

Comparison of physicochemical and functional properties of surimi and protein isolate obtained from mechanically deboned meat of chicken

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Abstract

The aim of this study was to evaluate the physicochemical and functional properties of the protein recovered from mechanically deboned chicken meat (MDM) obtained by surimi and pH shift processes. The final products were characterized for proximate chemical composition with the protein isolate show in low value of lipids and a higher value of proteins than the surimi. Among these proteins, fractions of myofibrillar proteins actin and myosin were identified by electrophoresis in both products. With these results, it was concluded that it is possible to obtain a protein isolate and surimi with desirable functional characteristics, with high protein value and less lipids and a chemical process applicable in the food industry.

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Introduction

Poultry meat is a very popular food commodity around the world, and its consumption has increased over the last decades in many countries. Some of the reasons for the popularity are the relatively low cost of production, low fat content and the high nutritional value of poultry meat. Considering the fact that poultry belongs to perishable foods, the main concern of industries is the shelf-life extension of the poultry products (Chouliara *et al.*, 2007).

Mechanical deboning is a procedure that leverages much of the meat remaining on bones after the removal of the meat by skilled meat cutters. Meat can be recovered from the neck, frame, and back bones of poultry; and bony fish trimmings after filleting, thus providing a new raw material for processed meat products: mechanically deboned meat (Sousa *et al.*, 2003; Luiz *et al.*, 2004).

Protein concentrates and isolates have been produced on a large scale to serve as functional ingredients in a wide and ever increasing range of application in foods. When it replaces conventional proteins, the concentrates and isolates developed should maintain or improve the quality and acceptability of the products that were incorporated (Hua *et al.*, 2005).

Chicken protein isolate is a product obtained by chemical solubilization of the protein from

chicken byproducts, recovering only myofibrillar and sarcoplasmic proteins (Nolsoe and Undeland, 2009). The knowledge of specific functional properties of protein isolates favors their adequate implementation, contributing to better technology use. A good solubility of proteins is necessary for many applications, especially for emulsions, foams and gels (Thiansilakul *et al.*, 2007). The oil holding capacity is of great importance in the formulation of food, being able to influence the order of addition of dry ingredients into the mixture, besides being used to determine the mixing times using a uniform distribution of oil or fat in the dry mixtures (Chaud and Sgarbieri, 2006). The aim of this study was to evaluate and compare the physicochemical and functional properties of surimi and protein isolate from mechanically deboned chicken meat, since they can be used as dietary supplements.

Material and Methods

Recovery of protein

Frozen mechanically deboned chicken meat (MDM) was supplied from a Brazilian regional poultry industry. It was transported under refrigerated conditions to the laboratory and kept at -18°C before use. The surimi was obtained from washing the MDM in three cycles using a washing solution: meat ratio of 4:1 (v/w), at 7°C, for 10 min. In each washing

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cycle, the stirring was kept constant at 220 rpm using a mechanical agitator (Marconi model MA-259, Piracicaba, Brazil). A 0.5% NaHCO₃ solution was utilized for the first and second cycles and 0.3% NaCl solution was used for the last one. After each washing cycle, the samples were centrifuged at 7°C (centrifuge Sigma model 6-15, Osterode, Germany). The first and second centrifugations were carried out at 3000 x g for 15 min, while the third one was at 7000 x g for 25 min. The supernatant containing fat and water-soluble proteins was discarded. The final slurry was sieved through a 1-mm mesh metal screen to remove connective tissues (Cortez-Vega *et al.*, 2013).

For the process of protein isolation, the MDM was homogenized with distilled water at 4°C, at a ratio of 1:9 (CMS: water) for 60 sec on agitation. Solubilization of protein was performed by adjusting the pH of the slurry to 11.0 with NaOH 1N for 20 minutes under 4°C. After solubilization, the suspension was centrifuged at 7500 x g at 7°C for 20 min with agitation. Three phases were obtained after centrifugation: the upper phase of lipids, soluble proteins in the intermediate phase and a lower phase of insoluble proteins. The intermediate phase was collected while the others were discarded. Soluble proteins were precipitated at the isoelectric point at pH 5.5 with the addition of HCl 1N for a period of 20 min on agitation. The proteins precipitated were separated by centrifugation at 7500 x g at 20°C for 7 min. Two layers were obtained: the top phase consisting of the residual liquid was discarded while the lower phase of the protein isolate was subjected to drying where the samples were maintained in ultra-freezer (Indrel, IULT 90-D, Brazil) at -70°C for 24 hours, and then lyophilized (Liotop, L108, Brazil) for 48 hours, then packaged in glass containers at room temperature.

Proximate chemical composition

The moisture content, proteins, lipids and ash were determined in triplicate, according to the method described by AOAC (2000). The moisture content was calculated according to the gravimetric method in an oven at 105°C (no 935.29); the total nitrogen content by the Kjeldahl method (no 920.87) and the crude protein content obtained by multiplying by a factor of 6.25; lipid content was obtained by the Soxhlet method (no 920.85) and the ash by the gravimetric method (no 923.03) in an oven at 500-600°C.

Functional properties

Protein solubility

Protein solubility was determined according to the methods of Chalamaiah *et al* (2010), and Tadpitchayangkoon *et al* (2010) adapted to laboratory conditions, varying the pH (3, 5, 7, 9 and 11). In general, 0.5 g of the sample was weighed into a 50 mL beaker and 2 mL of 0.1 M NaCl and 48 mL of distilled water were added. The pH was adjusted with HCl 1 N and NaOH 1 N. The dispersion was stirred for 30 minutes on a magnetic stirrer (QUIMIS, model 261-2), then centrifuged for 20 minutes at 8,667 x g in centrifuge tubes (Biosystems, Model: MPW-350R). The soluble protein content in the supernatant was determined by the Folin-Ciocalteu method according to Lowry *et al* (1951). The solubility of protein was calculated according to the equation below. For the calculation of protein in the supernatant albumin the standard curve was used.

$$\%S = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100 \quad (1)$$

Determination of water holding capacity (WHC)

WHC was determined according to the method by Regenstein *et al.* (1984) adapted to the laboratory conditions. Protein dispersion was prepared at 1% with pH variation (3, 5, 7, 9 and 11), at each pH value was determination of WHC in duplicate. Also 2 mL of NaCl 0.1 M was added to the dispersion to obtain a smooth paste, and the corresponding buffer solution was added according to the corresponding pH up to the volume of 40 mL, the dispersion was kept under stirring for 15 minutes and centrifuged at 8,667 x g for 20 minutes. Soluble proteins in the supernatant were quantified by the method of Bradford (1976), and deducted from the total protein of the original sample. The WHC was determined as shown below.

$$\text{WHC} = \frac{\text{Amount of water retained (mL)}}{\text{Original mass of protein (g)}} \quad (2)$$

Determination of the oil holding capacity (OHC)

OHC was determined according to the method described by Fonkwe and Singh (1996) where 0.5 g of protein was weighed and mixed with 10 mL of soybean oil in centrifuge tubes and shaken for 10 min in a tube agitator at speed 4 (PROENIX AP 56). Later, the mixture was centrifuged at 8,667 x g for 20 minutes, and the difference between the added oil and the unretained oil was considered as the amount of oil retained by the isolates. The determination of OHC was in duplicate. The result was obtained using the equation below.

$$\text{OHC} = \frac{\text{Oil retained (mL)}}{\text{Protein mass (g)}} \quad (3)$$

In vitro digestibility

The determination of in vitro protein digestibility was performed by enzymatic digestion with pepsin (specific activity of 107 µg tyrosine/min/mg protein) in 0.1N HCl and pancreatin (specific activity of 24 µg tyrosine/min/mg protein) in a phosphate buffer pH 8.0, and the determination of released amino acid was accomplished by the method of Lowry *et al.* (1951). Concentrations were calculated based on standard tyrosine curve, whose concentration ranged between 3 and 11 µg.mL⁻¹ (Feddern *et al.*, 2008).

Electrophoresis of recovered protein

SDS-PAGE (sodium dodecyl sulfate-PolyAcrylamide Gel Electrophoresis) was performed according to the method of Laemmli (1970). The characterization of the recovered protein fractions was performed by polyacrylamide gel electrophoresis in presence of sodium dodecyl sulfate. The electrophoretic analysis was performed on a vertical electrophoresis unit (GSR/300STS). SDS-PAGE separation was performed in a continuous buffer system consisting of 1.5 M Tris buffer and 10% SDS (w/v). The gel was prepared with a 12% separating gel and a 4% gel concentration. The samples were dissolved in 1.5 mL distilled water to form a solution containing 0.2% protein. Samples were thermally denatured at 95°C for 4 min in a solution of β-mercaptoethanol, 0.5 M Tris (pH 6.8), glycerol, 10% SDS (w/v) and 0.1% bromophenol blue (w/v). To identify the proteins present in the samples, Bio-Rad marker ladders were used. Bands were revealed with Coomassie Brilliant Blue R-250 (Vetec Química Fina LTDA, Rio de Janeiro, Brazil). The determination of protein fractions was performed by molecular weight. A mixture of standard proteins (Bench Mark™ Protein Ladder, California, USA), ranging in molecular mass from 10 to 220 kDa, was used.

Statistical analysis

Statistical analysis was performed by comparing the means through analysis of variance (ANOVA) and Tukey's test at 5% significance using Statistica 7.0 (Statsoft™, Inc., Tulsa, USA).

Results and Discussion

Proximate chemical composition

Considering that the raw material (MDM) is presented in its composition of 39.5% protein and

Table 1. Proximate composition of protein isolate and surimi of MDM from chicken (in dry basis)

Component	MDM*	Protein isolate**	Surimi**
Protein	39.5 ± 0.55	89.0 ± 2.07	78.0 ± 0.4
Lipids	54.9 ± 0.66	5.3 ± 0.59	16.5 ± 0.09
Ash	3.4 ± 0.10	1.6 ± 0.02	3.5 ± 0.02

*MDM: mechanically deboned chicken meat **Mean result was by triplicate with standard deviation in dry basis

54.9% lipids, when compared with surimi and protein isolate, one can verify that the protein is concentrated and that the percentage of fat is reduced, which leads to a product with higher added value for application in food.

Table 1 shows the proximate chemical composition of protein isolate compared to surimi from mechanically deboned chicken meat (MDM). A high percentage of protein (89%) in the protein isolate and lower percentage of lipids (5.3%) was observed in comparison with surimi, which has 78% protein and 16.5% fat. The surimi had a lower protein content than protein isolate because it had a higher lipid content and this difference may be due to the protein isolate contains myofibrillar and sarcoplasmic proteins while surimi only has myofibrillar protein. The number of washing cycles and the compressive force exerted has a major effect in reducing the protein content (Mira and Marquez, 2005), the surimi had three cycles while the protein isolate had two. The surimi had higher ash content than protein isolate, 3.8% vs 1.8% (dry weight basis). Therefore it is concluded that the protein isolate is a more efficient method for recovering MDM protein, which has seen increased protein percentage.

In comparison with the literature it is observed similarities, Moraes *et al.* (2011) found approximate values of 82% protein and 4% lipids for the protein isolate of mechanically deboned meat from chicken, and Rossi *et al.* (2009) found 61.9% protein and 1.8% lipids. For surimi from mechanically deboned chicken meat, Mira and Marquez (2005) found 87.2% protein; Jin *et al.* (2008) found 20% protein and 1% lipids. Therefore, it can be observed that protein values superior to those of the cited authors were found in the present study. This generates a recovery protein content of the final product, since the protein is the most abundant compound in the raw material.

Solubility

The solubility of proteins is the result, among other factors, of the polar interaction with the solvent, ionic interactions with the salt present in the solution

and the electrostatic forces of repulsion (Neves *et al.*, 2001). The effect of pH variation on solubility of surimi and protein isolate is presented in Figure 1 and it can be seen that acid pH values result in increased solubility and the same effect occurs on surimi.

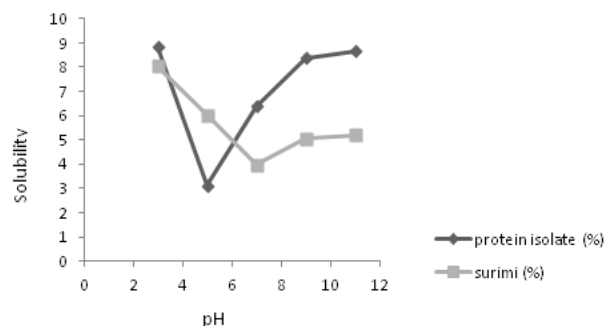


Figure 1. Protein solubility curve of acid and alkaline protein isolate and surimi from MDM of chicken.

In the present study, both products of solubilization have low solubility at pH near the isoelectric point of the protein. For the isolate the low solubility (3%) was at pH 5 and the maximum solubility (8.6%) was reached at pH 11.0 for the isolate. Different behavior than surimi, due methods are extracted in different solvents can influence the solubility. The low solubility of the protein is probably caused by denaturation of the muscle protein induced by pH change process (Rawdkuen *et al.*, 2009).

Other authors also showed lower solubility of isolates in the range of the isoelectric point of proteins. Costa *et al* (2007) observed that the isolate has solubility of 2.8% at pH 4.0 for pink shrimp (*Farfantepenaeus paulensis*), Freitas *et al* (2011) found lower solubility (2.96%) at pH 5.0 for Argentine anchovy (*Engraulis anchoita*). It is important to determine these solubility values, since the proper functionality of the isolate and surimi depend on higher protein solubility for gelation and emulsification of these products during their applicability.

Water holding capacity (WHC) and oil holding capacity (OHC)

The results for the water holding capacity of the protein isolate and surimi studied at different acid and alkaline pH are presented in Figure 2. It can be observed that the retention of water at pH near the isoelectric point was lower. The capacity was 3.66 g H₂O/g protein for protein isolate and 4.03 g H₂O/g protein at a pH of 5.0. Near the isoelectric point of the protein, the ability of the protein to bind with the water is lower due to intermolecular interactions and formation of a large protein cluster. At pH distant from the isoelectric point, the protein has charges

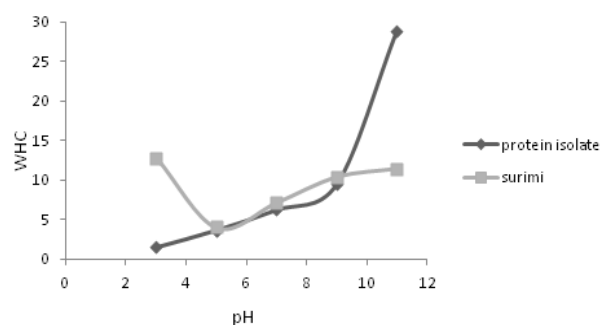


Figure 2. Water holding capacity (WHC) of the protein isolate and chicken surimi.

of the same sign, and causes repulsion between molecules, leaving more space to be filled by water molecules, thus increasing the capacity of retaining water. The WHC increased with increasing pH, from the pH of the isoelectric point.

The WHC was obtained in the greater pH extremes, because at pH below 5.0 and above 7.0, the water molecules combine with the polar groups of proteins and the WHC tends to increase. The water holding capacity of the meat is a key property in products derived from meat due to its implication in the final product quality and production yields. The low water retention capacity implies the loss of other important factors for the quality of the processed product.

Oil holding capacity values of the protein isolate was 1,96 g oil/g of protein and for chicken surimi was 4,01 g oil/g of protein. Of its constituents, fat deserves attention due to variation in the content, which is directly reflected in the stability of emulsion as well as in oxidative processes, thus the importance of knowing the oil holding capacity (Terra, 2000). The higher oil holding capacity displayed by surimi protein is related to the amount of lipids present in the initial sample. In this case, while the protein isolate had approximately 5% lipids, the surimi had 26%. A low fat retention capacity is important to avoid the formation of pockets of fat, which besides not being pleasant for the consumer, can bring the of degradation products.

Electrophoresis

The results of electrophoresis analysis are presented in Figure 3 where one can see that the protein isolate and surimi have a similar electrophoretic profile, with the difference being that no bands of myosin of low molecular weight were observed in surimi, probably because that low molecular weight proteins were washed out during the surimi preparation.

The results of the analysis by densitometry of

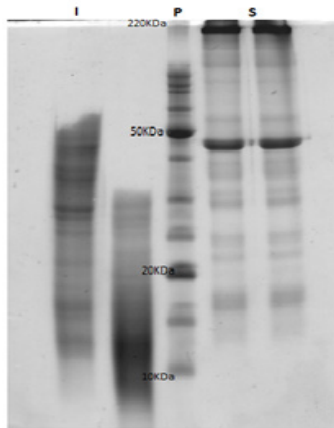


Figure 3. Electropherogram of protein samples of surimi and protein isolate of chicken. (I: protein isolate; P: standard; S:surimi).

gels and by electrophoresis allow the identification of the various fractions of proteins in the samples. These are characterized by chains of myosin at high molecular weight (50 kDa to 220 kDa), actin (20 kDa) and myosin light chain (10 kDa). Among these protein fractions, myofibrillar proteins actin and myosin were identified in both samples. In the protein isolate, high molecular weight proteins myosin and actin and a band of protein with weight less than 20kDa were identified. These smaller fraction masses present in the recovered protein are possibly due to the process of alkaline solubilization, in which parts of the proteins are hydrolyzed to proteins of lower molecular weight, and therefore bands with lower molecular weight appear. The surimi also identifies proteins of 50 to 220 kDa, nevertheless low molecular weight proteins are not found.

The number and intensity of the bands corresponding to fragments of myosin of high molecular weight showed the preservation of myofibrillar proteins (Quintero and Sobral, 2000). The protein isolate obtained has a higher percentage of proteins (80%), this result from the electropherogram therefore confirms the quality of these protein bands.

In vitro digestibility

The values obtained for the digestibility of the samples studied were around 93% (protein isolate) and 90% (surimi). The meat products present digestibility values of approximately 90% (Berno *et al.*, 2007). Both feature myofibrillar proteins (actin and myosin), and only the protein isolate presents a larger amount of myosin of high and low molecular weight. Pires *et al.* (2006) reported that mechanically deboned meat had a digestibility of approximately 92.57%. Therefore, this protein concentrates are within the standard digestibility for animal proteins.

Conclusions

In study the protein content of the protein isolate is higher than that of surimi. Both the surimi as well as the isolate from the solubilizing process has low solubility at pH near the isoelectric point of the protein and the highest solubility was reached at pH 11.0. It can be observed that the water holding capacity, at pH near the isoelectric point was smaller, and values of oil holding capacity were low in the same pH for both products. The isolate and surimi have a similar electrophoretic profile, with the difference being that no bands of myosin of low molecular weight were observed in surimi. Among these proteins, fractions of myofibrillar proteins actin and myosin were identified in both samples.

Both protein products are technologies that increase the commercial value and utilization of chicken meat, with low manufacturing. The results show that the products of chicken meat that are usually used for the production of animal feed or simply discarded can contribute to decrease environmental pollution and may be used to produce products with a greater added value.

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