Exploring Protein Composition in Stickwater From Tuna by-products Processing as a Preliminary Approach for the Recovery of a Low Molecular Weight Fraction With Potential Algicidal Activity

Ofmara Maria Ponce Moreno

Center for Food Research and Development: Centro de Investigacion en Alimentacion y Desarrollo AC

Maria Elena Lugo-Sanchez

CIAD: Centro de Investigacion en Alimentacion y Desarrollo AC <https://orcid.org/0000-0002-1472-5438>

Juan Carlos Ramírez-Suarez

CIAD: Centro de Investigacion en Alimentacion y Desarrollo AC <https://orcid.org/0000-0001-8512-1271>

Christine Johanna Band-Schmidt

CICIMAR: Instituto Politecnico Nacional Centro Interdisciplinario de Ciencias Marinas

<https://orcid.org/0000-0002-8251-9820>

Jesús Aarón Salazar-Leyva

Universidad Politécnica de Sinaloa: Universidad Politecnica de Sinaloa <https://orcid.org/0000-0002-2234-7422>

Rogelio Sotelo-Mundo

CIAD: Centro de Investigacion en Alimentacion y Desarrollo AC <https://orcid.org/0000-0001-5543-6889>

Ramón Pacheco-Aguilar (✉ rpacheco@ciad.mx)

CIAD: Centro de Investigacion en Alimentacion y Desarrollo AC <https://orcid.org/0000-0002-1669-8007>

Research Article

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Abstract

The use of by-products for fishmeal production is constantly rising. During this process, stickwater is generated, an effluent that contains organic matter in soluble, colloidal or particulate form. It has been shown that stickwater contains an important amount of protein and that its characterization is the first step into achieving its full valorization. Tuna canning by-product's stickwater was centrifuged and fractionated by ultrafiltration to bring awareness to its protein quality. Stickwater had a net protein content of 61.4%, centrifuged stickwater maintained a similar protein content meanwhile the protein content in ultrafiltered fractions decreased as their molecular weight range decreased as well. Stickwater, centrifuged stickwater and the fraction R10 presented gel-like characteristics that could position this effluent as a potential source of gelatin. The electrophoretic profile of stickwater, centrifuged stickwater and centrifuged solids demonstrated that a high amount of protein in stickwater was soluble. This first glance at protein/peptides from tuna canning by-products is part of the ongoing effort to propose their recovery an alternative and sustainable use of a fish processing effluent with potential application as a source of peptides with algicidal bioactivity against harmful algal blooms (HABs).

Statement Of Novelty

Enormous quantity of stickwater results from global fishmeal production, which poses as an environmental contaminant if not discarded properly. Despite the industry efforts to recover proteins from stickwater to boost fishmeal quality, this is just one of the possible uses for the significant amount of protein in this effluent. Consequently, this work aims to explore the characteristics of stickwater's protein content in order to broaden the discussion for their potential use as algicidal peptides. This study could pose as new insight for possible distinct and novel purposes for the protein/peptide content in this effluent and exploit characteristics that can position them as value-added compounds.

1. Introduction

The fish industry's presence in the global market accounts for its huge production quantities, which in 2018 was approximately 179 million tons (MT) [1]. However, considering that the approximate species-dependent fillet yield is 50% [2], this gives us 89.5 MT of discards or by-products. A by-product is generally understood as raw material that results from the production of a main marketable product and that after proper treatment can be recycled [2]. In Mexico, this definition is well managed among the scientific community; however, there is a lack of legislation on this subject, since it is only vaguely mentioned on the Official Mexican Standard 242 (NOM-242-SSA1-2009). The fish industry's growing production gives a rising quantity of by-products, which makes it imperative to expand their applications increasing benefits in the producer's economy and decreasing its environmental impacts.

An important quantity of the fish industry's production is used to produce fishmeal (FM) and fish oil [1]. Originally, FM and fish oil were produced from small pelagic species or fish processing by-products; the latter being responsible for approximately 35% of their production in recent years [3, 4]. FM was originally

considered to be a low value product; nonetheless, the rise in prices seen in the last decade demonstrate its increased market demand [5]. The production of FM and fish oil involves many steps and high energy input, which in turn generates an ascending amount of effluents. Stickwater (SW), the main effluent generated in this process, represents approximately 60% of the initial weight of the processed raw material [4]. Since SW maintains significant protein content and other soluble components, they are normally added back to the press cake to enhance the quality of FM [6].

The protein content in SW gives it the potential to broaden its applications. Previous studies confirm that the protein content in SW ranges from 5–9% [3, 7]; this includes protein from SW made with by-products, since they can contain up to 20% (w/w) of total fish protein [8]. Fish protein is recognized as high quality protein, since it has a better balance of essential amino acids in comparison to that from other animal sources [9]. The Food and Agriculture Organization of the United Nations (FAO) has found that fish protein accounted for a 17% of the global population's intake of animal protein in 2017 [1]. Knowing this, the recovery of fish by-products' protein in order to create added-value compounds has been an ongoing research interest [8, 10, 11].

Recovering added-value compounds from fish industry's by-products is a well-documented area of study. It is estimated that 60% of fish industry's production results in by-products, therefore the rise in added-value compounds is seen as the right path towards a more sustainable use of fish resources [12, 13]. By-products have been used as raw material for production of protein concentrates valuable in the food sector, bioactive peptides for the pharmaceutical industry, and collagen hydrolysates for the cosmetic industry, among others [11, 14].

Initially, the protein recovered from SW was reincorporated into the FM production process. Currently, the studies of SW's protein recovery and application are starting to broaden, employing it for microbial lipid and single cell protein production along with a polypeptide bioactive properties screening [15–17]. However, studies redirecting SW protein towards algicidal activity is not common.

Although algicides have been used for control of Harmful Algal Blooms (HABs) for many years, algicidal activity of peptides is a recent line of investigation. Commonly, algicides were of chemical or physical nature, like copper sulfate and clay flocculation, respectively [18, 19]. However, their drawbacks in real-world applications has expanded the interest in other options, such as algicidal peptides. Studies on hybrid cationic peptides showed algicidal activity against HABs without affecting other marine organisms [20, 21]. Moreover, initial studies on lab-produced SW revealed a peptide with inhibitory effect against HABs [22, 23]. This premise allows for the selection of low molecular weight range peptides in SW in order to evaluate its algicidal activity.

The use of membranes to recover value-added compounds such as protein has proven beneficial since they can simultaneously recover and concentrate the protein while maintaining its integrity, which is a priority for the production of value-added compounds [24, 25]. Hence, this study aims to explore the protein content of the soluble fraction of SW from tuna by-products processing. This preliminary

approach will allow for complementary research regarding these peptides and their potential bioactivity as algicides, which in turn will broaden the range their applications as a high value-added product.

2. Material And Methods

2.1. Materials

The tuna processing plant Mazindustrial-Pinsa, located in Mazatlán, México, provided the SW made from tuna canning by-products. SW samples were collected before they exited the centrifuge. The samples were collected in 20 L containers, transported in ice and maintained at -20°C until further analyses.

2.2. Stickwater preparation for ultrafiltration

SW was defrosted at 4°C for two days. The samples were put on a warm water bath and centrifuged at $7400 \times g$ at $20 \pm 1^\circ\text{C}$ for 30 minutes in a refrigerated centrifuge (Model Avanti J-26S XPI, Beckman Instruments Inc. Palo Alto, CA). The supernatant represented the centrifuged portion of each sample, named CSW and the starting sample in the ultrafiltration sequence. The precipitated sample (centrifuged solids, CS) were reserved for further analyses not included in this study. SW, CSW and CS were frozen at -80°C and freeze-dried (Labconco™ Freezone 12, Labconco, Kansas City, MO).

2.3. Sample ultrafiltration

CSW was fractionated successively by ultrafiltration. Experiments were carried out using tubular membranes composed of regenerated cellulose (EMD Millipore™ Prep/Scale) with molecular weight cut-off (MWCO) of 30 kDa, 10 kDa, 5 kDa and 1 kDa (Millipore Corporation, Billerica, MA) using the Prep/Scale support module (Millipore Corporation, Billerica, MA) at room temperature. The permeate from each fractionation step was used as the feed solution for the following membrane after being fractionated fifteen times in order to obtain higher yields (Fig. 1). A Manostat Preston Model # 72-370-000 peristaltic pump (Barnant Co.) controlled the flow of the samples with 1-1.5 bar pressure at a flow rate of 13 mL / min. A 500 mL aliquot from each permeate was reserved for freeze-drying and further analyses. Five fractions were obtained and identified as follows: P30 (≤ 30 kDa), R10 (30 – 10 kDa), P10 (≤ 10 kDa), R5 (10 – 5 kDa), P5 (≤ 5 kDa).

2.4. Protein content and moisture of SW, CSW, CS and fraction P5

Quantification of protein and NPN were carried out by AOAC methodology and Woyewoda et al., respectively [26, 27]. NPN was deducted from total protein to obtain net protein content. Moisture of the samples was determined by weight difference after freeze-drying.

2.5. Electrophoretic profile of SW, CSW, CS and ultrafiltered fractions

SDS-PAGE profiles were determined for SW, CSW and CS according to Laemmli [28] with slight modifications. Samples were suspended in SDS solution (5% (w/v) and 0.1% β -ME (w/v)) and incubated for 1h at 95% °C. Afterward, the mixture was centrifuged at 3,000 rpm for 15 min. The treated sample was mixed with loading buffer at 1:1 ratio, incubated at 95°C for 5 min and protein concentration was determined based on the Biuret method, 60 μ g of protein was loaded per well. Electrophoresis was carried out in 0.75mm gels with 12% resolution gel and 4% stacking gel and a wide range standard (14–200kDa) was used to estimate the molecular weight of proteins.

The electrophoretic pattern of ultrafiltered fractions was determined by Tricine SDS-PAGE [29] at 125 V and 140 mA. Protein concentration loaded per fraction ranged from 20 μ g – 70 μ g. A polypeptide standard (1–27kDa) was used to estimate the molecular weight of peptides. Resulting bands were analyzed with software Image Lab™ 4.0 (Bio-Rad. Lab., Hercules, CA).

2.6. Statistical analysis

Descriptive statistical analyses were used to describe protein content and moisture of SW, CSW and fraction P5. All experiments were conducted in two replicates.

3. Results And Discussion

3.1. Protein and moisture content of SW, CSW and ultrafiltered fraction

The protein and moisture of SW, CSW and P5 is shown in Table 1. Chemical composition of SW is dependent of the species used as raw material for the production of FM, as well as time of year and manipulation [3]. SW from two types of sardine (*Opisthonema libertate* Günther 1867 and *Sardinops sagax* Jenyns 1842) presented net protein content of 3.7% and 1.6% respectively (wet basis) [3] which was lower than the net protein obtained from SW used in the present study (5.9%). It is also important to note that CSW showed a total protein content of 71% (dry basis), which is slightly higher than the total protein recovered by acid precipitation in CSW from pelagic species [30].

Table 1
Partial chemical characterization of SW, CSW and ultrafiltered fraction

Sample	Chemical composition on dry basis (%)			
	Moisture	Net protein	NPN	Protein content with respect to SW (wet basis, %)
SW	89.9 ± 0.9	61.4 ± 4.2	8.2 ± 1.8	-
CSW	90.8 ± 2.1	61.9 ± 1.6	9.1 ± 1.8	67
P5	94.8 ± 1.5	47.9 ± 7.5	8.3 ± 0.3	0.8
NPN: non-protein nitrogen. Results are shown as mean ± standard deviation (n = 2).				

Protein content in FM is one of its quality indicators, categorized as high quality FM when it contains 60–72% crude protein [31]. Reportedly, the FM source of the SW used in this study generally has a 58–60% average protein content (dry basis). The total protein content from the specific batch of FM used to produce this SW is presumed to be higher, according to the effluent's protein content.

The complementary centrifugation step was an important unit operation in the process to obtain the ultrafiltered fractions. This centrifugation separated suspended particles and oil from the soluble fraction of SW, which would have otherwise rendered it difficult to separate protein fractions given the complexity of this effluent [3]. This unit operation has previously been incorporated into the recovery of valuable components from SW and other fish by-products [11, 32].

Solids represented 10% in initial SW, which is similar to that of sardine SW [3]. Ultrafiltration permits the passing of monovalent and divalent salts through its pores [33] Given that one specific characteristic of this effluent is its high salinity [34] with an average initial 22% of ashes, these are concentrated as they progress through the ultrafiltration sequence.

A qualitative aspect of SW was its gelification capacity, evaluated in another of our studies. It was noted that when defrosted SW remained in gel-like form and that this characteristic remained after centrifugation (Fig. 2). This is to our knowledge, the first report of gelatin characteristics in SW. The heating of the tuna canning by-products to produce FM denatured the collagen present and produced gelatin, which remained in SW. Gelatin is normally extracted from mammalian sources; however, derived from socio-cultural reasons, there is a demand for other gelatin sources [35]. There have been reports of the characterization of gelatin from fish skins such as cod, salmon, hake, squid and hoki [36–38]. Gelatin from fish sources is less preferred than gelatins from land animals since they have been found to be less stable and with inferior rheological properties [37]. However, gelatin from yellowfin tuna skin showed the highest gel strength (426 Bloom) compared to bovine and porcine gelatins (216 and 295 Bloom, respectively) [39]. This gives an indication that tuna SW gelatin could be a good alternative source of gelatin and further studies are necessary to evaluate the quality of gelatin present in this particular effluent.

3.2. SDS-PAGE profile of SW, CSW and CS

The electrophoretic profile of SW, CSW and CS is shown in Fig. 3. SW and CS have a very similar profile, highlighting an intense band at approximately 15.1 kDa molecular weight that is not present in CSW. This band is similar to that reported by Sanmartin et al., [40] who identified it as myoglobin after the proteomic analysis of tuna processing by-products. A band with molecular weight of 39.3 kDa is also present in SW. A band with similar molecular weight was identified as tropomyosin of bluefin tuna, which is also present CSW and this confirms its solubility.

The electrophoretic profile shown in Fig. 3 clearly demonstrates the effect of a complementary centrifugation of SW. This unit operation has a great effect in removing proteins/peptides as sediments, which can be recovered for their further analyses and valorization.

3.3. Tricine SDS-PAGE profile of ultrafiltered fractions

The ultrafiltration of CSW resulted in five fractions: P30 (≤ 30 kDa), R10 (30 – 10 kDa), P10 (≤ 10 kDa), R5 (10 – 5 kDa) and P5 (≤ 5 kDa). The electrophoretic profile is shown in Fig. 4. Ultrafiltration was effective in the fractionation of CSW into ranges of specific molecular weights, except for samples P30 and R10. These fractions show bands above the respective molecular weights, which can be due to protein aggregation during the ultrafiltration process [41]. The proteins/peptides present in SW gives certainty in their potential functionality, and considering that the low molecular weight fractions also had an important protein content, bioactivity analyses could be also relevant.

3.4. Protein content in industrial SW production

The previous results strengthen the need for an understanding of the amount of high quality protein that is contained in effluents such as SW. A protein balance calculation was done for the samples of SW, CSW and the fraction P5 (Table 1). It was seen that 67% of SW protein was still present in CSW, and 0.8% of this protein remained in fraction P5. This means that a FM industrial production of 10 tons/h of tuna canning by-products will produce 6 ton/h of SW, which in turn will equal to 438 kg net protein. Likewise, CSW will contain 291.4 kg of net protein and 3.5 kg of net protein remain in fraction P5. This shows the importance of recovering this protein and directing it to the food industry or use it as a source of bioactive peptides in low molecular weight fractions. Other sources like sardine CSW also contains a significant amount of protein (57.6% dry basis, data not shown) therefore it's also a potential source of high quality protein. Previous research shows that mackerel SW can be redirected towards the control of HABs since low molecular peptides exert selective algicidal activity towards these species [23].

4. Conclusions

Stickwater from the processing of tuna canning by-products was processed for an initial evaluation of its protein content and to obtain the low molecular weight fraction with special interest in P5. Stickwater presented 61.4% net protein. Considering the protein content remaining in fraction P5, it can be a good source of algicidal peptides. This first approach to characterize the protein in SW will expand its potential applications and valorization by bringing environmental and economic achievements and a sustainable use of fish resources. Further studies needed would revolve around the algicidal activity of SW peptides in P5 and their mode of action over HAB producing dinoflagellates.

Declarations

Author contributions

Ramon Pacheco-Aguilar: resources, funding acquisition. Ofmara Maria Ponce Moreno: investigation, data analysis. Maria Elena Lugo Sanchez: supervision, methodology. Jesus Aaron Salazar Leyva: resources,

validation. Rogerio Sotelo Mundo and Johanna Christine Band Schmidt: validation. Juan Carlos Ramirez Suarez: resources, funding acquisition.

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Conflicts of interest/Competing interests

The authors declare no conflicts of interest.

Availability of data and material

All data generated or analysed during this study are included in this manuscript.

Code availability (software application or custom code)

Not applicable.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

References

1. FAO, *The State of World Fisheries and Aquaculture 2020. Sustainability in action*. 2020: Rome.
2. Rustad, T., Storrø, I., and Slizyte, R., Possibilities for the utilisation of marine by-products. *International Journal of Food Science & Technology*. **46**, 2001-2014. (2011) 10.1111/j.1365-2621.2011.02736.x
3. Valdez-Hurtado, S., Goycolea-Valencia, F., and Márquez-Ríos, E. J. B., Efecto de una centrifugación complementaria en la composición química y reológica del agua de cola. **20**, 95-103. (2018) 10.18633/biotecnia.v20i2.606
4. García-Sifuentes, C. O., Pacheco-Aguilar, R., Valdez-Hurtado, S., Márquez-Rios, E., Lugo-Sánchez, M. E., and Ezquerro-Brauer, J. M., Impacto del agua de cola de la industria pesquera: tratamientos y usos. *CyTA - Journal of Food*. **7**, 67-77. (2009) 10.1080/11358120902850412

5. Olsen, R. L., Toppe, J., and Karunasagar, I., Challenges and realistic opportunities in the use of by-products from processing of fish and shellfish. *Trends Food Sci. Technol.* **36**, 144-151. (2014) 10.1016/j.tifs.2014.01.007
6. Bechtel, P. J., Properties of Stickwater from Fish Processing Byproducts. *Journal of Aquatic Food Product Technology.* **14**, 25-38. (2005) 10.1300/J030v14n02_03
7. Pacheco-Aguilar, R., Leyva-Soto, P., Carvallo-Ruiz, G., García-Carreño, L. F., and Márquez-Ríos, E. J. I., Efecto de la concentración de quitosano y pH sobre la remoción de sólidos en agua de cola de la industria sardinera. *Interciencia.* **34**, 274-279. (2009)
8. Zamora-Sillero, J., Gharsallaoui, A., and Prentice, C., Peptides from fish by-product protein hydrolysates and its functional properties: An overview. *J Marine Biotechnology.* **20**, 118-130. (2018) 10.1007/s10126-018-9799-3
9. Ghaly, A., Ramakrishnan, V., Brooks, M., Budge, S., and Dave, D., Fish Processing Wastes as a Potential Source of Proteins, Amino Acids and Oils: A Critical Review. *J Microbial Biochemical Technology.* **5**, 107-129. (2013) 10.4172/1948-5948.1000110
10. Gehring, C., Gigliotti, J., Moritz, J., Tou, J., and Jaczynski, J., Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: A review. *Food Chem.* **124**, 422-431. (2011) 10.1016/j.foodchem.2010.06.078
11. He, S., Franco, C., and Zhang, W., Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). *Food Res. Int.* **50**, 289-297. (2013) 10.1016/j.foodres.2012.10.031
12. Chalamaiah, M., Hemalatha, R., and Jyothirmayi, T., Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. *Food Chem.* **135**, 3020-3038. (2012) 10.1016/j.foodchem.2012.06.100
13. Venugopal, V. and Sasidharan, A., Seafood industry effluents: environmental hazards, treatment and resource recovery. *J. Env. Chem.* (2020) 10.1016/j.jece.2020.104758
14. Ferraro, V., Carvalho, A. P., Piccirillo, C., Santos, M. M., Castro, P. M., and Pintado, M., Extraction of high added value biological compounds from sardine, sardine-type fish and mackerel canning residues—A review. *Materials Science and Engineering: C.* **33**, 3111-3120. (2013) 10.1016/j.msec.2013.04.003
15. Kam, S., Kenari, A. A., and Younesi, H., Production of single cell protein in stickwater by *Lactobacillus acidophilus* and *Aspergillus niger*. *Journal of Aquatic Food Product Technology.* **21**, 403-417. (2012) 10.1080/10498850.2011.605539
16. Huang, L., Zhang, B., Gao, B., and Sun, G., Application of fishmeal wastewater as a potential low-cost medium for lipid production by *Lipomyces starkeyi* HL. *Environ. Technol.* **32**, 1975-1981. (2011)

17. Martínez-Montaña, E., Osuna-Ruiz, I., Benítez-García, I., Osuna, C. O., Pacheco-Aguilar, R., Navarro-Peraza, R. S., Sánchez, M. E. L., Hernández, C., Spanopoulos-Hernández, M., and Salazar-Leyva, J. A., Biochemical and antioxidant properties of recovered solids with pH shift from fishery effluents (sardine stickwater and tuna cooking water). *Waste Biomass Valor.* **12**, 1901-1913. (2021) 10.1007/s12649-020-01147-6
18. Anderson, D. M., Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean & Coastal Management.* **52**, 342-347. (2009) <https://doi.org/10.1016/j.ocecoaman.2009.04.006>
19. SENGCO, M. R. and ANDERSON, D. M., Controlling Harmful Algal Blooms Through Clay Flocculation1. *Journal of Eukaryotic Microbiology.* **51**, 169-172. (2004) <https://doi.org/10.1111/j.1550-7408.2004.tb00541.x>
20. Park, S.-C., Moon, J. C., Kim, N.-H., Kim, E.-J., Jeong, J.-E., Nelson, A. D., Jo, B.-H., Jang, M.-K., and Lee, J. R. J. B. I., Algicidal effect of hybrid peptides as potential inhibitors of harmful algal blooms. **38**, 847-854. (2016) 10.1007/s10529-016-2052-0
21. Park, S. C., Lee, J. K., Kim, S. W., and Park, Y., Selective algicidal action of peptides against harmful algal bloom species. *PloS one.* **6**, e26733. (2011) 10.1371/journal.pone.0026733
22. Cho, J. Y. and Kim, J. K. J. J. o. C. B., Isolation and identification of a novel algicidal peptide from mackerel muscle hydrolysate. **1093**, 39-46. (2018)
23. Cho, J. Y., Jung, H. Y., and Kim, J. K., Biodegraded mackerel wastewater selectively inhibits harmful algal blooms. *J. Hazard. Mater.* **364**, 349-355. (2019) <https://doi.org/10.1016/j.jhazmat.2018.10.053>
24. Afonso, M. D. and Borquez, R., Review of the treatment of seafood processing wastewaters and recovery of proteins therein by membrane separation processes—prospects of the ultrafiltration of wastewaters from the fish meal industry. *Desalination.* **142**, 29-45. (2002) 10.1016/S0011-9164(01)00423-4
25. Navarro-Peraza, R. S., Osuna-Ruiz, I., Lugo-Sánchez, M. E., Pacheco-Aguilar, R., Ramírez-Suárez, J. C., Burgos-Hernández, A., Martínez-Montaña, E., and Salazar-Leyva, J. A., Structural and biological properties of protein hydrolysates from seafood by-products: a review focused on fishery effluents. *Food Science and Technology.* **40**, 1-5. (2020) 10.1590/fst.24719
26. AOAC, *Official Methods of analysis*. 2000, Association of Official Analytical Chemists: Washington.
27. Woyewoda, A., Shaw, S., Ke, P., and Burns, B., *Recommended laboratory methods for assessment of fish quality*. 1986.

28. Laemmli, U. K. J. n., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. **227**, 680-685. (1970) 10.1038/227680a0
29. Schägger, H., Tricine SDS-PAGE. *Nature Protocols*. **1**, 16-22. (2006) 10.1038/nprot.2006.4
30. Garcia-Sifuentes, C., Pacheco-Aguilar, R., Lugo-Sánchez, M., Garcia-Sánchez, G., Ramirez-Suarez, J. C., and Garcia-Carreño, F., Properties of recovered solids from stick-water treated by centrifugation and pH shift. *Food Chem.* **114**, 197-203. (2009) 10.1016/j.foodchem.2008.09.064
31. Cho, J. H. and Kim, I. H., Fish meal – nutritive value. **95**, 685-692. (2011) 10.1111/j.1439-0396.2010.01109.x
32. Sathivel, S., Bechtel, P. J., Babbitt, J., Prinyawiwatkul, W., Negulescu, I. I., and Reppond, K. D., Properties of Protein Powders from Arrowtooth Flounder (*Atheresthes stomias*) and Herring (*Clupea harengus*) Byproducts. *J. Agric. Food. Chem.* **52**, 5040-5046. (2004) 10.1021/jf0351422
33. Cheryan, M., *Ultrafiltration and microfiltration handbook*. 1998: CRC press.
34. Guerrero, L., Omil, F., Méndez, R., and Lema, J. M., Protein recovery during the overall treatment of wastewaters from fish-meal factories. *Bioresour. Technol.* **63**, 221-229. (1998) [https://doi.org/10.1016/S0960-8524\(97\)00140-5](https://doi.org/10.1016/S0960-8524(97)00140-5)
35. Wasswa, J., Tang, J., and Gu, X., Utilization of Fish Processing By-Products in the Gelatin Industry. *Food Reviews International*. **23**, 159-174. (2007) 10.1080/87559120701225029
36. Arnesen, J. A. and Gildberg, A., Extraction and characterisation of gelatine from Atlantic salmon (*Salmo salar*) skin. *Bioresour. Technol.* **98**, 53-57. (2007) 10.1016/j.biortech.2005.11.021
37. Gómez-Guillén, M. C., Turnay, J., Fernández-Díaz, M. D., Ulmo, N., Lizarbe, M. A., and Montero, P., Structural and physical properties of gelatin extracted from different marine species: a comparative study. *Food Hydrocolloids*. **16**, 25-34. (2002) [https://doi.org/10.1016/S0268-005X\(01\)00035-2](https://doi.org/10.1016/S0268-005X(01)00035-2)
38. Mohtar, N. F., Perera, C., and Quek, S.-Y., Optimisation of gelatine extraction from hoki (*Macruronus novaezelandiae*) skins and measurement of gel strength and SDS–PAGE. *Food Chem.* **122**, 307-313. (2010) <https://doi.org/10.1016/j.foodchem.2010.02.027>
39. Cho, S. M., Gu, Y. S., and Kim, S. B., Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocolloids*. **19**, 221-229. (2005) <https://doi.org/10.1016/j.foodhyd.2004.05.005>
40. Sanmartín, E., Arbolea, J. C., Iloro, I., Escuredo, K., Elortza, F., and Moreno, F. J., Proteomic analysis of processing by-products from canned and fresh tuna: Identification of potentially functional food proteins. *Food Chem.* **134**, 1211-1219. (2012) 10.1016/j.foodchem.2012.02.177

41. Bechtel, P. J., Chantarachoti, J., Oliveira, A. C. M., and Sathivel, S., Characterization of Protein Fractions from Immature Alaska Walleye Pollock (*Theragra chalcogramma*) Roe. **72**, S338-S343. (2007) 10.1111/j.1750-3841.2007.00396.x

Figures

Figure 1

Stickwater (SW) ultrafiltration sequence CSW: centrifuged stickwater, P30: permeate 30 kDa, R10: retentate 10 kDa, P10: permeate 10 kDa, R5: retentate 5 kDa, P5: permeate 5 kDa

Figure 2

Stickwater (SW) and centrifuged stickwater (CSW) maintain gel-like form A: CSW, lateral view. B: CSW, top view

Figure 3

SDS-PAGE of stickwater (SW), centrifuged stickwater (CSW) and centrifuged solids (CS) a: broad-range molecular weight marker. b: stickwater (SW). c: centrifuged stickwater (CSW). d: centrifuged solids (CS).

Figure 4

Tricine SDS-PAGE profile of low molecular weight fractions a: polypeptide molecular weight marker. P30: ≤ 30 kDa, R10: 30-10 kDa, P10: ≤ 10 kDa, R5: 10-5 kDa, P5: ≤ 5 kDa