristics of tuna livers. *Food Science and Biotechnology*, 16(3), 367-373.

Kang, S.W., Kim, H.M., Rahman, M.S., Kim, A.N., Yang,

H.S. and Choi, S.G. (2017). Nutritional quality and physicochemical characteristics of defatted bovine liver treated by supercritical carbon dioxide and organic solvent. *Food Science of Animal Resources*, 37(1), 29-37. https://doi.org/10.5851/kosfa.2017.37.1.29

Kang, S., Zhang, J., Guo, X., Lei, Y. and Yang, M. (2022). Effects of ultrasonic treatment on the structure, functional properties of chickpea protein isolate and its digestibility *in vitro*. *Foods*, 11, 880.

Kaur, L., Lamsar, H., López, I.F., Filippi, M., Min, D.O.S., Ah-Sing, K. and Singh, J. (2021). Physico-chemical characteristics and *in vitro* gastro-small. *Foods*, 10(2), 331. https://doi.org/10.3390/foods10020331

Klettenhammer, S., Ferrentino, G., Morozova, K. and Scampicchio, M. (2020). Novel technologies based on supercritical fluids for the encapsulation of food grade bioactive compounds. *Foods*, 9(10), 1395. https://doi.org/10.3390/foods9101395

Kristinsson, H.G. and Rasco, B.A. (2000). Fish protein hydrolysates: production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition*, 40(1), 43-81. https://doi.org/10.1080/10408690091189266

Lowry, O., Rosebrough, N., Farr, A.L. and Randall R. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Nazari, B., Mohammadifarb, M.A., Shojaee-Aliabadia. S., Feizollahia, E. and Mirmoghtadaiea, L. (2018). Effect of ultrasound treatments on functional properties and structure of millet protein concentrate. *Ultrasonics Sonochemistry*, 41, 382-388. https://doi.org/10.1016/j.ultsonch.2017.10.002

Panpipat, W. and Chaijan, M. (2017). Functional properties of pH-shifted protein isolates from bigeye snapper (*Priacanthus tayenus*) head by-product. *International Journal of Food Properties*, 20(3), 596-610. https://doi.org/10.1080/10942912.2016.1171778

Pires, C., Costa, S., Batista, A.P., Nunes, M.C., Raymundo, A. and Batista, I. (2012). Properties of protein powder prepared from cape hake by-products. *Journal of Food Engineering*, 108(2), 268–275. https://doi.org/10.1016/j.jfoodeng.2011.08.020

Pohlman, F.W., Dikeman, M.E. and Zayas, J.F. (1997). The effect of low-intensity ultrasound treatment on shear properties, color stability and shelf-life of vacuum-packaged beef s*emitendinosus* and *biceps femoris* muscles. *Meat Science*, 45(3), 329-337. https://doi.org/10.1016/S0309-1740(96)00106-4

Przybylski, W., Jaworska, D., Kajak-Siemaszko, K., Sałek, P. and Pakuła, K. (2021). Effect of heat treatment by the sous-vide method on the quality of poultry meat. *Foods*, 10(7), 1610. https://doi.org/10.3390/foods10071610

Sarojnalini, C. and Hei, A. (2019). Fish as an important functional food for quality life. In Lagouri, V. (Eds.). Functional foods. InTech Open E-Book. https://doi.org/10.5772/intechopen.81947

Shaviklo, A.R. (2015). Development of fish protein powder as an ingredient for food applications: a review. *Journal of Food Science and Technology*, 52(2), 648–661. https://doi.org/10.1007/s13197-013-1042-7

Shen, K., Mu, W., Xia, S., Chen, Y., Ren, H., Xie, X., Fang, Y. and Huang, G. (2021). Preparation of protein powder from the liver of Yellowfin tuna (*Thunnus albacores*): a comparison of acid- and alkali-aided pH-shifting. *Food Science and Technology*, 42, e40120. https://doi.org/10.1590/fst.40120

Solana, M., Teel, J., Hojilla-Evangelista, M., Bertucco, A. and Eller, F. (2016). Counter-current carbon dioxide extraction of fat from soy skim. *The Journal of Supercritical Fluids*, 113, 106-111. https://doi.org/10.1016/j.supflu.2016.03.022

Sparks, D., Hernandez, R., Zappi, M., Blackwell, D. and Flaming, T. (2006). Extraction of rice bran oil using supercritical carbon dioxide and propane. *Journal of the American Oil Chemists' Society*, 83(10), 885-891. https://doi.org/10.1007/s11746-006-5042-x

Thai Industrial Standards Institute (TISI). (2014). Standard of product: fermented fish powder. Retrieved on August 12, 2022 from TISI website: https://rb.gy/tvm6r6.

Tian, Y., Wang, W., Yuan, C., Zhang, L., Liu, J. and Liu, J. (2017). Nutritional and digestive properties of protein isolates extracted from the muscle of the common carp using pH-shift processing. *Journal of Food Processing and Preservation*, 41(1), e12847. https://doi.org/10.1111/jfpp.12847

Wang, Q., Wang, Y., Huang, M., Hayat, K., Kurtz, N.C., Wu, X., Ahmad, M. and Zheng, F. (2021). Ultrasound-assisted alkaline proteinase extraction enhances the yield of pecan protein and modifies its functional properties. *Ultrasonics Sonochemistry*, 80, 105789. https://doi.org/10.1016/j.ultsonch.2021.105789

Yuen, F., Watson, M., Barker, R., Grillo, I., Heenan, R.K., Tunnacliffe, A. and Routh A.F. (2019). Preferential adsorption to air–water interfaces: a novel cryoprotective mechanism for LEA proteins. *Biochemical Journal*, 476(7), 1121-1135. https://doi.org/10.1042/BCJ20180901

Zou, Y., Zhang, W., Kang, D. and Zhou, G. (2017). Improvement of tenderness and water holding capacity of spiced beef by the application of ultrasound during cooking. *International Journal of Food Science and Technology*, 53(3), 828-836. https://doi.org/10.1111/ijfs.13659

