Heliyon 10 (2024) e26669

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Protein and water recovery from tuna defrosting wastewater

Jirapa Hinsui [a,*], Kornkanok Krasae [a], Nuttapong Jantaratch [a], Nopparat Mahae [b]

[a] Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand
[b] Department of Aquaculture and Fishery Products, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Mai Fad-district, Sikao District, Trang, 92150, Thailand

ARTICLE INFO

Keywords:
Protein recovery
Fish protein
Wastewater
Waste utilization
Water recycling

ABSTRACT

The objective of this research was to recover protein and water from tuna defrosting wastewater. Tuna defrosting wastewater (TDW) was concentrated, and salt protein residue (PR) was separated from concentrate TDW (cTDW). Protein in the cTDW was precipitated (PP). Salt was removed from PR and PP by using hot water (60 °C). PR and PP were dried at 50 °C before analysis for total protein, amino acid profile and salt content. Salty protein solution (PS) following salt removal from the precipitate was collected and concentrated. Then salt cPS was desalted by Sephadex G-25, and the elution was collected and concentrated. The resulting cPS was analyzed for pH, total protein, salt content, amino acid profile and antioxidant properties. Water from the protein recovery procedure was collected and analyzed for biological qualities (heterotrophic plate count, coliform bacteria, *E. coli*, *Staphylococcus aureus, Salmonella* spp. and *Clostridium perfringens*), physical qualities (apparent color, turbidity, pH) and chemical qualities (total dissolved solids, total hardness and sulfate).The results showed that cTDW contained 11.57 ± 0.03 % protein and 3.36 ± 0.03% NaCl. After salt was removed, the dried PR and PP contained 33.10 ± 0.16% and 6.92 ± 0.13% protein, respectively, and 0.23 ± 0.00% and 0.05 ± 0.00% NaCl, respectively. Dried PR contained 9 essential amino acids at higher concentrations than in PP. Concentrated PS contained 3.15 ± 0.12% protein and no NaCl. Histidine (254.15 mg/100 g) was the dominant amino acid in cPS. Antioxidant properties are shown by values for DPPH, ABTS and FRAP. The physical, chemical and bacterial parameters of recovered water met the guidelines for drinking water quality. These results indicate that recovery of protein and water is possible in fish processing, which could reduce costs for processors and benefit the environment.

1. Introduction

The main processes used during industrial fish processing are defrosting, washing, cooking, canning and cleaning. These processes require high quantities of water leading to the release of significant volumes of wastewater [1]. The characteristics of wastewater are determined by the operations and production lines of the factory. The polluting effect of these wastes is due to their high content of soluble organic matter and suspended solids, which vary depending on the raw material and the characteristics of the industrial process [2]. Defrosting wastewater contains protein, giving it a high organic nitrogen concentration, which is converted anaerobically into ammonium and can cause water pollution. This wastewater can also contain high concentrations of salts (Na^+, K^+, Ca^{2+}, Mg^{2+})

* Corresponding author.
E-mail addresses: jirapa_h@hotmail.com (J. Hinsui), sbbam77@gmail.com (K. Krasae), nuttapong.ju@ku.th (N. Jantaratch), nopparat.rmutsv@gmail.com (N. Mahae).

https://doi.org/10.1016/j.heliyon.2024.e26669
Received 29 August 2023; Received in revised form 8 February 2024; Accepted 16 February 2024
Available online 19 February 2024

due to onboard freezing of fish and can lead to high-salinity waste. A tuna processing plant may consume 400 m^3/day of water for defrosting. Outgoing wastewater contains high salinity and organic matter (protein, carotenoids, minerals and flavor) that presents a serious environmental problem [1]. These plants therefore need a suitable wastewater treatment system for pollution control. Presently, wastewater treatment systems consume a large portion of the operating budget for processors, while no byproducts are recovered from the waste, and the treated water is released to natural waterways. Thus, fish processing plants must continuously take in large volumes of clean freshwater to support their activities.

Recently, two-thirds of the world's population was found to be under stressful conditions due to water shortages. Fish processing industries both contribute to and are impacted by declining supplies of clean and fresh water. Some researchers attempted to conserve water during canned tuna processing by educating staff about water conservation and modifying three processing steps, namely spray cooling, can washing and floor washing. Following these measures, net water consumption was reduced from 13 m^3/ton of raw material to 8.8m^3/ton, representing a 32% reduction in overall water consumption [3]. Many researchers have tried to recycle water from fish processing. Raw sardines were washed to stimulate wastewater from fish industries, and then the wastewater was passed through a 500 Da MWCO polyamide membrane. Protein recoveries of 19–58% were observed [4]. Skipjack tuna wash water was precipitated with different pH and temperature combinations and found that the best processing conditions for protein precipitation were 4 °C and pH 4.5, 5.5 or 6.5, with protein recoveries of appoximately 94% [5]. In contrast, 63% the protein in surimi wash water was precipitated at pH 5 and heat treatment at 60 °C precipitated almost all of the remaining protein [6].

Microbiological safety issues are associated with water recycling during the production of shrimps in brine, but these hazards may be effectively controlled using a HACCP approach. Following these procedures, processed water recovered from peeling during shrimp processing and treated by means of reverse osmosis could be recycled within the same food unit operation [7]. A continuous ohmic heating system has been developed to coagulate protein from surimi wastewater to reduce the biological oxygen demand of the wastewater. After heating the samples were centrifuged and the remaining protein in the supernatants was measured and compared with the results from previous batch experiments. Heating under a higher electric field strength and lower flow rate resulted in higher temperatures of the samples. The lab-scale ohmic heating system possessed good performance in coagulating protein (60%) from surimi wastewater [8]. Water use (water balance) was measured at six fish processing steps, and physicochemical and bacteriological parameters of the wastewater were analyzed. Water recycling and reuse can potentially be applied after a simple primary treatment of the wastewater and disinfection from the freezing tunnel and cooling chamber defrosting. This practice may reduce the total average water consumption of the processing unit by 11%, and, if the wastewater from the cooling tower purges were also reused for other purposes, this reduction could reach 21.9%, enhancing the competitiveness of this industry and conserving fresh drinking water [9].

The objective of this research was to recover protein and water from tuna defrosting wastewater. The results can be applied in tuna processing plants to create value-added products and to save costs by reducing water consumption. These modifications would benefit the tuna processing industry and other fishery industries.

2. Materials and methods

2.1. Tuna defrosting wastewater characterization

Tuna defrosting wastewater (TDW) from a tuna processing plant in Samutsakorn Province, Thailand, was used in this study. The TDW was first filtered through cotton fabric to remove any fish particle. The pH was measured using a Metrohm 744 pH meter. The dissolved solids in the liquid fractions were measured using an RHB-32ATC refractometer. Total protein was determined according to Ref. [10], and salt (sodium chloride) content was determined according to Ref. [11].

2.2. Crude protein harvesting

TDW was concentrated by using Buchi Rotavapor R-220 Pro/V-600 Pro until the solid content of an aqueous solution was 30 °Bx. Salty protein residue (PR) was separated from concentrated TDW (cTDW) by using Tomy Refrigerated Centrifuge Suprema 25 at 10,000×*g* and 4 °C for 30 min and then dried in a hot air oven at 50 °C for 3 h. The pH, total protein [10] and salt content [11] in cTDW and dried salty PR were determined.

2.3. Protein precipitation

Concentrated TDW was precipitated by ethanol and heat precipitation. The salty protein precipitate (PP) was collected by using a Hettich Universal 32 centrifuge at 6000×*g* and 4 °C for 30 min. Salty PP was dried in hot air oven at 50 °C for 3 h. The total protein [10] and salt content [11] in the dried salty PP were determined.

Ethanol precipitation: Absolute ethanol was chilled at −20 °C for 3 h before use. Cold ethanol was added to cTDW at a ratio of 1:3 and kept at −20 °C for 3 h.

Heat precipitation: cTDW was heated in a water bath at 60 °C for 30 min.

2.4. Salt removal from precipitate

Salt was removed from salt PR and PP by using hot water. Water (60 °C) was added to salt PR and PP at a ratio 9:1 and heating continued at 60 °C in a water bath for 30 min. The mixture was then centrifuged at 6000×*g* and 4 °C for 30 min, and washed 2–3 times

until no sodium chloride was detected. PR and PP were dried at 50 °C in a hot air oven for 3 h. Dried PR and PP were analyzed for total protein [10], amino acid profile [12] and salt content [11].

2.5. Desalting of protein solution

Salty protein solution (PS) following salt removal from precipitate was collected. Salty PS was concentrated by using a BuchiR-124 evaporator until the solid content of an aqueous solution was 30 °Bx. Concentrated salty PS was desalted as follows.

Ten milliliters of salty cPS was loaded into a desalting column (1.2 × 58.5 cm) packed with Sephadex G-25 at a flow rate of 1.3 ml/min and equilibrated with distilled water. The fractions were eluted from the column using distilled water and 1.5 ml fractions were collected until no additional fractions contained salt. The absorbance at 280 nm and salt content [11] of each fraction were determined. The fractions with high absorption at 280 nm were pooled and then concentrated by using a Buchi R-124 evaporator.

Concentrate protein solution (cPS) was analyzed for pH, total protein [10], soluble protein [13,14], salt content [11], and amino acid profile [12]. The antioxidant properties DPPH [15], ABTS [16], and FRAP [17] were also determined using Trolox as a positive control. The metal chelating assay was modified from Ref. [18], using EDTA as a positive control.

2.6. Water recovery from tuna defrosting wastewater

Water from the protein recovery procedure was collected and analyzed according to Ref. [19] for biological qualities, namely standard plate count, coliform bacteria, *E. coli*, *Staphylococcus aureus, Salmonella* spp. and *Clostridium perfringens*; physical qualities, namely apparent color using an Agilent 8454 UV–Vis spectrophotometer, turbidity using a Hach 2100Q turbidity meter, pH and chemical qualities, namely, total dissolved solids, total hardness as $CaCO_3$, and sulfate, soluble protein [13,14] and salt content [11].

2.7. Statistical analysis

A completely randomized design (CRD) was used, with three replications. Data are presented as the mean ± standard deviation (SD). Means among treatments were compared by Duncan's multiple range test and analysis of variance (ANOVA) at a statistical significance of 95%.

3. Results and discussion

3.1. Tuna defrosting wastewater characterization

The characteristics of the tuna defrosting wastewater (TDW) are summarized in Table 1. The pH of TDW (7.45 ± 0.1) was in the range of 6.45–8.65 reported from other fish processing plants for tuna wastewater [1], surimi wastewater [4] and shrimp deicing water [7]. Ammonia emission and proteinaceous matter decomposition from different fish processing activities are mostly pH dependent [20]. The amount of dissolved solids in TDW was 0.20 ± 0.00 °Bx, and was related to the protein and sodium chloride content in TDW (0.17 ± 0.01 % protein and 0.14 ± 0.02 % NaCl). The small amount of dissolved solids in TDW indicated that water recovered from

Table 1
Total protein and salt content in material, crude protein, protein precipitation, salt removal and desalting.

Sample	% protein	% Salt
Characterization of material		
TDW	0.17 ± 0.01^{a}	0.14 ± 0.02^{a}
Crude Protein harvesting		
cTDW	11.57 ± 0.03^{e}	3.36 ± 0.03^{b}
Dried salty PR	22.11 ± 1.50^{g}	78.36 ± 7.78^{e}
Protein precipitation		
Dried ethanol precipitate	16.28 ± 0.79^{f}	79.32 ± 0.99^{e}
Dried heat precipitate	25.73 ± 0.28^{h}	43.40 ± 0.02^{d}
Salt removal from precipitate		
Dried PR	33.10 ± 0.16^{i}	0.23 ± 0.00^{a}
Dried PP	6.92 ± 0.13^{c}	0.05 ± 0.00^{a}
Desalting of protein solution		
Salty cPS	8.05 ± 0.08^{d}	24.49 ± 0.44^{c}
cPS	3.15 ± 0.00^{b}	ND

ND: Not detected.
TDW: tuna defrosting wastewater; cTDW: concentrated tuna defrosting wastewater; Dried salty PR: Dried salty protein residue; Dried PR: Dried protein residue; Dried PP: Dried protein precipitation; Salty cPS: Salty concentrated protein solution; cPS: concentrated protein solution.
The data are presented as the mean ± standard deviation.
^{abc} different superscript letters within a column for each category represent significant differences between samples ($P \leq 0.05$).

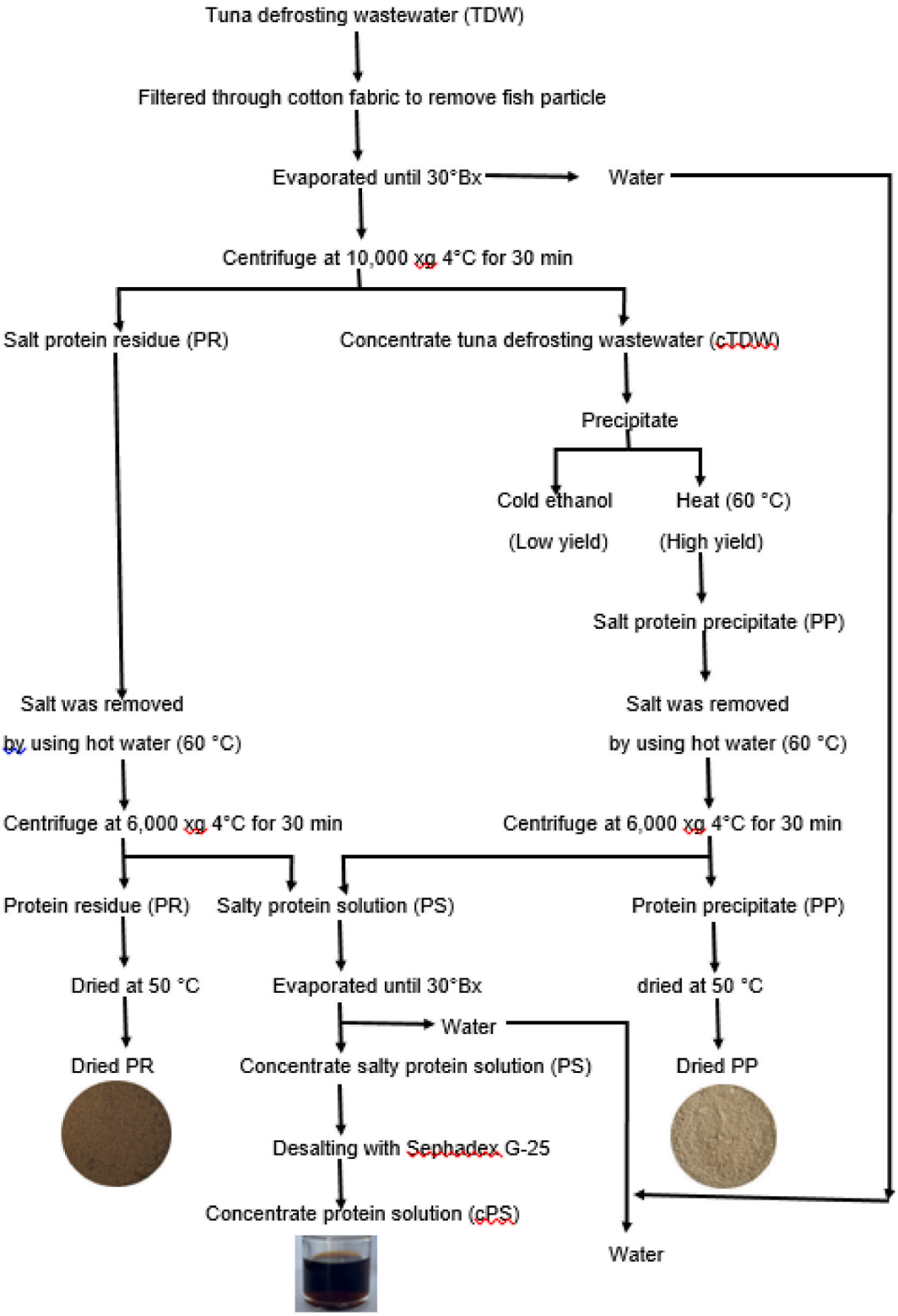

Fig. 1. Process of protein and water recovery from tuna defrosting wastewater.

TDW is the best potential source for water recycling. Fish processing wastewater contains small particles of fish, soluble protein, blood and other elements that produce a C: N: P ratio approximately 100:4:1, which is favorable for promoting microbial growth under optimum conditions and causing water pollution [1].

3.2. Crude protein harvesting

The protein and salt contents in cTDW were 11.57 ± 0.03% and 3.36 ± 0.03%, respectively as shown in Table 1. Concentrate TDW may cause serious environmental problems due to its high concentration of protein and high salinity [1]. Salty PR contained high concentrations of protein (22.11 ± 1.50%) and salt (78.36 ± 7.78%). General sewage sludge requires treatment at plants due to its high nutrient levels [21]. The characteristic of cTDW and salt PR were similar to those of sewage sludge from tuna processing waste treatment. This sludge may impair the waste water treatment process due to the effects of high salinity on plant growth and other physiological processes [22]. Salt removal is an important process for protein utilization.

3.3. Protein precipitation

Concentrate TDW was precipitated by ethanol and heat methods. The percentage of dried yield from ethanol precipitation was 4.95 ± 0.32%. It contained 16.28 ± 0.79% protein and 79.32 ± 0.99% salt (Table 1). Ethanol, an organic solvent, affects protein solutions by lowering protein solubility [23]. Heat precipitation gave a higher dried yield (7.64 ± 0.22%) than ethanol. It contained 25.73 ± 0.28 % protein and 43.4 ± 0.02 % salt. Tuna protein was denatured at 60 °C, similar to the temperature for denaturing protein in Atlantic cod (58–68 °C) [24], then protein was coagulated and separated from aqueous solution. Heat protein precipitation showed higher potential than alcohol both in yield and protein content due to the higher sodium chloride solubility in water than in ethanol [25]. Both precipitates contained high salt content, and would pose a risk for human and animal health.

3.4. Salt removal from precipitate

After salt was removed, the dried PR and PP contained 33.10 ± 0.16% and 6.92 ± 0.13% protein, respectively and 0.23 ± 0.00% and 0.05 ± 0.00% salt, respectively (Table 1). The salt content in both PR and PP was less than 1%, a level that is considered safe for human and animal intake [26].

Water, salt and blood are found in defrosting wastewater [27]. Tuna is a vertebrate, and the hemoglobin in red cells gives defrosting wastewater its red color [28]. The color of the dried PR was dark brown, the protein pigment was harvested and the resulting dried PP was light brown, as shown in Fig. 1. The amino acid profiles of PR and PP are shown in Table 2. Dried PR contained 20 amino acids, and the concentrations of 9 essential amino acids were much higher than those in PP. Protein and amino acids were water soluble because the concentration of protein and amino acids in PP was lower than that in PR after salt removal. The concentrations of protein and amino acids in PR were similar to those of tuna flesh [29], and make them potentially useful in human food or animal feed.

Table 2
Amino acid profile of dried protein residue and protein precipitate.

Amino acid profile (mg/100g sample)	Dried sample	
	Protein residue (PR)	Protein precipitate (PP)
Histidine	2245.98	1521.48
Isoleucine	1240.75	<100
Leucine	2677.95	126.92
Lysine	2035.69	207.99
Methionine	745.89	ND
Phenylalanine	1753.13	<250
Threonine	1315.18	<200
Tryptophan	451.94	ND
Valine	1838.20	<100
Sum of EAA	14304.7	1856.39
Aspartic acid	3000.13	364.15
Alanine	1988.6	171.09
Arginine	1435.57	<250
Cystine	367.77	ND
Glutamic acid	3218.34	1153.05
Glycine	1270.61	251.9
Hydroxylysine	ND	ND
Hydroxyproline	ND	ND
Proline	1011.11	<200
Serine	1238.28	<200
Tyrosine	1163.07	ND
Total AA	28998.19	3796.58

ND: Not detected.

3.5. Desalting of protein solution

Histidine (661.18 mg/100 g), was the dominant amino acid in salty cPS, it is important for human health and may be necessary as a supplement in some populations [30]. Moreover, histidine is a pharmaceutical agent because of its antioxidant and anti-inflammatory properties [31]. A high concentration of salt (24.49 ± 0.44%) may pose a human health risk. After desalting, the color of cPS was dark brown, as shown in Fig. 1.

The concentrated PS remained protein (3.15 ± 0.12%) and histidine (254.15 mg/100 g). Its antioxidant properties shown in terms of DPPH, ABTS and FRAP were 48.34 ± 1.78, 602.73 ± 8.14 and 672.36 ± 4.71 μmol TE/mg protein, respectively, as shown in Table 3. The odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from histidine to the corresponding hydrazine [32], and histidine donates a hydrogen atom to the ABTS radical [33]. Histidine donates electrons to Fe^{3+} for ferric reducing antioxidant power (FRAP) [34], whereas no metal chelating activity is observed for histidine. No research has examined amino acids in TDW, but the amount of histidine in this research was higher than that in tuna protein hydrolysate (136.99 mg/100 g sample) [35], which showed antioxidant activities in FRAB, ABTS and DPPH.

3.6. Water recovered from tuna defrosting wastewater

The physical, chemical and microbiological qualities of the tap water and water remaining following the protein recovery procedure are shown in Table 4. The pH of water recovered from TDW was 7.53, it contained 0.04 ± 0.00% protein and 0.03 ± 0.00% salt. These small amounts of protein and salt would not have an effect on food processing due to GHP chlorinated water sanitation [36]. Physical parameters of appearance color and turbidity were measured as < 0.1 Pt–Co Unit and 0.43 NTU, respectively. The chemical parameters, namely, total dissolved solid, total hardness and sulfate, of the recovery water were <0.5 mg/l, <2.5 mg/l and <0.68 mg/l, respectively. Bacteria were not detected. The physical, chemical and bacterial parameters of recovered water were less than those of tap water, and met the guidelines for drinking water quality [37]. Recovered water was suitable for reuse in fish processing. The yield of water recovered from this research was approximately 82% of the total TDW. The quality of water is better than that of water from other wastewater treatment, which would benefit recycling in processing plants.

4. Conclusion

Our experiments have shown that it is possible to recover protein from tuna defrosting wastewater, and that the protein is suitable as a food ingredient in seasoning or protein powder. Water was a by-product generated from this process, and was clean enough for reuse in food processing. This finding could be applied by the food industry to achieve zero waste release and to recycle water and reduce production costs.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Jirapa Hinsui: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Kornkanok Krasae:** Methodology, Investigation, Formal analysis. **Nuttapong Jantaratch:** Methodology, Investigation, Formal analysis. **Nopparat Mahae:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jirapa Hinsui reports financial support was provided by Kasetsart University Research and Development Institute. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 3
Antioxidant properties of salty concentrated protein solution (Salty cPS) and concentrated protein solution (cPS).

Sample	Antioxidant properties (μmol TE/mg protein)			
	DPPH	ABTS	FRAP	Metal chelating
Salty cPS	58.57 ± 1.36^{b}	97.14 ± 10.13^{b}	1442.99 ± 20.24^{b}	ND
cPS	48.34 ± 1.78^{a}	602.73 ± 8.14^{a}	672.36 ± 4.71^{a}	ND

ND: Not detected.
The data are presented as the mean ± standard deviation.
^{abc} different superscript letters within a column for each category represent significant differences between samples (P ≤ 0.05).

Table 4
Physical, chemical and microbiological qualities of tap water and water remaining following the protein recovery procedure.

Parameters	Unit	Drinking water quality (FAO, 2011)	Tap water	Recovery water
1. Physical				
Appearance color	Pt–Co Unit	<15	8.33	<0.1
Turbidity	NTU	<4	0.58	0.43
pH	–	6.5–8.5	7.0	7.53
2. Chemical				
Total dissolved solid	mg/L	<600	156	<0.5
Hardness as $CaCO_3$	mg/L	<300	87.34	<2.5
Sulfate	mg/L	<250	30.74	<0.68
3. Bacterial				
Standard plate count	CFU/mL	500	<1	2.3
Total coliform bacteria	MPN/100 mL	ND	<1	<1
E. coli	MPN/100 mL	ND	<1	<1
Staphylococcus aureus	CFU/100 mL	ND	<1	<1
Salmonella spp.	CFU/100 mL	ND	<1	<1
Clostridium perfringens	CFU/100 mL	ND	<1	<1

ND: Not detected.
Total coliform bacteria, *E. coli*, *Staphylococcus aureus*, *Salmonella* spp. and *Clostridium perfringens* < 1 means not detected.

Acknowledgement

This research was funded by Kasetsart University Research and Development Institute (KURDI), FF (KU) 7.65.

References

[1] S. Hamimed, T. Barkaoui, I. Trabelsi, A. Landoulsi, A. Chatti, High-performance biological treatment of tuna wash processing wastewater using *Yarrowiali polytica*, Environ. Sci. Pollut. Res. 28 (2021) 1545–1554, https://doi.org/10.1007/s11356-020-10586-6.
[2] M.C. Veiga, R. Mendez, J.M. Lema, Waste water treatment for fisheries operations, in: A.M. Martin (Ed.), Fisheries Processing, Springer, Boston, 1994, pp. 344–369.
[3] M. Uttamangkabovorn, P. Prasertsan, A.H. Kittikun, Water conservation in canned tuna (pet food) plant in Thailand, J. Clean. Prod. 13 (2005) 547–555, https://doi.org/10.1016/j.jclepro.2003.12.003.
[4] T.Y. Wu, A.W. Mohammad, N. Anuar, R.A. Rahman, Potential use of nanofiltration membrane in treatment of wastewater from fish and surimi industries, Songklanakarin J. Sci. Technol. 24 (2002) 977–987. https://www.thaiscience.info/journals/Article/SONG/10462305.pdf.
[5] P. Elizondo-Garza, S.O. Serna-Saldívar, C. Chuck-Hernandez, Protein recovery from skipjack tuna (*Katsuwonus pelamis*) wash water with different pH and temperature combinations, Rev. Mex. Ing. Quim. 16 (2017) 91–99. https://www.redalyc.org/pdf/620/62049878010.pdf.
[6] K. Iwashita, M. Sumida, K. Shirota, K. Shiraki, Recovery Method for surimi wash-water protein by pH shift and heat treatment, Food Sci. Technol. Res. 22 (2016) 743–749, https://doi.org/10.3136/fstr.22.743.
[7] S. Casani, T. Leth, S. Knochel, Water reuse in a shrimp processing line: safety considerations using a HACCP approach, Food Control 17 (2006) 540–550, https://doi.org/10.1016/j.foodcont.2005.03.002.
[8] K. Kanjanapongkul, S. Tia, P. Wongsa-Ngasri, T. Yoovidhya, Coagulation of protein in surimi wastewater using a continuous ohmic heater, J. Food Eng. 91 (2009) 341–346, https://doi.org/10.1016/j.jfoodeng.2008.09.010.
[9] J.T. Guimarães, A.L.M. Souza, A.I.S. Brígida, A.A.L. Furtado, P.C.M.S. Chicrala, V.R.V. Santos, R.R. Alves, D.B. Luiz, E.F.M. Mesquita, Quantification and characterization of effluents from the seafood processing industry aiming at water reuse: a pilot study, J. Water Proc. Eng. 26 (2018) 138–145, https://doi.org/10.1016/j.jwpe.2018.10.006.
[10] AOAC, Official, Method of the Association of Official Analytical (2000) Chemists, seventeenth ed., Association Official Analytical Chemists, Washington DC, 2000.
[11] S.S. Nielsen, Food Analysis Laboratory: Food Science Text Series, second ed., Springer, London, 2010.
[12] AOAC, Official, Method of the Association of Official Analytical Chemists, sixteenth ed., Association Official Analytical Chemists, Arlington, 1997.
[13] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275, https://doi.org/10.1016/S0021-9258(19)52451-6.
[14] G.L. Peterson, A simplification of protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem. 83 (1977) 346–356, https://doi.org/10.1016/0003-2697(77)90043-4.
[15] S. Sukkwai, K. Kijroongrojana, S. Benjakul, Extraction of gelatin from bigeye snapper *(Priacanthus tayenus)* skin for gelatin hydrolysate production, Int. Food Res. 18 (2011) 1129–1134. http://www.ifrj.upm.edu.my/18%20(03)%202011/39)IFRJ-2010-200.pdf.
[16] S. Ketnawa, A.M. Liceaga, Effect of microwave treatment on antioxidant activity and antigenicity of fish frame protein hydrolysates, Food Bioprocess Technol. 10 (2017) 582–591. https://link.springer.com/article/10.1007/s11947-016-1841-8.
[17] H.C. Wu, H.M. Chen, C.Y. Shiau, Free amino acid and peptide as related to antioxidant properties in protein hydrolysate of mackerel *(Scomber austriasicus)*, Food Res. Int. 36 (2003) 949–957, https://doi.org/10.1016/S0963-9969(03)00104-2.
[18] R.F. Boyer, C.J. McCleary, Superoxide ion as a primary reductant in ascorbate-mediated ferretin iron release, Free Radical Biol. Med. 3 (1987) 389–395, https://doi.org/10.1016/0891-5849(87)90017-7.
[19] APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 23rd ed., American Public Health Association, American Water Works Association and Water Environment Federation, Washington, D.C, 2017.
[20] P. Chowdhury, T. Viraraghavan, A. Srinivasan, Biological treatment processes for fish processing wastewater – a review, Bioresour. Technol. 101 (2010) 439–449, https://doi.org/10.1016/j.biortech.2009.08.065.
[21] A.F. Santos, P.V. Almeida, P. Alvarenga, L.M. Gando-Ferreira, M.J. Quina, From wastewater to fertilizer products: alternative paths to mitigate phosphorus demand in European countries, Chemosphere 284 (2021) 131258, https://doi.org/10.1016/j.chemosphere.2021.131258.
[22] M.H. Kalaji, S. Pietkiewicz, Salinity effects on plant growth and other physiological processes, Acta Physiol. Plant. 15 (1993) 89–124, https://doi.org/10.1016/j.plaphy.2020.08.042.
[23] D.M. Bollag, M.D. Rozycki, S.J. Edelstein, Protein Method, second ed., New York: John Willey & Sons, Inc, 1996.
[24] D. Skipnes, I. Van der Plancken, A. Van Loey, M.E. Hendrickx, Kinetics of heat denaturation of proteins from farmed Atlantic cod (*Gadus morhua*), J. Food Eng. 85 (2008) 51–58, https://doi.org/10.1016/j.jfoodeng.2007.06.030.

[25] S.P. Pinho, E.A. Macedo, Solubility of NaCl, NaBr, and KCl in water, methanol, ethanol, and their mixed solvents, J. Chem. Eng. Data 50 (2005) 29–32, https://doi.org/10.1021/je049922y.
[26] A.M. Pearson, A.M. Wolzak, Salt-its use in animal products-a human health dilemma, J. Animal Sci. 54 (6) (1982) 1263–1278, https://doi.org/10.2527/jas1982.5461263x.
[27] R.O. Cristovao, C.M. Botelho, R.J.E. Martins, J.M. Loureiro, R.A.R. Boaventura, Primary treatment optimization of a fish canning wastewater from a Portuguese plant - a review, Water Resour, Ind 6 (2014) 51–63, https://doi.org/10.1016/j.wri.2014.07.002.
[28] P.C. De Souza, G.O. Bonilla-Rodriguez, Fish hemoglobins, Brazilian, J. Med. Biol. Res. 40 (2007) 769–778, https://doi.org/10.1590/s0100-879x2007000600004.
[29] S. Peng, C. Chen, Z. Shi, L. Wang, Amino acid and fatty acid composition of the muscle tissue of yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus obesus*), J. Food Nutr. Res. 1 (2013) 42–45, https://doi.org/10.12691/jfnr-1-4-2.
[30] A. Thalacker-Merce, M.E. Gheller, Benefits and adverse effects of histidine, J. Nutri. Supplementation (2020) 1–5, https://doi.org/10.1093/jn/nxaa229.
[31] A.M. Wade, H.N. Tucker, Antioxidant characteristic of L-histidine, J.Nutri. Biochem. 9 (1998) 308–315, https://doi.org/10.1016/S0955-2863(98)00022-9.
[32] S.B. Kedare, R.P. Singh, Genesis and development of DPPH method of antioxidant assay, J. Food Sci. Technol. 48 (2011) 412–422, https://doi.org/10.1007/s13197-011-0251-1.
[33] C.V. Ratnavathi, V.V. Komala, Chapter1-Sorgym grain quality, Sorgum Biochem. (2016) 1–61, https://doi.org/10.1016/B978-0-12-803157-5.00001-0.
[34] R. Amarowicz, R.B. Pegg, Chapter One - natural antioxidants of plant origin, Adv. Food Nutr. Res. 90 (2019) 1–81, https://doi.org/10.1016/bs.afnr.2019.02.011.
[35] K. Krasae, W. Worawattanamateekul, J. Hinsui, Effects of peptide fractions and amino acids on antioxidant properties of autolyzed tuna viscera protein hydrolysate, Food Res. 7 (2023) 156–163, https://doi.org/10.26656/fr.2017.7(5).270.
[36] CODEX ALIMENTARIUS, General Principles of Food Hygiene, World Health Organization, Geneva, 2017.
[37] WHO, Guidelines for Drinking-Water Quality, fourth ed., World Health Organization, Geneva, 2011.