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ARTICLE

Influence of Adding Recovered Protein from Processing Wastewater on the Quality of Mechanically Separated Chicken Meat Surimi Like-Material

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Abstract

Functional and nutritional soluble proteins can be recovered from surimi (and surimi-like material) processing wastewater, reducing environmental problems and the cost of an irresponsible waste disposal. Recovered proteins may be added back at a low percentage to surimi products. The aim of this work was to evaluate the effect of the addition of soluble recovered proteins (RP), obtained from mechanically separated chicken meat surimi-like material (MSCM-SLM) processing wastewater by acidic pH-shifting, on the composition and texture of RP-MSCM-SLM, with RP contents of 0, 10, 20 and 30% (w/w) in the mixture. For that, proximate composition and gel properties were evaluated. The fat content of the MSCM-SLM was significantly reduced to 11.98% and protein increased to 83.64% (dry basis) after three washing cycles. The addition of 30% RP in the MSCM-SLM significantly augmented the protein content to 93.45% and reduced fat content from to 2.78%. On the other hand, the addition of RP was responsible for a drastic decrease in texture parameters, reaching 252.36 g, 185.23 g.cm, and 6.97 N for breaking force, gel strength and cutting strength, respectively, when 30% of RP was included in the MSCM-SLM. It was concluded that the obtained intermediary product (RP-MSCM-SLM) is a good option to applications in processed meat products where high texture parameters are dispensable, e.g., emulsified inlaid frankfurter-type sausages, but high protein content and low fat content desired.

Keywords chicken, surimi, texture properties, proximate composition, characterization

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Introduction

Surimi (and surimi-like material) processing water contains high organic loads that may represent an environmental problem, e.g., poultry processors (Velazquez *et al.*, 2007). Therefore, it is essential to find out new efficient technologies for these by-products, with environmentally sound procedures requiring low energy consumption (Dumay *et al.*, 2008; Stine *et al.*, 2012).

The processing water generated in the surimi processing contains 0.5 to 2.3% of total proteins which are composed of sarcoplasmic proteins with small amounts of myofibrillar proteins (Morrisey *et al.*, 2000; Velazquez *et al.*, 2007). The wash water is generally discarded back into the plant's waste stream, resulting in the loss of valuable components, especially soluble proteins with good functional and nutritional properties that could be recovered and fractionated (Sanmartín *et al.*,

2009; Tacharatanamanee *et al.*, 2004). This is an irresponsible waste of raw-material that is not acceptable in a world with increasing populations that needs proteins (Nølsoe and Underland, 2009). Recovering proteins would reduce the negative environmental impact and the cost of waste disposal, beyond generate potential profits (Bourtoom *et al.*, 2009).

The recovery of proteins may potentially improve yield beyond recycle the water from surimi obtaining processes (Lin *et al.*, 1995) and them added back at a low percentage to surimi products (Stine *et al.*, 2012). Numerous studies have been published on recovering proteins from surimi process (Bourtoom *et al.*, 2009; Dumay *et al.*, 2008; Lin *et al.*, 1995; Rawdkuen *et al.*, 2009; Sanmartín *et al.*, 2009; Stine *et al.*, 2012; Velazquez *et al.*, 2007).

Functional proteins can be efficiently recovered from low-value muscle processing by-products using isoelectric solubilization/precipitation and subsequently be used in value-added human foods (Ramirez *et al.*, 2007). The extraction mechanism is characterized by acid- or alkaline-aided solubilisation of the muscle protein at low or high pH to separate soluble proteins of non-soluble materials, like bones, skin, connective tissue, cellular membranes, and neutral storage lipids through centrifugation (Nølsoe and Underland, 2009; Rawdkuen *et al.*, 2009). Then, precipitated proteins are recovered by centrifugation and decanting (Sanmartín *et al.*, 2009). The benefits of each method with reference to quality, quantity and end use of the recovered proteins need thorough investigation (Bourtoom *et al.*, 2009).

The extracting efficiency to isolate muscle proteins depends mainly on the nature of the raw material to be treated, as well as on the final application, once that the species respond differently to acid and alkaline solubilization (Sanmartín *et al.*, 2009). The increase in the mechanical properties may induce a higher level of protein-protein interaction and thus stronger gels (Velazquez *et al.*, 2007). The importance of high gel strength is dependent on the intended use of the product. The acid and/or alkaline methods provide higher protein yields due to the additional recovery of sarcoplasmic proteins and increased lipid removal caused by the high-speed centrifugation step. However the temperature should be kept below 15°C during the entire isolation process to retain important functional properties like gel strength (Sanmartín *et al.*, 2009).

The aim of this work was to evaluate the effect of adding soluble proteins, recovered by acidic pH-shifting, from mechanically separated chicken meat surimi like-material (MSM-SLM) wastewater on the composition and texture of MSM-SLM.

Material and Methods

Mechanically separated chicken meat (MSCM)

Fresh MSCM was supplied from a local poultry processing plant and transported under refrigerated conditions to our laboratory. It was produced in 3 mm particle size using a meat-bone separator, (Model 694 Baader, Germany) operating at inlet 6°C and outlet 10°C, from broiler's necks, frames and backs, 24 h after the slaughtering.

Surimi-like material, recovered protein and surimi-like material gel

Mechanically separated chicken meat (MSCM) was washed in 3 cycles, utilizing in each cycle a washing solution: MSCM ratio of, 4:1 (w/v) for 10 min at 7°C. The stirring was kept constant at 220 rpm. It was utilized 0.5% $NaHCO_3$ solution for the first and second washings and 0.3% NaCl solution for the last one. The first and second centrifugations were carried out at 3,000 *g* for 15 min, while the third one at 7,000 *g* for 25 min. (Cortez-Vega *et al.*, 2015).

The fractions of supernatant containing fat and water-soluble proteins obtained from each washing cycle were mixture. Then the soluble protein was isolated by the process of pH variation at the isoelectric point in pH 5, according to methodology adapted from elsewhere (Nølsoe and Underland, 2009). The homogenate was ice-cooled and the pH adjusted to 10.8 for alkaline protein isolation by drop-wise addition of 2 M NaOH. The pH-adjusted homogenates were centrifuged at 10,000 *g* for 20 min at 4°C. The precipitation was performed by adjusting the pH of the supernatant to pH 5.0 by drop-wise addition of 2 M NaOH. After that, the material was again centrifuged at 10,000 *g* for 20 min at 4°C. Then, the supernatant was poured off and the recovered protein was collected and maintained at 4°C until all batches were processed.

To prepare the gel, surimi-like material (SLM) was added of recovered protein (RP) in order to obtain 0, 10, 20 and 30% (w/w) of RP in the mixture. The RP-SLM mixture was added of 2.5% NaCl and chopped for 5 min at 4°C to obtain the homogenous sol. The sol was then stuffed into stainless steel cylinders (30 mm i.d., 30 mm ht) and both ends of casing were sealed tightly. Two-step heated gels were prepared by setting the sol at 40°C for 30 min, followed by heating at 90°C for 20 min. The gels were

then cooled in iced water and stored for 24 h at 4°C prior to analysis.

Proximate composition

Moisture, crude protein, crude fat and crude ash contents were determined in triplicate according to the methods described by AOAC (1995). Moisture was determined by the oven drying method at 105°C until constant weight (method 950.46), protein by the Kjeldhal method (method 928.08), fat by the Soxhlet method (method 960.39) and ash by using the muffle oven technique (method 920.153).

pH

pH was measured using a digital pH meter (Model PA 200, Marconi Instruments, Inc., Brazil). About 10 g of sample (MSCM, MSCM-RP, MSCM-SLM, MSCM-RP-SLM) was cut into small pieces to which 50 mL of distilled water was added and slurry was made using a blender and the pH was recorded.

Myofibrillar protein

The myofibrillar proteins was determined according to the method described by Jin *et al.* (2007) and Kuo and Chu (2003). Myofibrilla proteins were isolated from samples by homogenizing 4 g of minced samples in a Metvisa homogenizer (Model TA-2, SC, Brazil) for 10 s in 10 vol. (v/w) of a 2°C isolating medium containing 100 mM KCl, 20 mM potassium phosphate (pH 7.0), 1 mM EDTA and 1 mM sodium azide. The homogenate was sedimented at 1,000 g for 15 min and the supernatant decanted. The sediment was resuspended at 1,000 g for 15 min and the supernatant decanted. The sediment was again resuspended in 5 vol. (v/w) of the original isolating medium and passed through a polyethylene strainer to remove connective tissue and debris. Five more volumes, resulting in 10 vol. (v/w) total of the original isolating medium, were used to further facilitate passage of myofibrilla protein through the strainer. Again, the supernatant was sedimented at 1,000 g for 15 min and the supernatants decanted. The sediments were washed three more times by suspending in 5 vol. (v/w) of the original isolating medium, and were sedimented at 1,000 *g* for 15 min. Finally, the sedimented myofibrilla proteins were resuspended in 5 vol. (v/w) of the original isolating medium. The protein concentration was determined by the biuret procedure described by Clark and Switzer (1977).

Gel properties

Texture analysis of surimi gel was carried out using a texture analyzer (Model 3 TA-XT2 plus; Stable Micro Systems, Surrey, England). Gels kept at 4°C were equilibrated at room temperature (28-30°C) before analysis. Cylindrical samples, 2.5×3.0 cm, were prepared and placed in the texture analyzer equipped with a spherical plunger (5 mm diameter; 60 mm/min depression speed). The results were expressed as breaking force (g) and deformation (mm) representing the hardness and cohesiveness of the surimi gels, respectively. Gel strength (g.cm) was expressed as the product of breaking strength and deformation. Analogously, samples were submitted to a cutting/shearing test using a knife blade. The cutting strength (N) is correlated to the firmness of the sample and the work of shear (N.s) indicated the total energy (work) required to shear (Cortez-Vega *et al.*, 2015).

Statistical analysis

The assumptions of normality and homogeneity at 5% of the residues were verified by the Shapiro-Wilk and Levene tests and analyzed by ANOVA, and means were compared by the Tukey test at 5% using the statistical software Statistica 7.0.

Table 1. Proximate composition and pH of mechanically separated chicken meat, surimi-like material and recovered protein

Treatment	Proximal composition					pH
	Moisture (%)	Crude protein* (%)	Crude fat* (%)	Ash* (%)	Myofibrillar protein (mg/g)	
UW MSCM	68.10 ± 0.50	40.31 ± 2.31	57.81 ± 1.83	1.88 ± 0.08	nd	6.62 ± 0.02
SLM W3	82.16 ± 0.08^{a}	83.64 ± 0.23^{d}	11.98 ± 0.10^{a}	4.13 ± 0.11^{b}	5.04 ± 0.01^{a}	7.26 ± 0.01^{a}
SLM+10%RP	79.97 ± 0.01^{b}	89.03 ± 0.37^{c}	4.46 ± 0.09^{b}	5.82 ± 0.02^{a}	5.01 ± 0.01^{a}	7.18 ± 0.01^{b}
SLM+20%RP	79.35 ± 0.02^{b}	92.38 ± 0.40^{b}	3.35 ± 0.51^{c}	4.26 ± 0.40^{b}	4.85 ± 0.02^{ab}	6.90 ± 0.01^{c}
SLM+30%RP	79.15 ± 0.01^{b}	93.45 ± 0.28^{a}	2.78 ± 0.28^{d}	3.76 ± 0.15^{c}	4.73 ± 0.01^{b}	6.34 ± 0.02^{d}

*Dry basis: UW MSCM, unwashed mechanically separated chicken meat; SLM, surimi-like material; SLM W3, SLM obtained after three washing cycles; RP, Recovered protein; nd, not determined.

Average and standard deviation calculated from triplicate analyses of five samples.

Different letters in the same column indicate significant differences at $p<0.05$.

Results and Discussion

Proximate composition, pH and myofibrillar protein

Proximate composition and pH of mechanically separated chicken meat (MSCM), mechanically separated chicken meat surimi like-material (MSCM-SLM), mechanically separated chicken meat recovered protein (MSCM-RP) and mechanically separated chicken meat surimi like-material added with recovered protein (MSCM-RP-SLM) at different levels are given in Table 1.

The composition of the unwashed MSCM is in accordance with results previously reported (Cortez-Vega *et al.*, 2015; De Oliveira *et al.*, 2014; Perlo *et al.*, 2006; Rivera *et al.*, 2000; Smyth and O'Neill, 1997). As the MSCM composition varies in function of the raw material utilized (Cortez-Vega *et al.*, 2015), a variable number of washings may be necessary to obtain surimi-like material with reduced fat contents. Here, the fat content of the MSCM-SLM was significantly reduced to 11.98% and protein increased to 83.64% (dry basis) ($p<0.05$) after three washing cycles (Table 1).

It is important to highlight that the protein recovery was very high for a conventional method for SLM obtaining. Rawdkuen *et al.* (2009), e.g., recovered protein from minced tilapia muscle using three methods. The highest protein recovery was obtained in the acid-aided process (85.4%), followed by the alkaline-aided process (71.5%) and conventional method (67.9%).

The MSCM-SLM was then added of sarcoplasmic soluble proteins left in the supernatant after the centrifugations by the acid-aided process. Thus, the most obvious finding observed was that the increase of the RP concentration in the MSCM-SLM significantly augmented the protein content from 83.64% to 93.45% and reduced fat content from 11.98% to 2.78%. This was expected since the majority of the water-soluble components, including sarcoplasmic proteins and inorganic salts, were removed during the leaching for MSCM-SLM obtaining. Despite the concentration of the myofibrillar proteins is increased during this process, the addition of recovered proteins from other nature has decreased the final myofibrillar protein content of the MSCM-SLM, as much as RP was added. In agreement, Rawdkuen *et al.* (2009) found decreases in myoglobin and lipid contents during protein recovery.

Ash and moisture contents were also reduced, but the difference was in most of the cases not statistically significant (Table 1). Washing increased the pH ($p>0.05$) of the MSCM, but the addition of recovered protein on the MSCM-SLM reduced it ($p>0.05$) (Table 1).

Gel properties

Texture is shown in Table 2. SLM prepared from MDCM has the texture parameters increased with the washings (Nowsad *et al.*, 2000). However, the addition of recovered to the SLM-MDCM showed a decrease in texture as the RP increased from 10 to 30% (Table 2).

Previous studies report breaking forces of 946 g (Cortez-Vega *et al.*, 2012) and 1003.4 g (Cortez-Vega *et al.*, 2015) and gel strengths of 838.2 g.cm (Cortez-Vega *et al.*, 2012) and 646.85 g.cm (Cortez-Vega *et al.*, 2015) for SLM-MSCM obtained after three washing cycles. The addition of recovered protein was responsible for a drastic decrease in both the parameters, reaching 252.36 g and 185.23 g.cm for breaking force and gel strength, respectively, when 30% of RP was included in the MSCM-SLM (Table 2). The same behavior was observed for the cutting strength. Cortez-Vega *et al.* (2015) reported a cutting strength of 9 N for MSCM-SLM obtained after three washing cycles while here the MSCM-SLM (from three washing cycles) added with 30% RP presented a cutting strength of 6.97 N.

It was reported that gels prepared from conventional surimi presents greater breaking force than that from acid-aided processes (Rawdkuen *et al.*, 2009). The reduction in the texture can be associated with the settling effect on the gel structure (Nowsad *et al.*, 2000) that decreased with

Table 2. Textural results of surimi-like material and recovered protein

Treatment	Knife blade		Spherical probe		
	Cutting strength (N)	Work of shear (N.s)	Breaking force (g)	Distance to rupture (mm)	Gel strength (g.cm)
SLM+10%RP	7.95 ± 0.18^{b}	19.78 ± 0.17^{b}	395.29 ± 8.24^{b}	7.04 ± 0.08^{a}	278.28 ± 7.13^{b}
SLM+20%RP	7.66 ± 0.40^{b}	18.60 ± 0.12^{b}	299.81 ± 7.78^{c}	7.34 ± 0.07^{b}	220.06 ± 4.01^{c}
SLM+30%RP	6.97 ± 0.40^{c}	17.56 ± 0.12^{c}	252.36 ± 7.78^{d}	7.34 ± 0.07^{b}	185.23 ± 4.01^{d}

SLM, surimi like material; RP, Recovered protein. Gel strength = breaking force x distance to rupture.
*Average and standard deviation calculated from triplicate analyses of five samples.
Different letters in the same column indicate significant differences ($p<0.05$) between treatments.

the addition of recovered protein, once that the gel-forming ability of surimi varies with the function of the myofibrillar proteins (Rawdkuen *et al.*, 2009). This is underlined by the pH decrease observed with the RP addition (Table 1). The obtained results suggest that the protein was extremely denatured due to the acidic pH-shift process (Rawdkuen *et al.*, 2009) and that the neutralization of acidified protein did not recover the level of gel strength found before acidification (Shikha *et al.*, 2006). Moreover, the sarcoplasmic proteins may have had adversely effected the strength of myofibril protein gels due bounding to the myofibrils during the heat treatment (Rawdkuen *et al.*, 2009).

Ramirez *et al.* (2007) related that the recovered proteins from surimi wash water retained the gel-forming ability of surimi, but failed to gel unless beef plasma protein was added, which was obtained with the addition of potato starch, transglutaminase, and phosphate to the recovered proteins.

The reduced characteristics of RP-MSCM-SLM gels are due the presence of tropomyosin, troponin and myosin light chain, which were removed with washings, but re-added in the formulations. These compounds interfere in protein-protein interactions involved in gel formation. However, it was also found in the literature that surimi with a substitution 10% of recovered proteins did not have diminished the texture properties (Lin *et al.*, 1995). Other authors observed the decrease in the mechanical properties only if high contents of recovered protein were added to the surimi (Velazquez *et al.*, 2007).

Conclusion

Several successful applications have been reported for fish protein recovered from the wastewater derived from the surimi obtaining processing. However the recovered protein from the surimi-like material obtaining processing from mechanically separated chicken meat was responsible to reduce the texture properties of the surimi-like material when added back even at the low percentage of 10%. On the other hand, the final protein content of the surimi-like material added of recovered protein has significantly increased and the crude fat significantly reduced, which turns the obtained intermediary product an option to applications in processed meat products where low gel strength is as suitable as high gel strength products.

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