Revista Mexicana de Ingeniería Química
ISSN: 1665-2738
amidiq@xanum.uam.mx
Universidad Autónoma Metropolitana
Unidad Iztapalapa
México

Elizondo-Garza, P.; Serna-Saldívar, S.O.; Chuck-Hernández, C.
PROTEIN RECOVERY FROM SKIPJACK TUNA (Katsuwonus pelamis) WASH WATER WITH DIFFERENT pH AND TEMPERATURE COMBINATIONS
Revista Mexicana de Ingeniería Química, vol. 16, núm. 1, 2017, pp. 91-99
Universidad Autónoma Metropolitana Unidad Iztapalapa
Distrito Federal, México

Vol. 16, No. 1 (2017) 91-99
Revista Mexicana de Ingeniería Química

PROTEIN RECOVERY FROM SKIPJACK TUNA (*Katsuwonus pelamis*) WASH WATER WITH DIFFERENT pH AND TEMPERATURE COMBINATIONS

RECUPERACIÓN DE PROTEÍNA DE BARRILETE (*Katsuwonus pelamis*) DEL AGUA DE PROCESO CON DIFERENTES COMBINACIONES DE pH Y TEMPERATURA

P. Elizondo-Garza, S.O. Serna-Saldívar, C. Chuck-Hernández*
Centro de Biotecnología FEMSA, Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, C.P 64849, Monterrey, N.L., México.
Received September 4, 2016; Accepted October 21, 2016

Abstract
The industrial processing of fish demands high usage of water and its main drawback is the production of effluents high in Chemical Oxygen Demand (COD). Surimi production from skipjack (*K. pelamis*) has not been reported and neither the separation and recovery of its sarcoplasmic proteins from the wash-water. The aim of this research was to find a simple process to recover solids and protein, using pH (2.5, 3.5, 4.5, 5.5, 6.5 or 9.5) and temperature (4, 25 or 90°C) combinations. The best processing conditions for protein precipitation were 4°C and pH 4.5, 5.5 or 6.5, with protein recoveries of about 94% and purities of 92%. This protein recovery represented an average of 60% of the protein that was originally present in the skipjack flesh, and around 50% of the initial solids. The essential amino acid profile for the best treatments exceeded the requirements for children or infants. The proposed process can contribute to 1) improve the economics of processing facilities and 2) produce isolates with high protein quality.
Keywords: proteins, fish, skipjack.

Resumen
En la producción de alimentos basados en pescados se usa una gran cantidad de agua, generando efluentes con alta Demanda Química de Oxígeno (COD). La producción de surimi a partir de barrilete (*K. pelamis*) no ha sido reportado ni tampoco la recuperación de proteínas sarcoplásmicas presentes en las aguas de descarte. El objetivo de esta investigación fue encontrar un proceso simple para recuperar sólidos y proteínas usando combinaciones de pH (2.5, 3.5, 4.5, 5.5, 6.5 o 9.5) y temperatura (4, 25 o 90°C). Las mejores condiciones de proceso para la precipitación proteica fueron 4°C y pH de 4.5, 5.5 y 6.5, con recuperación de proteína del 94% y pureza del 92%. Para estos tratamientos, se recuperó en promedio un 60% de la proteína presente originalmente en el barrilete, lo cual representa alrededor del 50% de los sólidos iniciales. Los mejores tratamientos excedieron el perfil de aminoácidos esenciales requerido para la alimentación para niños o infantes. El proceso propuesto puede contribuir a: 1) mejorar la economía de las empresas de procesamiento y 2) producir aislados de alta calidad proteica.
Palabras clave: proteínas, pescado, barrilete.

1 Introduction

Worldwide members of the family *Scombridae* commonly known as tunas and mackerels are the basis of important commercial and recreational fisheries (Lucano-Ramírez *et al*., 2011). The main tuna species include: albacore, Atlantic bluefin, bigeye, Pacific bluefin, Southern bluefin, yellowfin and skipjack. They have a high demand in the international market for canning and sashimi manufacturing (FAO, 2016). In Mexico, tuna represents the fourth place in fish production and the second regarding its economic value (Global Biotech Consulting Group, 2016).

Skipjack (*Katsuwonus pelamis*), with 10,000 to 30,000 tons yearly produced, represents 1 to 2% of the national fishing and around 20% of total tuna harvested. The comparatively main withdraw of skipjack is that contains a muscle tissue darker

* *Corresponding author. E-mail*: cristina.chuck@itesm.mx

compared to other tuna species which significantly lowers its economic market value. Skipjack from Mexican fisheries is a market opportunity because it is underexploited (SAGARPA, 2009). Despite this significant opportunity, compared to other fisheries its economic value is relatively low, opening the possibility to industrialization and production of value added processed foods. One method to process skipjack and therefore to increase its value is through its use as raw material for surimi, in which the dark color is reduced because of the water affinity of myoglobin. Surimi is one of the major fish transformations (Martín-Sánchez *et al*., 2009), and can be described as minced fish thoroughly washed to remove sarcoplasmic proteins obtaining a highly refined product. Fishery industries, both artisanal and industrial, same as other food industries (as cheese producers), generates waste and residual streams of around 29 million ton worldwide (Spanopoulos-Hernandez *et al*., 2010; Vicencio-de la Rosa *et al*., 2015). This figure means a waste of high quality protein and an elevated environmental burden. Solids concentration in the discarded water from fish processing reaches up to 40 to 50 g per each 100 grams of processed flesh tissue. Solids are commonly recovered for production of animal feeds or fishmeal (Bourtoom *et al*., 2009; Spanopoulos-Hernandez *et al*., 2010). Due to its high nutritional and economical value, the recovery of this protein at high concentrations represents an opportunity, mainly if the devised process is cost-efficient. Gildberg (2002) sustains that when components are recovered from processing by-products and they are used in the development of human foods, their value increases about five times. Besides, the recovery of water-soluble proteins is required to reduce the environmental impact of food processing plants.

Methods to recover protein from fish processing by-products include pH shifting and thermal treatment. The former consists in an isoelectric precipitation followed by a centrifugation in order to recover the curd rich in protein (Sanmartín *et al*., 2009). The latter, as reported by Sathivel *et al*. (2004), is protein separation from different fish by-products using high temperatures (85 °C), followed by protein separation via centrifugation. Other methods are ultrafiltration and aqueous two-phase systems, both reported when the recovery of highly valuable protein products or the specificity of extraction is the goal (Jaouen and Quenmeneur, 1992; Martín-Sánchez *et al*., 2009; Sanmartín *et al*., 2009; Stine *et al*., 2012; Vázquez-Villegas *et al*., 2015). Ultrafiltration is a neat and selective method but the cost and excessive processing time limits its use to some food industrial applications (Sanmartín *et al*., 2009). pH shifting on the other hand has the advantage of high recovery yield and compatibility with the industrial environment. Bourtoom *et al*. (2009), precipitated proteins from surimi's threadfin bream (*Nemipterus hexodon*) wash-water at different pHs (3.0 to 6.0), temperatures (4, 17 and 30°C) and ethanol concentrations (10 to 50 g of ethanol / 100 g of wash-water). They found that the highest precipitation was obtained using the highest ethanol concentration, pH of 3.5 and that the amount of precipitated protein was directly related to temperature. The processing conditions (pH and temperature for example) are specific for each fish genus, because of differences in protein properties (Gehring *et al*., 2011), even in related species. Rehbein (1995) found important differences in the electrophoresis patterns, isoelectric focusing, titration curve and native polyacrylamide gel electrophoresis (PAGE) for sarcoplasmic proteins obtained from Scombroid species.

These variations have an impact in pH based process, making necessary the search for optimum processing conditions to reach a better separation of the proteins in wash-water by-products. For skipjack fish or *Barrilete* as commonly known in Mexico, there are not reports regarding the use of its flesh for industrial processing nor the recovery of its soluble proteins from the wash-water. Being this fish important from the economic point of view, the study and development of processing strategies for its maximum protein recovery are required. Therefore, the aim of this investigation was the evaluation of different pHs and temperatures in the wash-water of skipjack surimi production in order to find the best processing conditions for protein precipitation and recovery. This information could enhance the economic benefit of skipjack's processing and reduce the environmental impact of the wastewater high in COD. Yields in relation to the total flesh weight, solids and protein were calculated as well as protein purity of the skipjack isolates obtained with the different treatment combinations. Furthermore, essential amino acid profile was obtained in the skipjack flesh, surimi and curds obtained after the proposed precipitation technologies.

2 Materials and methods

2.1 Surimi production and recovery of wash-water

Whole skipjack tuna from Chiapas Mexico was received frozen and was thawed at 4°C during two days before beheading, gutting and deboning. The clean flesh tissue was minced in a food processor and immediately washed to produce surimi following the process recommended by Hall and Ahmad (1997) with slight modifications. Briefly the minced tissue was washed with iced water (5-10°C; 3:1 ratio water: flesh) three times at pH 10.5 (50% NaOH) during 10 min in continuous agitation (75 rpm). The last washing was made with a 0.2% NaCl solution at the same conditions, except for the pH adjustment. Between washes, a filtration was made using cheesecloth in order to recover the surimi before the next washing cycle. All filtrates were collected, pooled and centrifuged at 15,275g during 15 min at 8°C (Model 5804R, Eppendorf, Germany) in order to recover the supernatant to perform precipitation tests. The yield of surimi solids and protein were calculated based on the original minced flesh weight.

2.2 Precipitation tests of water-soluble proteins from wash-water

The supernatant previously obtained (wash-water) was used to precipitate water-soluble proteins using pH shifting (six levels: 2.5, 3.5, 4.5, 5.5, 6.5 or 9.5, being the latter the original pH of the samples-) and temperatures (4, 25 or 90°C). pH was adjusted with a potentiometer (BenchTop Meter, HI 2550, Hanna Instruments, USA) and 4N HCl. Treatments at 4°C were processed in an ice-water bath (30min), samples at 25°C were maintained in an incubator (Model RF 1575, VWR, USA) during 30 min and samples processed at 90°C were treated in a water-bath over an electrical heater (Model DT, MiChef, Mexico) for 10 min. After pH and temperature treatments, the suspension was placed inside a plastic tube (50mL) and centrifuged at 1300g during 10 minutes at 4°C (Model 5804R, Eppendorf, Germany). The resulting pellet or curd and supernatant obtained by triplicates were collected for protein and moisture analyses.

2.3 Moisture and protein determination

Moisture and protein content of the pellet and supernatant of the treatments in which precipitation was achieved, were determined. Moisture was assayed by the gravimetric oven drying test AOAC Method 925.10 (AOAC, 1992) and protein content using the micro Kjeldahl procedure AOAC Method 984.13 (AOAC, 1992). The percentage nitrogen was multiplied by 6.25 to convert to protein.

2.4 Yield calculation of precipitated protein from wash-water

Three yield parameters were obtained. The percent crude yield was the ratio between final pellet or curd weight (precipitate material) and initial wash-water weight. The percent solid yield was the relationship between final solids (precipitate or pellet) and initial total solids in the wash-water, whereas the percentage protein extraction yield was computed as the final protein weight in the pellet divided by the initial protein weight in the wash-water (in dry basis).

2.5 Statistical analysis

Crude, total solids and protein yields were calculated and differences analyzed by Analysis of Variance (ANOVA) using Minitab Statistical Software (version 16, Minitab, USA). Differences among means were obtained with Tukey's test (*alpha*=0.05).

3 Results and discussion

3.1 Proximal composition of raw material

The skipjack contained 72.5% of moisture, 24.2% of protein, 1.8% fat, 1.36% ash and 0.1% of carbohydrates. The composition was similar to the reported by the USDA (70.6, 22.0, 1.0, 0.73 and 0.0 %) and Mazorra-Manzano *et al*. (2000) (73.2, 21.8, 1.0, 1.4 and 2.6), respectively. Moisture contents of fish related to skipjack were similar, for instance the moisture of yellowfin tuna and Bluefin were 74.3% and 68.0%, being skipjack in the middle of this range.

The skipjack protein content was similar to Bluefin and yellowfin tuna (23.3 and 24.4% respectively). Total lipids, on the other hand, were higher in Bluefin tuna specimens (4.9%) compared to skipjack and yellowfin, which contained 1.8 and 0.5%, respectively. As expected, the carbohydrates present in the flesh tissue were in very low amounts. Tuna varieties or species compared with other fish as Alaska Pollock (used for surimi manufacturing), contained considerably less water and more protein and lipids

(86.8, 12.2 and 0.41% respectively). The composition of the raw material affects extraction conditions especially in terms of protein recovery.

3.2 Carcass and surimi yields of skipjack

The yield from the skipjack carcass to deboned flesh was 38.15±3.09%, close to 40.8% reported previously by Mazorra-Manzano *et al*. (2000). One kilogram of minced skipjack produced 373 g of washed surimi or 48.83 g of dry solids which represented 13.09% of the initial flesh solids. The extraction of surimi generated 12 kg of wash-water which contained 2% of insoluble solids and 1.4% of soluble solids. The amount of water used for tissue washing was similar to the quantity reported by Ramírez *et al*. (2007) for surimi manufacturing (around 12.4 kg if a conversion of 35% of raw fish to minced fish is considered). As stated above, the wash-water contained fine insoluble solids that accounted for 0.24 kg plus 0.17 kg of soluble solids. The first fraction was effectively removed through centrifugation that in an industrial scale could be made with robust and fully automated equipment. The wet and dry soluble solids contained 1.30 and 87.74% protein respectively. The wet protein value is within the range commonly found in the surimi wash-water (0.5-2.3%) previously researched by Stine *et al*. (2012). This protein represents 63.2% of the initial protein of the minced flesh of skipjack, a percentage that makes worthy to devise economical processes to recover these nitrogenous compounds. Table 1 summarizes the mass balance of the surimi and pooled wash-water in terms of solids and protein.

3.3 Protein precipitation from wash-water

In Table 2, the protein content as well as the crude, solids and protein yields of the supernatants and recovered pellets from wash-waters subjected to the proposed different treatments are presented. Independently of the temperature applied, the control (pH 9.5) and experimental wash-waters treated at acidic pHs of 2.5 or 3.5 did not yield curds. This observation does not agree with (Bourtoom *et al*., 2009), who reported the maximum precipitation for sarcoplasmic proteins of threadfin bream (*Nemipterus hexodon*) at pH of 3.5 treated at 4, 17 or 30°C, indicating the differences among solubility properties of skipjack and threadfin bream sarcoplasmic proteins. It is well known that sarcoplasmic proteins are composed of globular and soluble proteins such as myoglobin, hemoglobin, globulins, albumins, and various enzymes found in extracellular fluids (Ramírez *et al*., 2007). These proteins are denatured by heat and pH shifting, factors that can enhance the precipitation or coagulation due to the loss of solubility.

Dry curds obtained from the different treatments can be considered protein isolates, because they contained more that 90% protein expressed in dry basis (Table 2). The only sample with protein content less than 90% was the obtained after treating the wash-water with pH 4.5 at 25°C (88.56%). The maximum protein extraction was achieved at 4°C, a temperature recommended to minimize microbial spoilage and keep protein functionality (Badui, 2006; Pelegrine and Gomes, 2008).

Table 1. Solid and protein mass balances from skipjack flesh processing

Skipjack fraction from surimi manufacturing	**Weight (kg)**	**Solids (kg)**	**Protein (kg)**
Minced flesh from Skipjack	1.00	0.28±2.26 [a]	0.24±0.92 [a]
Washed flesh	0.37	0.05±0.28 [c]	0.03±0.24 [c]
Wash-Water	11.76	0.17±1.83 [b]	0.15±0.50 [b]
Flesh recovered after centrifugation (insoluble solids)	0.24	0.02±0.09 [c]	0.01±0.00 [c]

Data are the average ± standard deviation. Different letters within columns indicate statistical difference with P<0.05.

Table 2. Protein and yield results of the protein precipitation from wash-water obtained from skipjack processing (*Katsuwonus pelamis*).

Treatments		Final protein %		Yield, %		
pH	Temperature (°C)	Supernatant, db	Pellet or isolated, db	Crude Yield final / initial weight, as is	Solids Yield final / initial solids	Protein Extraction final/ initial protein
4.5	4	61.73 ± 5.01 ^{b}	92.94 ± 1.40 ^{a}	7.61 ± 0.29 ^{ab}	83.15 ± 13.10 ^{a}	94.10 ± 22.2 ^{a}
	25	68.59 ± 2.93 ^{ab}	88.56 ± 2.54 ^{b}	6.66 ± 1.11 ^{bcd}	47.19 ± 2.44 ^{cd}	60.17 ± 1.83 ^{bcd}
	90	57.89 ± 10.15 ^{bc}	90.86 ± 2.46 ^{ab}	7.72 ± 0.54 ^{abc}	54.65 ± 5.43 ^{cd}	67.46 ± 4.04 ^{bc}
5.5	4	57.39 ± 2.50 ^{bc}	94.07 ± 2.32 ^{a}	6.15 ± 0.64 ^{bcd}	73.95 ± 6.77 ^{ab}	97.04 ± 15.17 ^{a}
	25	62.68 ± 4.69 ^{b}	92.31 ± 0.83 ^{ab}	4.37 ± 0.42 ^{d}	43.18 ± 2.24 ^{d}	56.13 ± 5.62 ^{cd}
	90	46.91 ± 2.01 ^{cd}	93.88 ± 2.31 ^{a}	9.77 ± 0.61 ^{a}	60.68 ± 2.92 ^{bc}	78.13 ± 4.83 ^{ab}
6.5	4	62.25 ± 1.12 ^{b}	92.32 ± 1.50 ^{ab}	8.61 ± 0.65 ^{ab}	78.07 ± 8.78 ^{ab}	99.31 ± 4.88 ^{a}
	25	75.76 ± 1.43 ^{a}	92.58 ± 2.39 ^{a}	4.96 ± 1.10 ^{cd}	39.94 ± 11.50 ^{d}	42.19 ± 0.98 ^{d}
	90	41.76 ± 1.05 ^{d}	93.22 ± 2.27 ^{a}	7.02 ± 1.87 ^{abcd}	37.64 ± 6.77 ^{d}	62.50 ± 3.59 ^{bcd}
C	90	40.23 ± 8.33 ^{d}	95.09 ± 0.74 ^{a}	10.00 ± 0.11 ^{a}	42.24 ± 3.51 ^{d}	58.53 ± 4.87 ^{bcd}
Main Effects						
pH		**	**	NS	*	**
Temperature		**	*	**	**	**
pH*Temperature		**	NS	**	*	NS

NS: Non significant; db: dry basis; * Significant with *alpha* < 0.05; ** Highly significant with alpha < 0.005.
Data are the average ± standard deviation. Different letters within columns indicate statistical difference with P<0.05.

Being temperature one of the factors that influences the most solubility, the best strategy when solubility want to be reduced for production of protein isolates, is to move temperature away from the 40 to 50°C range. In this case the use of 4°C was the most effective to protein recovery.

According to results shown in Table 2, when 4°C could not be reached in an industrial setup, other possible and effective alternative is to increase temperature to 90°C. Under these conditions, the precipitated material (pH 5.5) represented 9.77% when expressed in terms of the initial sample weight (slightly higher than wash-water treated at 4°C). Despite this high crude yield, the solid and protein recoveries were not the highest, but average compared to the rest of the treatments (Table 2). This difference is related to moisture content of the pellets (data not shown). The wash-water treated at 90°C and pH 5.5 contained 89.45% moisture, higher compared with the average of the rest of treatments (around 88%) and even higher than the treatment with the lowest crude and solids yield (83.47% moisture for treatment with pH 5.5 and 25°C).

The precipitation mechanism at 90°C and pH 5.5 was related to the denaturalization of the sarcoplasmic proteins which in turn had an effect on the quantity of moisture trapped into the protein matrix. Denaturation implies a change in structure and aggregation of protein chains with an increased water-trapping capacity of the new assembly as denoted in the pellet moisture. Treatments at 90°C were far beyond the 64.7°C, limit reported as the denaturalization temperature for sarcoplasmic proteins. These results indicate that pH and mainly temperature have an effect on the protein precipitation from the skipjack surimi wash-water. Bourtoom *et al*. (2009) concluded that protein precipitation increased considerably with a pH reduction and a high temperature but a very low pH and excessive high temperature lead to massive denaturation. In the case of skipjack, the best pH range to reach precipitation was from 4.5 to 6.5. This result is related to the sarcoplasmic protein solubility that was minimum near to the isoelectric point.

As can be seen in information depicted in Table 2, most of the protein was obtained during the pH shifting process; the least efficient treatment was 42.2%, below the 69% reported by Lee *et al*. (2014), which in turn was lower than the average depicted in Table 2 (71.6%). The same authors tested a pretreatment process of micro (MF) and ultrafiltration (UF), which is different indeed to pH shifting. UF has some advantages as the non-change in protein structure, but disadvantages as protein fouling and membrane blockage, making in some cases very

inefficient its industrial use. On the other hand, the least efficient treatments in terms of protein precipitation were at two pHs (5.5 and 6.5) at 25°C. These are near to the isoelectric point reported by Kim *et al*. (2003).

3.4 Changes in amino acid profile

Amino acid composition in food products or ingredients determines their nutritional value and also their functional properties. Amino acid profile for skipjack flesh, skipjack surimi and pellets (produced under the best recovery treatments from the surimi wash water) are depicted in Table 3. There are several interesting traits to be observed, first of all the essential amino acid profile for skipjack flesh was (as expected), way over the requirements for a 2 to 5 years old child. This is because the high quality of fish proteins, one of the most perfect among food sources (FAO/WHO, 2007; Mohanty *et al*., 2014). Other interesting characteristic was the change inflicted to the skipjack flesh: 1) with the washing process for surimi production and 2) with the protein precipitation from the wash-water subjected to different processing conditions. In the surimi manufacturing step from skipjack, the amino acid profile did change but only for histidine (378% to 160%, Table 3). Histidine is the most abundant free amino acid in dark-colored fish flesh (Belitz *et al*., 2009) and since the surimi process employs extensive washing it was clear that the amount of this amino acid greatly diminished in the skipjack surimi compared to the initial fish flesh.

Table 3. Essential amino acids content (% of the amino acid requirement for a 2 to 5 years old child) for different skipjack fractions and precipitated pellets from surimi wash water

Amino acid	Skipjack flesh	Skipjack Surimi	Pellet			
			pH alkaline and 90°C	pH 4.5 and 4°C	pH 5.5 and 4°C	pH 6.5 and 4°C
	% of the amino acid requirement for 2 to 5 years old children (FAO/WHO)					
Threonine	133.27	139.73	149.22	143.38	144.63	148.22
Valine	148.72	143.58	170.64	157.96	156.45	160.05
Isoleucine	167.63	163.80	193.80	183.20	183.05	195.43
Leucine	122.18	123.02	130.38	134.46	128.43	129.12
Lysine	146.35	153.42	155.57	161.03	157.29	150.84
Histidine	378.43	160.54	245.05	188.34	198.28	176.44
Tryptophan	105.54	93.76	129.41	105.76	111.51	119.94
Methionine + Cysteine	150.29	155.80	152.41	153.78	163.40	168.27
Phenylalanine+Tyrosine	130.18	120.00	133.71	128.55	130.20	135.44

Table 4. Sequential solids and protein yields of skipjack muscle tissue after recovery of surimi, insolubles in wash-water and precipitated curds at different pHs and temperatures

Treatments		Whole yields that include surimi, insolubles and curds from wash-water	
pH	Temperature (°C)	Solids yield (final / initial solids in minced meat)	Protein yield (final / initial protein in minced meat)
4.5	4	77.20 ± 7.87 ^{a}	77.88 ± 14.01 ^{ab}
	25	54.76 ± 1.54 ^{cd}	56.43 ± 1.15 ^{c}
	90	59.48 ± 3.43 ^{cd}	61.04 ± 2.56 ^{bc}
5.5	4	71.70 ± 4.29 ^{ab}	79.74 ± 9.59 ^{a}
	25	52.23 ± 1.42 ^{d}	53.87 ± 3.55 ^{c}
	90	63.30 ± 1.85 ^{bc}	67.79 ± 3.05 ^{abc}
6.5	4	74.31 ± 5.56 ^{ab}	81.18 ± 3.08 ^{a}
	25	50.18 ± 7.27 ^{d}	36.17 ± 13.78 ^{d}
	90	48.73 ± 4.29 ^{d}	57.90 ± 2.27 ^{c}
C	90	51.64 ± 2.22 ^{d}	55.39 ± 3.08 ^{c}

Data are the average ± standard deviation. Different letters within columns indicate statistical difference with $P<0.05$.

Regarding the amino acid profile of pellets obtained after precipitation of the skipjack surimi wash-water, all products had an excellent essential amino acid profile which exceeded the human nutrimental requirements. The results displayed in Table 3 are different from other assayed processes where changes in methionine and the oxidation of histidine to aspartic acid have been reported (Hrynets *et al*., 2011). These results describe the suitability of precipitation treatments for high quality protein production from a waste stream (surimi wash-water).

3.5 Whole process yield

In Table 4, the solids and protein recovery during the total process is depicted, 24.9% solids and 18.4% of the original protein from the initial minced tissue ended up in the surimi. The surimi yield was 61.3% when expressed in terms of the total initial minced flesh weight, close to 55% of surimi yield reported by Martín-Sánchez *et al*. (2009). When the solids and protein were precipitated from the wash-water at 4°C and pH 4.5, 5.5 and 6.5, the total solids recoveries reached 77.2, 71.7 and 74.3% respectively. The least efficient treatments were pH 6.5 at 90 and 25°C, respectively where only 48.7 and 50.2% solids were recuperated. Despite these inefficiencies 25% more solids with a higher protein purity were obtained compared with a regular surimi process. When the most effective precipitation conditions were employed more than 50% of solids were recovered from the wash-water. Interestingly, there was a close relationship between recoveries of solids and proteins (Table 4). Thus, wash-waters treated at pH 4.5, 5.5 or 6.5 were to most effective.

Results summarized in Table 4 clearly imply that the highest yields of solids and proteins were obtained in wash-waters treated at pH below 6.5 at 4°C. These treatments were capable of recuperating about 75 to 80% of the original solids and proteins associated to the skipjack minced flesh. The experimental results obtained herein can be used to devise new processes that will allow the recovery of shelf-stable high nutritious proteins from minced fish muscle tissue by simply using reagents to change pH of wash-waters and unit operations of centrifugation and drying. The economics of the additional processing steps should be studied taking into consideration environmental issues such as the disposal of wash-waters rich in COD.

Conclusions

In this work the use of different pHs and temperature combinations applied to wash-water from skipjack surimi manufacturing were tested. The best pH range for protein precipitation from the surimi process wash-water was between 4.5 to 6.5 and the best temperature 4°C. Following these, the next efficient treatments for total weight recovery was at pH 5.5 and 90°C. Both temperatures can prevent pathogen and spoilage microbial growth favoring food safety issues. On the other hand, the least efficient treatments, where no precipitation occurred, were the ones with the most acidic pH´s (2.5 and 3.5). With the use of pH shifting for protein precipitation of the wash-waters around 60% of protein and 50% of solids from the original minced meat originally used for surimi manufacturing were recovered. These findings and conclusions open the possibility to implement new processes aimed towards the recovery of added value and high nutritious proteins for food or feed applications.

Acknowledgements

This research was supported by the Research Chair Funds 0020IDP001 from Centro de Investigación y Desarrollo de Proteínas (CIDPRO), Tecnológico de Monterrey-Campus Monterrey.

References

AOAC Official Methods. (1992). Methods 925.10 and 984.13 Solids (Total) and Moisture and Protein (Crude) in animal feed and pet food. AOAC International, Washington DC USA.

Badui, S. (2006). *Química de los Alimentos*. Editorial Pearson, México, pp 176.

Belitz, H.D., Grosch, W. and Schieberle, P. (2009). *Food Chemistry*. Editorial Springer, Germany, pp. 1070.

Bourtoom, T., Chinnan, M.S., Jantawat, P. and Sanguandeekul, R. (2009). Recovery and characterization of proteins precipitated from surimi wash-water. *LWT- Food Science and Technology 42*, 599-605.

FAO/WHO. Food and Agriculture Organization, World Health Organization. (2007). *Protein and amino acid requirement in human nutrition*.

WHO Technical Report Series 935. World Health Organization, Geneva Switzerland, pp. 265.

FAO. Food and Agriculture Organization. (2016). *Tuna and tuna-like species-Global*. Available at: http://firms.fao.org/firms/resource/16001/en. Accessed: 03 September 2016.

Global Biotech Consulting Group. (2016). Tuna fish. Resource document. Available at: http://www.gbcbiotech.com/genomicaypesca/en/especies/peces/atun.html. Accessed 03 September 2016.

Gehring, C.K., Gigliotti, J.C., Moritz, J.S., Tou, J.C. and Jaczynski, J. (2011). Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: A review. *Food Chemistry 124*, 422-431.

Gildberg, A. (2002). Enhancing returns from greater utilization. In: *Safety and Quality Issues in Fish Processing*, (H.A. Bremner, ed.), Pp. 425-449. Woodhead Publishing Limited, Cambridge, UK.

Hall, G.M. and Ahmad, N.H. (1997). Surimi and fish-mince products. In: *Fish Processing Technology*, (G.M. Hall, ed.), Pp. 74-92. Springer Science & Business Media, London, UK.

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*, 335-343.

Jaouen, P. and Quenmeneur, F. (1992). Membrane filtration for waste-water protein recovery. In: *Fish Processing Technology*, (G.M. Hall, ed.), Pp. 212-248. Springer Science & Business Media, London, UK.

Kim, Y.S., Park, J.W. and Choi, Y.J. (2003). New approaches for the effective recovery of fish proteins and their physicochemical characteristics. *Fisheries Science 69*, 1231-1239.

Lee, J.K., Li-Chan, E.C.Y., Jeon, J.K. and Byun, H.G. (2014). Development of functional materials from seafood by-products by membrane separation technology. In: *Seafood Processing by-products: Trends and Applications*, (S.K. Kim, ed.), Pp. 35-62. Springer, New York, USA.

Lucano-Ramírez, G., Ruiz-Ramírez, S., Palomera-Sánchez, F.I. and González-Sansón, G. (2011). Reproductive biology of the Pacific sierra *Scomberomorus* sierra (Pisces, *Scombridae*) in the central Mexican Pacific. *Ciencias Marinas 37*, 249-260.

Martín-Sánchez, A.M., Navarro, C., Pérez-Álvarez, J.A. and Kuri, V. (2009). Alternative for efficient and sustainable production of surimi: a review. *Comprehensive Reviews in Food Science and Food Safety 8*, 359-374.

Mazorra-Manzano, M.A., Pacheco-Aguilar, R., Díaz-Rojas, E.I. and Lugo-Sánchez, M.E. (2000). Postmortem changes in black skipjack muscle during storage in ice. *Food and Chemical Toxicology 65*, 774-779.

Mohanty, B., Mahanty, A., Ganguly, S., Sankar, T.V., Chakraborty, K., Rangasamy, A., Paul, B., Sarma, D., Mathew, S., Asha, K.K., Behera, B., Aftabuddin, M., Debnath, D., Vijayagopal, P., Sridhar, N., Akhtar, M.S., Sahi, N., Mitra, T., Banerjee, S., Paria, P., Das, D., Vijayan, K.K., Laxmanan, P.T. and Sharma, A.P. (2014). Amino acid compositions of 27 food fishes and their importance in clinical nutrition. *Journal of Amino Acids*. 7 pages. Article ID 269797.

Pelegrine, D.H.G., Gomes, M.T. (2008). Whey proteins solubility curves at several temperatures values. *Ciência e Natura 30*, 17-25.

Ramírez, J.A., Velazquez, G., López, G., Torres, J.A. (2007). Effect of adding insoluble solids from surimi wash-water on the functional and mechanical properties of pacific whiting grade A surimi. *Bioresource Technology 98*, 2148-2153.

Rehbein, H. (1995). Differentiation of *Scombroid* fish species (tunas, bonitos and mackerels) by isoelectric focusing, titration curve analysis and native polyacrylamide gel electrophoresis of sarcoplasmic proteins. *Electrophoresis 16*, 820-822.

SAGARPA. Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. (2009). SAGARPA: Oportunidades de Mercado para Sinaloa 2009. Available at: http://2006-2012.sagarpa.gob.mx/agronegocios/Documents/ONSinaloa.pdf. Accessed: 03 September 2016.

Sanmartín, E., Arboleya, J.C., Villamiel, M. and Moreno, F.J. (2009). Recent advances in the recovery and improvement of functional proteins from fish processing by-products: use of protein glycation as an alternative method. *Comprehensive Reviews in Food Science and Food Safety 8*, 332-344.

Sathivel, S., Bechtel, P.J., Babbitt, J., Prinyawiwatkul, W., Negulescu, I.I. and Reppond, K.D. (2004). Properties of protein powders from arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupea harengus*) byproducts. *Journal of Agricultural and Food Chemistry 52*, 5040-5046.

Spanopoulos-Hernandez, M., Ponce-Palafox, J.T., Barba-Quintero, G., Ruelas-Inzunza, J.R., Tiznado-Contreras, M.R., Hernández-González, C., Shirai, K. (2010). Producción de ensilados biológicos a partir de desechos de pescado, del ahumado de atún aleta amarilla (*Thunnus albacares*) y del fileteado de tilapia (*Oreochromis* sp), para la alimentación de especies acuícolas. *Revista Mexicana de Ingeniería Química 9*, 167-178.

Stine, J.J., Pedersen, L., Smiley, S. and Bechtel, P.J. (2012). Recovery and utilization of protein derived from surimi wash-water. *Journal of Food Quality 35*, 43-50.

Vázquez-Villegas, P., Acuña-González, E., Mejía-Manzano, L.A., Rito-Palomares, M. and Aguilar, O. (2015). Production and optimization of a chlorophyl-free leaf protein concentrate from alfalfa (*Medicago sativa*) through aqueous two-phase system. *Revista Mexicana de Ingeniería Química 14*, 383-392.

Vicencio-de la Rosa, M.G., Valencia-Vázquez, R., Pérez-López, M.E., Martínez-Prado, M.A. and Ramírez-Ramírez, E.S., (2015). Stabilization by co-composting of solids removed from wastewater treatment plant of a cheese factory. *Revista Mexicana de Ingeniería Química 14*, 61-71.