Characterization of hydrolysates of collagen from mechanically separated chicken meat residue

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Abstract

Obtaining collagen and hydrolysates from mechanically separated chicken meat residue is an excellent way to add value to this waste. The aims of this study were to obtain collagen hydrolysates from chicken MSM residue using Alcalase® and Flavourzyme® enzymes, as well characterizing the functional, structural and thermal stability properties. The highest degree of hydrolysis was obtained using Alcalase®, 36.11%, while using Flavourzyme® resulted in 12.02%. The DSC analysis of the collagen indicated a denaturation temperature of 46.47 °C. The FTIR spectra of the crude collagen showed absorption peaks that were characteristic of amide bands A, B, I, II and III. In the spectra of the hydrolysed the area of the amide bands was reduced, and some peaks appeared between 800 and 1,200 cm^{-1}. The disappearance of high molecular weight bands in the SDS-PAGE analysis also confirmed the hydrolysis of collagen. After the hydrolysis, the collagen presented reduced viscosity, the capacity to form foam, and foam stability. The emulsifying activity index was high in the hydrolysates in relation to the crude collagen. Thus, the use of Alcalase® and Flavourzyme® to obtain hydrolysates from collagen derived from chicken MSM residue was shown to be viable, and potentially useful for industrial applications.

Keywords: Alcalase®; electrophoresis; Flavourzyme®; FTIR; functional properties.

Practical Application: The hydrolysates presented good functionality and potential for industrial application.

1 Introduction

Brazil stands out in the international market in terms of production and export of poultry products, is the second largest producer of chicken in the world, with 13.056 million tons in 2017, of which, 32.3% was destined for exportation (ABPA, 2017; Campos et al., 2018). About 20% of chicken production in Brazil is transformed into mechanically separated meat (MSM) (Negrão et al., 2005) and the residue generated in the extraction process is in the order of 15% to 45%. Much of this waste is used to produce feed because of its low value. Consequently, obtaining collagen and hydrolysates from this raw material would add considerable value to this waste. Some studies have shown promisingly the extraction of collagen and hydrolyzed from chicken slaughter residues, such as feet (Araújo et al., 2018; Chakka et al., 2017), skin (Aksun Tümerkan et al., 2019; Oechsle et al., 2016), bones (Oechsle et al., 2016), among others, however chicken CMS residue has not yet been explored.

Enzymatic hydrolysis, when carried out under mild and controlled conditions, guarantees the maintenance of the nutritional quality of hydrolysates and a defined peptidic profile. Hydrolysis is capable of modifying, and even improving, the functional characteristics of proteins due to the formation of peptides and free amino acids (Bhaskar, et al. 2007; Halim et al., 2016). In addition, it is known that some of these peptides and amino acids have antioxidant, antimicrobial and antihypertensive capacity (Farvin et al., 2016; Fu et al., 2016; Halim et al., 2016; Cheung & Li-Chan, 2017).

The enzymes Alcalase® and Flavourzyme® have been widely used to obtain protein hydrolysates. Alcalase® is produced by the submerged fermentation of the microorganism *Bacillus licheniformis*; it is used at high temperatures and in moderate alkalinity (Jung et al., 2006). Flavourzyme® is a complex fungal protease produced by the submerged fermentation of a selected lineage of *Aspergillus oryzae* which has not been genetically modified; it is used in neutral or slightly acidic conditions (Šližyte et al., 2005). In a study by Vidal et al. (2018) enzymatic hydrolysis, with Flavourzyme®, provided structural rupture and improved the functionality to the different bovine collagen hydrolysates.

The aims of this study were to obtain collagen hydrolysates from chicken MSM residue using Alcalase® and Flavourzyme® enzymes, as well characterizing the functional, structural and thermal stability properties of that collagen.

2 Materials and methods

The frozen raw material (residue from the MSM extraction process) was donated by the Aurora Central Cooperative (Aurora Alimentos) in the city of Erechim, RS, Brazil. The MSM was produced using High Tech, model HT-6.0 SS, equipment with a capacity of 6,000 kg/h, power of 50 CV and weight of 1,680 kg. The MSM residue was kept in a freezer (Metalfrio, VF50F) at -22 °C until use.

The enzymes Flavourzyme® 1000 L and Alcalase® 2.4 L (Novozymes®, Araucária PR, Brazil) were supplied by Tovani Benzaquen Ingredientes (São Paulo, SP, Brazil) and the other reagents that were used were of analytical grade (PA).

2.1 Obtaining the collagen

The chicken MSM residue was pre-treated following the methodology of Li et al. (2013a) and Duan et al. (2009), with adaptations. First, using 0.1 M sodium hydroxide solution (NaOH) for 48 hours, with a sample/alkaline solution ratio of 1:20 (w/v), and the solution changed every 12 hours. Then, 0.5M EDTA-2Na (Ethylenediaminetetraacetic acid disodium) solution (pH 7.5) was used for five days with a 1:10 (w/ v) sample/solution ratio. Finally, 10% butyl alcohol was used for 48 hours with a solid/ solvent ratio of 1:10 (w/v) and the solvent changed every 12 hours.

After this pre-treatment the collagen was extracted with pepsin following the methodology proposed by Kittiphattanabawon et al. (2010), with modifications. The MSM residue was added to 0.5 M acetic acid containing the enzyme pepsin (extracted from powdered, porcine gastric mucosa, $\geq$ 400 units/mg protein, Sigma, St. Louis, USA) at a 1:2.50 enzyme/protein ratio, with a solid/solvent ratio of 1:15 (w/v). The mixture was stirred continuously for three days, followed by filtration through two layers of gauze. All the pre-treatment and extraction procedures were performed using Shaker Incubator (Solab SL-223, Piracicaba, Brazil) equipment at 150 rpm and 4 °C.

The collagen was subsequently precipitated in the presence of 0.05 M tris (hydroxymethyl) aminomethane, at pH 7.5, by adding NaCl to a final concentration of 2.6 M. The precipitated collagen was centrifuged at 12,000 rpm for 30 minutes in a centrifuge (Hitachi, CR22GIII) at 4 °C, and then dialysis using a cellulose membrane was performed (typical molecular weight cut-off 14,000 KDa, Sigma, St. Louis, USA) for two days with 0.1 M acetic acid, and for two days with distilled water, both at 4 °C under agitation and with the solution changed every 12 hours. Finally, the collagen was frozen in a freezer (Metalfrio, VF50F) at -22 °C and lyophilized in a lyophilizer (Terroni, LS 3000, São Carlos, Brazil).

2.2 Obtaining the collagen hydrolysates

To perform the enzymatic hydrolysis the methodology of Schmidt & Salas-Mellado (2009) was followed, with some modifications. The hydrolysis was performed in closed glass bottles, which were immersed in an ultrathermostatic bath (Model SL152, power of 2,000 W, SOLAB, Piracicaba, SP, Brazil) for temperature control.

For the hydrolysis using the enzyme Flavourzyme®, the collagen was homogenized in a phosphate buffer solution of pH 7.0 containing the enzyme and placed in the bath at 50 °C for two hours; the inactivation of the enzyme was performed for 15 minutes at 75 °C . For the hydrolysis using Alcalase®, phosphate buffer of pH 7.5 and a temperature of 55 °C for two hours was used; the inactivation was performed for 15 minutes at 85 °C. For both the hydrolyses the amount of added collagen was 4% (w/v) relative to the volume of the buffer and 8% (v/m) of enzyme to the mass of collagen. At the end of the hydrolysis the Alcalase® and Flavourzyme® hydrolysates were frozen in freezer (Metalfrio, VF50F) at -22 °C and lyophilized in a lyophilizer (Terroni, LS 3000, São Carlos, Brazil).

2.3 Characterization of collagen and hydrolysates

Degree of hydrolysis

To determine the degree of hydrolysis of the hydrolysates obtained using Alcalase® and Flavourzyme®, the method of Lowry et al. (1951) was used. The degree of hydrolysis was calculated based on the amount of protein in the crude collagen from the chicken MSM residue; bovine albumin was used as standard (Sigma-Aldrich Brasil Ltda, São Paulo, SP, Brazil).

Fourier-transform Infrared Spectroscopy (FTIR)

In order to evaluate the structural characteristics, Fourier-transform infrared spectroscopy (FTIR) analysis was performed using Shimadzu IR Prestige-21 equipment (Shimadzu Corporation, Japan). The attenuated total reflectance (ATR) technique was used in the range of 400 to 4,000 cm^{-1}; 16 scans per spectrum were performed at 2 cm^{-1} resolution.

Differential Scanning Calorimetry (DSC)

The DSC curves were obtained using DSC-Q200 equipment (TA-Instruments, USA), which was pre-calibrated with high purity indium (99.99%, mp = 156.6 °C, $\Delta H = 28.56$ J g^{-1}). The parameters used in the denaturation process were as follows: air flow of 50 mL min^{-1}, heating rate of 10 °C min^{-1} and heating range of -20 to 100 °C. For the analysis, about 3.0 mg of each sample was mixed with deionized water at a ratio of 6:1 (water:sample, m/m) and the mixture was held at 7 °C for 120 minutes to balance the moisture content and to hydrate the sample. The analysis was performed in sealed aluminum crucibles.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis (SDS-PAGE)

The gel electrophoresis analysis was based on the method proposed by Laemmli (1970), with modifications. Resolution gel (15%) and stacking gel were used (5%); 20 µL of the samples to be analyzed and 15 µL of the molar mass standard were added and subjected to electrophoresis at 150 V and 30 mA for approximately two hours in a vertical electrophoresis cell. The gel was subsequently stained with 0.1% Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories Inc., Richmond, VA, USA) for approximately twelve hours. The gel was then discolored by

heating in distilled water in microwaves until perfect visualization of the bands was possible.

Viscosity

The viscosity was determined following the methodology described by Kittiphattanabawon et al. (2010), with modifications. First, 500 mL of 0.03% (w/v) solutions of collagen or hydrolysate dissolved in 0.1 M acetic acid were prepared. The viscosity of the solutions was measured using a Brookfield viscometer (RV-DV-II+ Pro) with a No 1 rod and speed of 100 rpm, at temperatures of 10, 25 and 50 °C. A thermostatic bath (Haake, Fisons F3 C) was used to control the temperature at each of the designated temperatures, and the solution was kept for 30 minutes before the viscosity was determined.

Foam properties

The foam expansion capacity (FEC) and foam stability (FS) were determined based on the method of Shahidi et al. (1995). Sample solutions of 0.5% concentration were shaken in a Falcon tube (50 mL) using Turrax ultra homogenizer (Tecnal TE-102 Piracicaba, Brazil) at a speed of 10,000 rpm to incorporate air for two minutes at room temperature (20-25 °C). The FCE was calculated as the % increase in volume based on the initial volume and the volume after foaming, according to the Equation 1.

$$FEC(\%) = \frac{(V - V_0)}{V_0} \times 100 \qquad (1)$$

where, V_0 is the volume before shaking (mL) and V is the volume after shaking (mL). The determination of the foam stability was performed by resting the sample at room temperature and then reading the volume after three and 10 minute intervals; the FS was calculated by the Equation 2.

$$FS = \frac{V_t}{V_0} \times 100 \qquad (2)$$

where, V_t is the final volume of foam after each time interval (mL), and V_0 is the initial volume of the foam that formed (mL).

Emulsifying properties

The Pearce & Kinsella (1978) method was used to evaluate the emulsifying activity index (EAI) and the emulsion stability index (ESI). First, 10 mL of soybean oil and 30 mL of 0.1% sample solution were mixed in a Turrax ultra homogenizer (Tecnal TE-102 Piracicaba, Brazil) at a speed of 12,000 rpm for one min. Then, an aliquot of the emulsion was pipetted from the bottom of the vessel at 0 and 10 min after homogenization, and diluted 100 times using 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted solution was determined at 500 nm using a spectrophotometer (Servylab, UV-M51, B, São Leopoldo, RS, Brazil). The absorbances were used to calculate the EAI (Equation 3) and the ESI (Equation 4).

$$EAI\left(\frac{m^2}{g}\right) = \frac{(2 \times 2.303 \times A_0)}{(0.25 \times \text{concentration of collagen})} \qquad (3)$$

$$ESI(min) = \frac{A_0 \times 10\ min}{A_0 - A_{10}} \qquad (4)$$

where, A_0 is the absorbance determined immediately after the formation of the emulsion (0 min) and A_{10} is the absorbance determined 10 min after the formation of the emulsion.

Statistical analysis

Three replications were performed for the collagen extraction and the hydrolysis processes. All the analyses were performed in triplicate. The results were submitted to analysis of variance (ANOVA) and Tukey's test, with a 5% significance level (p <0.05) using Statistica® 8.0 (StatSoft Inc., Tulsa, OK, USA) software. After obtaining the FTIR spectra, each spectrum was treated with normalized absorbance between 0 and 1, smoothed (15 points), corrected at the baseline, and the CO_2 zone was removed using Shimadzu IRsolution 1.40 software. Then, a table with all the data was constructed using the wavelength as a column and the samples as lines. The FTIR spectra were then prepared using SAS® (Statistical Analysis System) version 9.4 software (SAS Institute Inc., Cary, NC, USA).

3 Results and discussion

3.1 Degree of hydrolysis

The collagen hydrolyzed using Alcalase® provided a higher degree of hydrolysis, 36.11% (± 0.34), compared to the collagen hydrolyzed using Flavourzyme®, 12.02% (± 0.23). This indicated that Alcalase® cleaved more peptide bonds than Flavourzyme®, resulting in peptides with lower molecular weight, which is beneficial for industrial applications.

Higher values for the degree of hydrolysis were also obtained using Alcalase® compared to Flavourzyme® in studies of the hydrolysis of protein from chicken thighs and breast (Schmidt & Salas-Mellado, 2009), tilapia (*Oreochromis niloticus*) (Foh et al., 2010) and bluewing searobin (*Prionotus punctatus*) (Santos et al., 2011).

Both Alcalase® and Flavourzyme® have very similar temperatures and pH levels; however, Alcalase® is a serine endoprotease, is able to hydrolyze most of the internal peptide bonds of protein molecules (Adler-Nissen, 1986), while Flavourzyme® contains endoprotease and exopeptidase activities (Nilsang et al., 2005), which guarantees a wide range of action.

3.2 Fourier-transform Infrared Spectroscopy (FTIR)

Figure 1 shows the infrared spectra of the collagen from the chicken MSM residue and the collagens hydrolyzed using Flavourzyme® and Alcalase®. The FTIR spectrum of the crude collagen showed characteristic absorption peaks in the regions of the amide bands A and B and I, II and III.

The amide band A was located at 3,325 cm⁻¹, which is associated with N-H stretching vibrations when the NH group of the peptide is involved in hydrogen bonds (Purna Sai & Babu, 2001). The amide band B (2,924 cm⁻¹) was related to the asymmetric stretching of the CH_2 stretching vibration and the absorption due to the CH_2 alkyl chain (Hsu et al., 2005). The amide I band, which has
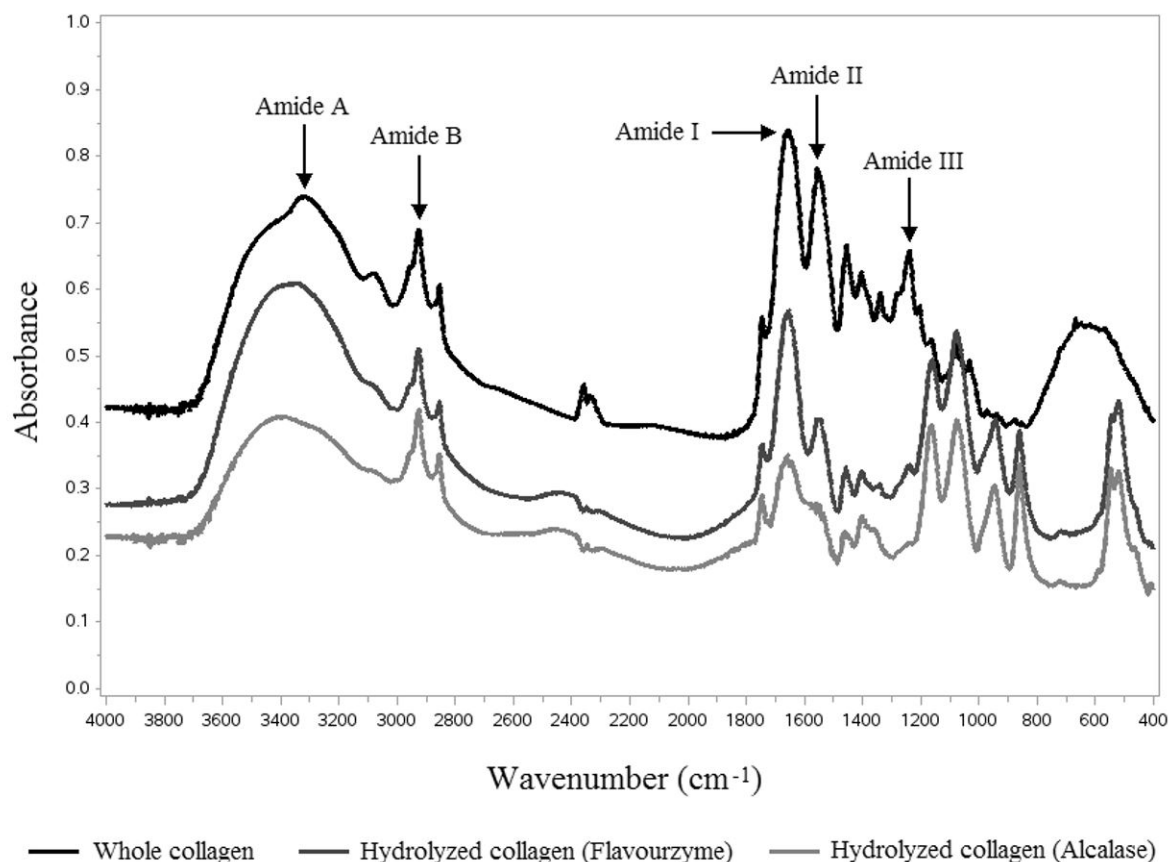
Figure 1. FTIR spectra of crude collagen from the chicken MSM residue and the hydrolysates obtained using Alcalase® and Flavourzyme®.

characteristic frequencies in the range of 1,600 to 1,700 cm^{-1}, is mainly associated with the stretching vibrations of the carbonyl groups along the polypeptide backbone (Payne & Veis, 1988). The amide bands II (1,556 cm^{-1}) and III (1,240 cm^{-1}) represented N-H flexural vibrations coupled with C-N stretch vibration (Payne & Veis, 1988; Jackson et al., 1995).

In the FTIR spectra of the collagen samples hydrolyzed using Alcalase® and Flavourzyme® the position of the amide bands remained practically unchanged, suggesting that the secondary structures were not completely destroyed in the hydrolysis process. In contrast, the area of the bands was reduced, indicating that part of the collagen was broken into polypeptides and the helical conformation of the collagen was partially destroyed (Li et al., 2013b).

The crude collagen from the chicken MSM residue showed a ratio between the amide III peak (1,240 cm^{-1}) and the peak at 1,450 cm^{-1} which was very close to 1.0, proving the presence of the triple helix structure which is characteristic of collagen (Guzzi Plepis et al., 1996). In the spectra of the hydrolysates, the peak at 1,450 cm^{-1} and the amide III peak showed a very low intensity; this was practically non-existent in the hydrolysate produced using Alcalase®, confirming that the triple helical structure of the collagen disappeared after enzymatic hydrolysis. Chi et al. (2014) obtained the same result in the hydrolysis of collagen from Spanish mackerel skin.

According to Lacerda et al. (1998) and Bet et al. (2001), the band close to 1,240 cm^{-1} is sensitive to changes in the secondary structure of the tropocollagen (triple helix), while the band at 1,450 cm^{-1} corresponds to the vibrations of the pyrrolidinic rings of proline and hydroxyproline.

In a study by Bryan et al. (2007), heating collagen from 12 to 60 °C resulted in a decrease in absorbance and a widening of the amide I peak due to the denaturation of the collagen. A similar effect was observed in the present study in the FTIR spectrum of the collagen hydrolyzed using Alcalase®.

Both the hydrolyzed collagen samples presented peaks of medium intensity at lower wavelengths, of 800 to 1,200 cm^{-1}, in their FTIR spectra, which were not observed in the spectrum of the crude collagen sample. This indicates structural changes in the collagen molecules that were brought about by the hydrolysis process; these changes are determinant of the functional properties of hydrolysates.

3.3 Differential Scanning Calorimetry (DSC)

Figure 2 shows the DSC thermograms for the collagen samples from chicken MSM residue and the collagen samples hydrolyzed using Flavourzyme® and Alcalase®. In the thermogram of the crude collagen sample (Figure 2a) an endothermic transition was observed at 46.47 °C, which corresponded to the denaturation
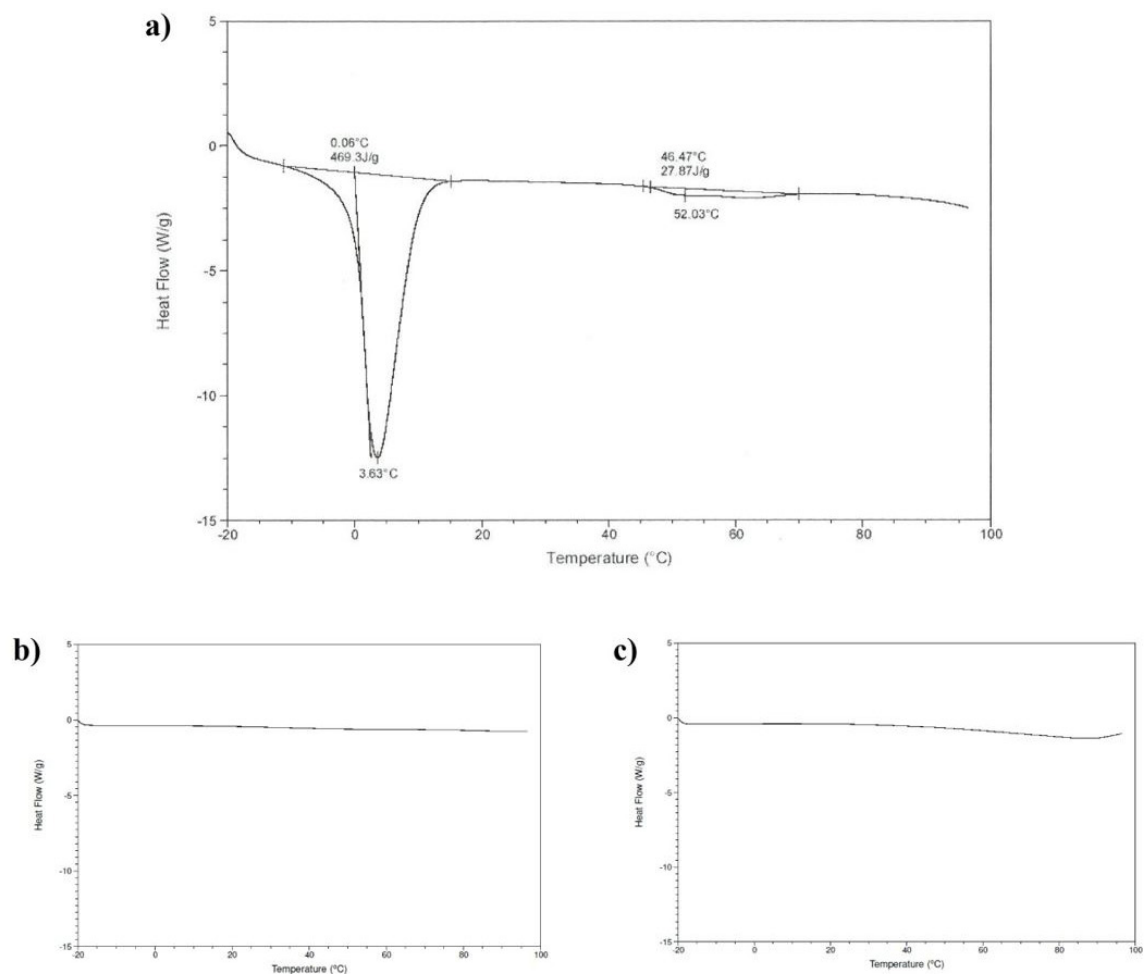
Figure 2. DSC thermogram for (a) crude collagen from the chicken MSM residue; (b) collagen hydrolyzed using Alcalase®; and (c) collagen hydrolyzed using Flavourzyme®.

of collagen; this was a similar value to that obtained for collagen from quail feet, 39.6 °C (Yousefi et al., 2017).

As expected, the thermograms of the hydrolyzed collagen samples (Figures 2b and 2c) did not show thermal transition phenomena, proving that the collagen molecules were broken down into smaller fractions during hydrolysis by the action of the enzyme and the heat.

3.4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis (SDS-PAGE)

Figure 3 shows the SDS-PAGE for the collagen from the chicken MSM residue and the collagen samples hydrolyzed using Alcalase® and Flavourzyme®. In the case of the crude collagen sample, two distinct bands (corresponding to the α_1 and α_2 chains, and close to 125 kDa) were observed, indicating that the collagen sample from the chicken MSM residue was mainly composed of type I collagen. It was also possible to verify the presence of some proteins of lower molecular weight between 100 and 30 kDa.

In the collagen samples hydrolyzed using Alcalase® and Flavourzyme® all the bands of medium-to-high molecular weight
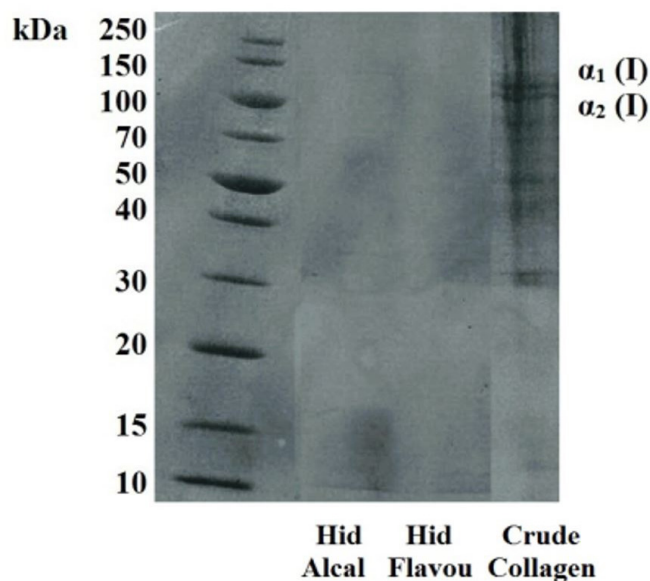

Figure 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis (SDS-PAGE) of crude collagen from the chicken MSM residue and the hydrolysates obtained using Alcalase® and Flavourzyme®.

proteins disappeared, leaving only light bands below 15 kDa, proving the hydrolysis of the collagen molecules into smaller peptides. Despite the difference in the degree of hydrolysis between the collagen samples hydrolyzed using Alcalase® and Flavourzyme®, no significant differences in molecular weight distribution were observed. In a study by Li et al. (2013b), the hydrolysis of collagen from fish cartilage using trypsin at neutral pH resulted in low molecular weight peptides which were less than 20 kDa.

According to Zhang et al. (2006), different processes lead to different molecular weight distributions. In the present study the collagen was extracted with pepsin in acid medium, which only attacked the non-triple helical domain of native collagen, while the collagen hydrolysates were obtained under more severe conditions, above the denaturation temperature. This destroyed the triple helix structure and part of the peptide bonds, resulting in lower molecular weights.

3.5 Viscosity

The viscosity results are presented in Table 1; they show that the viscosity was lower in the collagen samples hydrolyzed using Alcalase® and Flavourzyme® than in the crude collagen sample from the chicken MSM residue at all the tested temperatures (10, 25 and 50 °C). According to Zhang et al. (2017), the functional properties of hydrolysates are generally related to their molecular weight; low molecular weight hydrolysates usually have lower viscosity. Furthermore, the FTIR spectra (Figure 1) and SDS-PAGE analysis (Figure 3) indicated the denaturation of the hydrolyzed collagen, which may have led to a decrease in viscosity due to the breakage of hydrogen bonds between the polypeptide chains adjacent to the collagen molecules, leading to the formation of individual chains (α) or dimers (β).

In general terms, the solutions (0.03%) of collagen and the hydrolysates showed high values for viscosity. The results were

Table 1. Viscosity of solution of the crude collagen from the chicken MSM residue and the hydrolysates obtained using Alcalase® and Flavourzyme®, at temperatures of 10, 25 and 50 °C.

Temperature (°C)	Viscosity (mPA.s or cP)		
	Crude Collagen	Hydrolyzed Alcalase®	Hydrolyzed Flavourzyme®
10	14.43[a] ± 0.59	10.30[b] ± 0.24	10.90[b] ± 0.22
25	12.35[a] ± 0.34	9.55[c] ± 0.24	10.25[b] ± 0.13
50	8.83[a] ± 0.56	7.48[b] ± 0.42	8.67[ab] ± 0.60

Averages in the same row with the same superscript letters do not differ significantly by Tukey's test (p > 0.05). Values expressed as mean ± standard deviation.

higher than those found for gelatin from lizard scale (*Saurida spp.*), which ranged from 3.14 to 5, 80 cP for 6.67% solutions at 25 °C (Wangtueai & Noomhorm, 2009), and gelatin from tilapia skin (*Oreochromis urolepis hornorum*), which ranged from 2.56 to 6.12 cP at a temperature of 60 °C (Alfaro et al., 2014).

3.6 Foam properties

Table 2 shows the results for foam expansion capacity (FEC) and foam stability (FS). The crude collagen sample from the chicken MSM residue had a higher FEC (22.87%) than the collagen samples hydrolyzed using Alcalase® (3.75%) and Flavourzyme® (4.38%). The foam from the crude collagen sample showed better stability at the tested times.

Other studies (Li et al., 2013b; Chi et al., 2014) have reported that peptides of higher molecular weight are beneficial for the formation of a stable film around air bubbles, which is the main factor for better foaming ability.

Although they were low, the FEC values for the hydrolysates of the collagen sample from the chicken MSM residue were close to those obtained for hydrolysates of gelatin from squid and sole, which were between 6 and 7% at the same tested concentration (0.5%) (Giménez et al., 2009).

3.7 Emulsifying properties

The results of the emulsifying properties (Table 2) showed that the hydrolysates of the collagen sample from the chicken MSM residue obtained using Alcalase® and Flavourzyme® had a high emulsifying activity index (EAI), 130.53 and 126.89 m²/g respectively, and the potential to be used as emulsifying agents. Chi et al. (2016) also found high EAI values for collagen hydrolysates from the cartilages of sphyrna lewini, Dasyatis akjei and Raja porosa (116.07, 91.04 and 123.85 m²/g, respectively); these hydrolysates had high average molecular weights.

The collagen sample hydrolyzed using Alcalase® had a high emulsion stability index (ESI), 38.68 min, which was very similar to the value found for gelatin from bovine skin, 39.77 min (Jellouli et al., 2011).

According to Wasswa et al. (2008), the high emulsifying capacity of hydrolysates can probably be attributed to increases in protein solubility, lower molecular weight and greater molecular flexibility, thus facilitating the diffusion of the protein to the oil-water interface.

Hydrolysis altered the functionality of the collagen, which showed a decrease in viscosity and foam expansion capacity, as

Table 2. Foam expansion capacity (FEC), foam stability (FS), emulsifying activity index (EAI) and the emulsion stability index (ESI) of crude collagen from the chicken MSM residue and the hydrolysates obtained using Alcalase® and Flavourzyme®.

	FEC (%)	FS (%)		EAI (m²/g)	ESI (mins)
		3 mins	10 mins		
Crude Collagen	22.87[a] ± 1.05	90.78[a]	74.28[a]	49.01[b] ± 0.82	14.05[b] ± 0.86
Hydrolyzed Alcalase®	3.75[b] ± 0.76	63.54[b]	47.36[b]	130.53[a] ± 1.81	38.68[a] ± 0.79
Hydrolyzed Flavourzyme®	4.38[b] ±0.87	67.43[b]	45.89[b]	126.89[a] ± 1.46	23.62[b] ± 0.95

Averages in the same row with the same superscript letters do not differ significantly by Tukey's test (p > 0.05). Values expressed as mean ± standard deviation.

well as an improvement in the emulsifying activity index and emulsion stability index. The hydrolysates showed potential for use as emulsifying agents.

4 Conclusion

The obtaining hydrolysates from collagen derived from chicken MSM residue using the enzymes Alcalase® and Flavourzyme® proved to be feasible. It was proven that the collagen cleaved into peptides of lower molecular weight and that the hydrolysates presented good functionality with potential for industrial application.

Acknowledgements

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