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Functional protein hydrolysate from goat by-products: Optimization and characterization studies

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ABSTRACT

The protein hydrolysates of goat viscera were obtained using the Alcalase® and Brauzyn® enzymes then characterized by their chemical composition, total and free amino acids, hydrophobicity profile, as well as their functional and antioxidants properties. Hydrolysates with higher and lower degrees of hydrolysis of both enzymes were selected for the experimental design. Regarding the functional properties, maximum solubility values were achieved for the sample with a higher degree of hydrolysis; oil retention capacity showed higher values for the hydrolysates with lesser degree of hydrolysis. The emulsifying property and emulsion stability showed no significant difference in the four protein hydrolysates. In determining the antioxidant activity, higher reduction percentages of free radical ABTS•+ were observed than for the free radical DPPH•. Based on the results, we conclude that the protein hydrolysates of goat viscera have great technological applicability to the food industry, in addition to being excellent sources of nutrients.

1. Introduction

By-products of animals have an economic impact on the meat industry due to their potential for technological transformation and energy utilization. Overall, the current volume of animal slaughter by-products generated by processing industries is about 24.5 million tons per year (Martinez-Alvarez, Chamorro, and Brenes, 2015). According to Toldrá, Mora, and Reig (2016), the efficient use of cattle and pig slaughter by-products can generate overall economic gains of 11.4% and 7.5% for the productive sector, respectively.

Studies have been developed aiming at producing protein hydrolysates of high nutritional value from non-conventional sources of animal origin, such as the liver, lungs, heart, kidneys, brain and guts (Aristoy & Toldrá, 2011). This is justified by the fact that these by-products are excellent sources of functional peptides, essential amino acids, vitamins and minerals. The production of biomolecules by enzymatic hydrolysis of proteins improves the performance of important biological activities according to the amino acid sequence, directing their use as nutraceutical ingredients, in addition to expanding the range of technological applications in the food industry as flavor enhancers and emulsifiers (Lafarga & Hayes, 2014; Mora, Reig, & Toldrá, 2014).

Enzymatic hydrolysis is a fast and safe technique for the production of protein hydrolysates and food grade peptides. This technique has been used to improve the expression of functional and biological properties of proteins, adding innovation to low commercial value products (Mora et al., 2014).

Enzymatic hydrolysis presents advantages compared to acidic and alkaline hydrolysis; among these, producing homogeneous protein hydrolysates compared to the size of the generated peptides by reducing compounds' secondary degradation formed during the process; making products with less bitter taste and low salt (He, Franco, & Zhang, 2013). The functional properties displayed by protein hydrolysates resulting from the enzymatic hydrolysis are dependent on various factors such as the nature of the substrate, specificity of the enzyme used, and the hydrolysis conditions (Kumar, Nazeer, & Ganesh, 2012). Thus, it is necessary that the process conditions meet the conditions of highest activity, and therefore they need to be strictly defined.

Most of the studies reported in the literature directed to the use of animal slaughter by-products using processes with enzymatic

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hydrolysis mainly refer to fish waste, like catfish guts (Klomklao, Kishimura, & Benjakul, 2013), tilapia processing residue (Dieterich et al., 2014), or sardine (*S. pilchardus*) and mackerel (*T. mediterraneus*) processing residue (Morales-Medina, Tamm, Guadix, Guadix, & Drusch, 2016). A few other studies refer to obtaining protein hydrolysates from bovine slaughter by-products (Bah, Bekhit, El-Din, McConnell, & Carne, 2016), pig bones (Pagán, Ibarz, Falguera, & Benítez, 2013), sheep bones (Bhaskar, Modi, Govindaraju, Radha, & Lalitha, 2007) or chicken bones (Lasekan, Bakar, & Hashim, 2013). However, no work has focused on the potential of using the residual viscera from slaughtered goat in obtaining new ingredients. Rosa, Pires, Silva, and Motta (2002) proposed studies involving the use of goat viscera or other by-products as the functional ingredients in processed meat products. Viscera represented by the heart, lungs, liver, kidneys, intestines, stomach, along with the brain and the blood represent on average 15–20% of the live weight of goats. Therefore, these percentages would have great economic impact if they were used as raw material in preparing new ingredients with technological application and/or nutrition. Given the above and considering the protein value of these viscera, this study aimed to enzymatically obtain protein hydrolysates of goat viscera using the Alcalase® and Brauzyn® enzymes, and evaluate their technological application by determining their functional properties, chemical composition, and their amino acid and hydrophilic profile.

2. Materials and methods

2.1. Materials

Goat viscera (liver, lungs and heart) were used to obtain protein hydrolysate, acquired at the Central Market in the city of Joao Pessoa (Paraiba, Brazil). Goat viscera were collected shortly after slaughter, placed in a refrigerator (2 ± 1 °C), transported to the laboratory where they were washed and weighed in equal parts. They were immediately crushed in a meat processor with 0.6 mm diameter disc (CAF machines, 5 model, São Paulo, Brazil), homogenized and put under commercial freezing (−15 ± 1 °C) for a period not exceeding 30 days, during which studies for obtaining protein hydrolysates were performed.

Two enzymes were used in the hydrolysis process: 2.4 L Alcalase® (Novozymes Latino Americana Ltda, Paraná, Brazil), a serine protease of microbial origin (*Bacillus licheniformis*) with endogenous activity; and Brauzyn® (Prozyn Biosolutions, São Paulo, Brazil) a protease cysteine from a plant (*Carica papaya*) with exogenous activity.

2.2. Optimization of enzymatic hydrolysis process of goat viscera

The best hydrolytic conditions for the array (goat viscera) were selected by a central composite design (CCRD) 2^2 with 11 experiments, 4 factorial points (levels ± 1), 3 central points (level 0) and 4 axials (levels ± α), for each of the selected enzymes. The independent variables were the hydrolysis time (T) and the enzyme load (E:S); and the dependent variable was the degree of hydrolysis of the proteins. Hydrolysis was carried out for 180 min under agitation, in optimal pH and temperature conditions of each enzyme according to information provided by the manufacturer, being pH 7.0 and 60 °C for Alcalase® and pH 6.5 and 70 °C for Brauzyn®. The response surface model was prepared using the following equations:

$$DHA = \beta 0 + \beta 1(E:S) + \beta 2(T) - \beta 3(E:S)^2 - \beta 4(T)^2 + \beta 5(T)(E:S) \quad (1)$$

$$DHB = \beta 0 + \beta 1(\mathrm{E{:}S}) + \beta 2(\mathrm{T}) - \beta 3(\mathrm{E{:}S})^2 + \beta 4(\mathrm{T})^2 + \beta 5(\mathrm{T})(\mathrm{E{:}S}) \quad (2)$$

Where:DHA and DHB are the response values of the predicted degree of hydrolysis using the model for Alcalase® and Brauzyn® enzymes, respectively.β_0 it is the average or constant coefficient;β_1 and β_2 is the linear; β3 and β4 the quadratic and β5 the interaction coefficients.

The comminuted mass goat viscera were thawed under refrigeration, weighed, homogenized in deionized water (1: 2 w / v) and transferred to a double-walled glass reactor coupled to pH stat equipment (Automatic Titrator Model LD 50 Grafix, Mettler Toledo, Schwerzenbach, Switzerland) with stirring and heating system carried out by means of a thermostat bath connected to the reactor. During the hydrolysis reaction, the pH was kept constant by adding a NaOH solution (0.25 N), and the base consumption was recorded by the equipment. The degree of hydrolysis (DH) is defined by Eq. (3) (Adler-Nissen, 1986).

$$DH\,(\%) = B{*}Nb{*}\left(\frac{1}{\alpha}\right){*}\left(\frac{1}{MP}\right){*}\left(\frac{1}{htot}\right){*}100 \quad (3)$$

Wherein: DH (%) = degree of hydrolysis; B = base consumption in ml; N_b = normality of base; 1 / α = average degree of dissociation of the α-NH_2; α = 0.5267; MP = protein mass (g); h_{tot} = total number of peptide bonds in the protein substrate – 7.6 to meat (Adler-Nissen, 1986).

The highest and lowest degrees of hydrolysis were selected from among 11 hydrolysates and then analyzed for its technological features (solubility, oil retention capacity, emulsifying capacity, emulsion stability and antioxidant activity) and its physico-chemical parameters (chemical composition, soluble protein profile of total and free amino acids and peptides of the hydrophobicity profile).

2.3. Protein hydrolysate characterization of goat viscera

2.3.1. Chemical composition and soluble protein

Moisture, ash and protein were determined using the methodology described in items N° 926.07B, 923.03 and 930.25, respectively, of AOAC (2010). Ether extract was determined by following the procedures described by Folch, Less, and Stanley (1957). Soluble protein was according to the methodology described by Lowry, Rosebrough, Farr, and Randal (1951).

2.3.2. Total amino acids (TAA)

TAA were quantified according to the method described by White, Hart, and Fry (1896), in which the total amino acids were determined in samples previously hydrolyzed at 110 °C/ 22 h., with 6 N hydrochloric acid, vacuum sealed. The resulting amino acids were derivatived by phenyl isothiocyanate (PITC) and amino acids separation and quantifications was carried out by high-resolution liquid chromatography (Varian 2690 Waters, California, USA) coupled with reverse phase C18 column (PICO-TAG, 3.9 × 150 mm). The sample injection (20 μL) was performed manually and detection was at 254 nm. Chromatographic separation was performed at a constant flow of 1 ml/min at 35 °C. The chromatographic run time was 21 min. Amino acids were identified by comparing retention times with ASA Standard-18 Sigma-Aldrich (Chemie GmbH, Steinheim, Germany) and the results were expressed as g amino acids (AA)/100 g samples.

2.3.3. Profile of free amino acids (FAA)

The free amino acids of the dry samples were extracted by orbital shaking for 60 min with 0.1 M chloric acid (g/ml) followed by pre-column phenyl isothiocyanate (PITC) derivatization according to White et al. (1986) and Hagen, Frost, and Augustin (1989). The separation and quantification was performed in an HPLC system (Shimadzu Corporation, Tokyo, Japan) and Luna C18 reversed-phase column (250 mm × 4.6 mm, 5 μm; Phenomenex Inc., Torrence, CA, USA). The mobile phases consisted of an acetate buffer at pH 6.4 and a 40% acetonitrile solution. The sample was injected automatically (50 μL), and detection was performed at 254 nm. Chromatographic separation was performed at a constant flow rate of 1 ml/min at a temperature of 35 °C. The chromatographic run time was 45 min; the results are expressed as g amino acids (AA)/100 g samples.

2.3.4. Peptide hydrophobicity analysis

The separation of peptides by hydrophobicity was performed using a Nova-Pak C18 column (4.6 m × 250 mm, 4 μm particle size, cartridge; Waters, Ireland) connected to a high-performance liquid chromatography system (Varian, Waters 2690, California, EUA). The injection volume of the soluble extract (0.2 g/ml) was 20 μL, and the mobile phase was composed of eluent A (ultrapure water with 1% trifluoroacetic acid) and eluent B (acetonitrile with 1% trifluoroacetic acid). A linear gradient of eluent A and eluent B was applied for 60 min with a flow rate of 1 ml/min, and detection was performed at 214 nm (Bezerra et al., 2016).

2.4. Functional properties of goat viscera protein hydrolysates

2.4.1. Protein solubility

The protein solubility was determined by the method of Morr et al. (1985) with some modifications. Quantification of soluble protein in the supernatant was determined by the method of Lowry et al. (1951). The percentage of soluble protein was calculated according to Eq. (4).

$$P.\ S(\%) = \left\{\frac{A*50}{W*\frac{S}{100}}\right\}*100 \quad (4)$$

where: P.S = soluble protein content present in the sample [%]; A = protein concentration of the supernatant [mg/ml]; W = weight of the sample [mg]; S = protein concentration in the sample [%].

2.4.2. Oil retention capacity (ORC)

This was determined according to the method of Fonkwe and Singh (1996). The results were expressed as amount of oil retained by the amount of total protein present in the sample.

2.4.3. Emulsifying capacity (EC) and emulsion stability

The EC was obtained according to the methodology described by Dench, Rivas, and Caygill (1981) with modifications. The emulsifying capacity was calculated by the ratio of emulsified layer and the total height of the sample in ml oil/g protein. To determine the stability, the emulsion was heated in a water bath at 80 °C for 30 min, cooled with running water for 15 min, followed by centrifugation at 253 g for 15 min. The emulsion stability was expressed in percentage of the ratio between the height of the layer remaining emulsified and total sample height.

2.4.4. Antioxidant activity

Studies of antioxidant capacity were realized using two methods: radical activity 2,2-difenil-1picrilhidrazila (DPPH$^{\bullet}$) and overall activity by 2,2- azino-bis(3-etilbezotiazolina) − 6-ácido sulfônico – (ABTS$^{\bullet+}$)."

2.4.4.1. Determination of antioxidant activity total for the capture of free radical DPPH$^{\bullet}$. The activity of DPPH• radical was measured using the method proposed by Brand-Williams, Cuvelier, and Berset (1995). The absorbance reading was performed at 517 nm in a UV–visible spectrophotometer (Quimis®, Q798U Model, Shanghai, China). The sequester free radical capacity was expressed as a percentage of the radical oxidation inhibition.

2.4.4.2. Determination of total antioxidant activity by ABTS•+ method. The total antioxidant activity by ABTS•+ method was determined using the method proposed by Re et al. (1999). The absorbance reading was performed at 734 nm in a UV–visible spectrophotometer (Quimis® Model Q798U). The ability to scavenge free radicals was calculated from the inhibition percentage based on the decrease in absorbance relative to background at time zero.

Table 1
Central composite rotatable design (2^2) for the enzymatic hydrolysis of goat viscera using the Alcalase® and Brauzyn® enzymes.

Hydrolysates	Time (min)	E:S (%, g Enzyme/100 g substrate)	DH %	
			Alcalase®	Brauzyn®
1	− 1 (60)	− 1 (0.3)	8.63	5.32
2	− 1 (60)	+ 1 (0.8)	13.03	8.63
3	+ 1 (180)	− 1 (0.3)	9.57	6.38
4	+ 1 (180)	+ 1 (0.8)	**17.63**	14.09
5	- α (35)	0 (0.5)	9.75	5.13
6	+ α (205)	0 (0.5)	16.32	8.50
7	0 (120)	-α (0.1)	**8.58**	**4.61**
8	0 (120)	+α (1.0)	14.60	**14.42**
9	0 (120)	0 (0.5)	12.76	8.44
10	0 (120)	0 (0.5)	13.54	10.48
11	0 (120)	0 (0.5)	14.01	7.92

DH = degree of hydrolysis; E:S - Substrate enzyme relationship in %, g Enzyme/100 g substrate.

2.5. Statistical analysis

The Two-way analysis of variance (ANOVA) was used to evaluate the characterization and functional properties of goat viscera protein hydrolysates, in order to determine the enzyme effects and the degree of hydrolysis using the Statistical Analysis System software (version 11.0) (SAS, 2014).

3. Results and discussion

3.1. Obtaining goat viscera protein hydrolysate

Experimental CCRD results are shown in Table 1 and the Pareto diagram (Fig. 1A and B), exposing the significance of the variables and their interactions.

The degree of hydrolysis (DH) is a primary response in determining the optimization parameters and obtaining protein hydrolysates with different functionalities (Adler-Nissen, 1986). In this study, the hydrolysates that showed the highest and lowest value of obtained DH in different processing conditions were selected. The resulting R^2 in the model was 0.95262 for Alcalase® and 0.95117 for Brauzyn®; and according to the F test, this was a significant regression, as the $F_{calculated} > F_{tabulated}$, and showed no lack of fit ($F_{calculated} < F_{tabulated}$). Significant variables, their interactions, and the models can be expressed in Eqs. (5) and (6):

$$DHA = \mathbf{13.\ 44} + \mathbf{1.\ 85}(\boldsymbol{E:S}) - 0.22(E:S)^2 + \mathbf{2.\ 62}(\boldsymbol{T}) - 0.95(T)^2 + 0.91(T)(E:S) \quad (5)$$

$$DHB = \mathbf{8.\ 95} + 1.41(E:S) - 0.96(E:S)^2 + \mathbf{3.\ 11}(\boldsymbol{T}) + 0.39(T)^2 + 1.10(T)(E:S) \quad (6)$$

Wherein: DHA and DHB are the response values of the predicted degree of hydrolysis using the model for Alcalase® and Brauzyn® enzymes, respectively. In this model E:S (enzyme load) and T (hydrolysis time) are independent variables and DH is the dependent variable.

DH resulting from the experiment using Alcalase® and Brauzyn® enzymes showed variation from 8.58% to 17.63%, and 4.61–14.42%, respectively. Based on response surfaces (Fig. 1C and D), we can confirm that the highest degree of hydrolysis was prepared from the hydrolysate using the Alcalase® enzyme. The results show that Alcalase® (microbial source) was faster in hydrolyzing the goat protein substrate, having a different kinetic reaction than Brauzyn® (of vegetable origin). At the end of the 180 min period, the enzymes retained a difference of approximately 4 DH units together.

Alcalase® is an isolated microbial enzyme selected from strains of *Bacillus licheniformes*, and has been identified with great effectiveness in

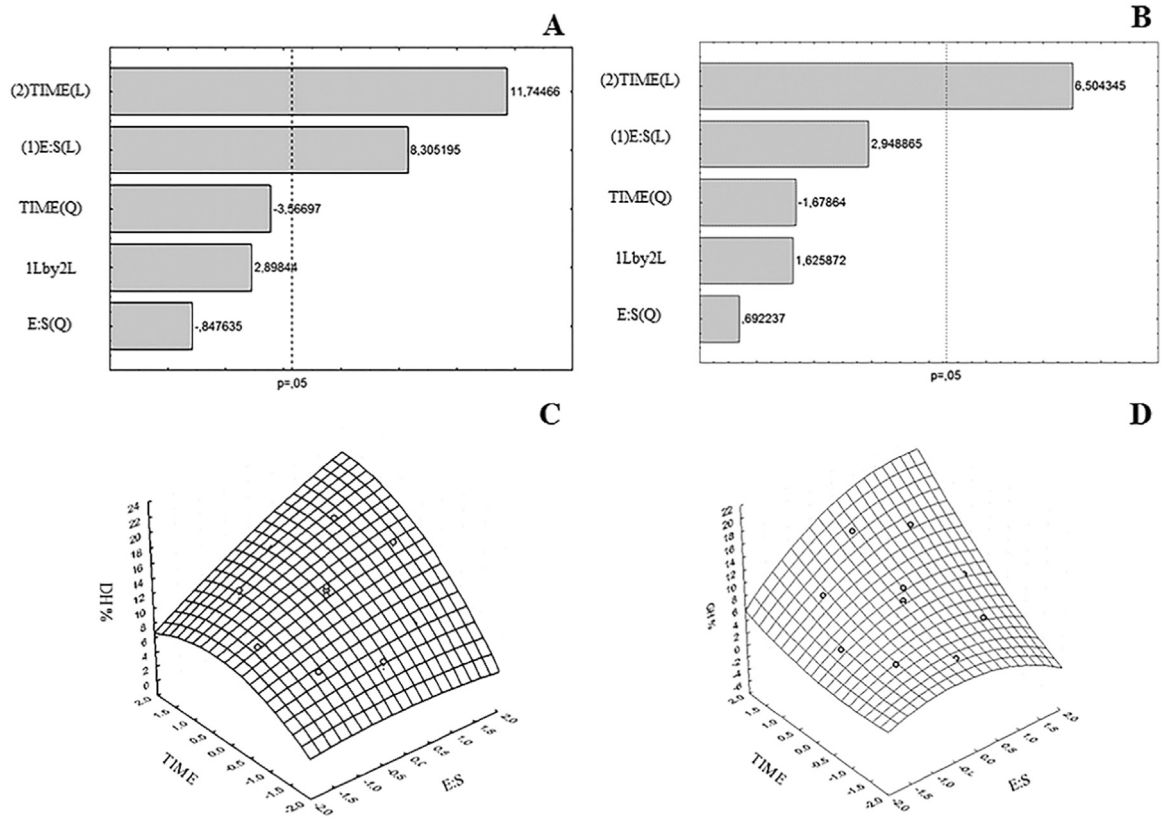

Fig. 1. Pareto Chart and response surface dependent variable using Alcalase® (A and C) and Brauzyn® (B and D).

Table 2
Total amino acid profile (g amino acids (AA)/100 g samples) of protein hydrolysates goat viscera ($p < 0.01$) on dry basis.

Amino acid	> DH		< DH		p	p	p
	Alcalase®	Brauzyn®	Alcalase®	Brauzyn®	Enzymes	DH	Enzymes*DH
Aspartic acid	10.63 ± 0.73	11.40 ± 1.59	18.67 ± 1.46	13.85 ± 0.00	< 0.001	< 0.001	< 0.001
Glutamic acid	23.88 ± 4.32	22.55 ± 5.33	24.63 ± 0.14	17.23 ± 0.05	< 0.001	< 0.001	< 0.001
Histidine	1.04 ± 0.20	1.95 ± 0.60	1.04 ± 0.01	1.14 ± 0.01	< 0.001	< 0.001	< 0.001
Arginine	4.78 ± 1.54	5.96 ± 1.22	2.96 ± 0.10	2.43 ± 0.05	< 0.001	< 0.001	< 0.001
Lysine	7.65 ± 0.00	7.87 ± 1.51	4.67 ± 0.11	3.52 ± 0.05	< 0.001	< 0.001	< 0.001
Hydrophilic							
Serine	3.98 ± 0.81	2.97 ± 0.85	1.90 ± 0.00	1.52 ± 0.00	< 0.001	< 0.001	< 0.001
Glycine	4.89 ± 0.76	5.53 ± 1.68	3.57 ± 0.01	2.77 ± 0.01	< 0.001	< 0.001	< 0.001
Threonine	4.93 ± 1.06	3.91 ± 1.03	2.65 ± 0.02	2.21 ± 0.01	< 0.001	< 0.001	< 0.001
Tyrosine	4.08 ± 0.56	3.28 ± 0.70	1.86 ± 0.02	1.40 ± 0.01	< 0.001	< 0.001	< 0.001
Hydrophobic							
Alanine	5.15 ± 0.99	4.26 ± 1.78	3.74 ± 0.12	2.45 ± 0.02	< 0.001	< 0.001	< 0.001
Proline	Traces	Traces	Traces	Traces	NA	NA	NA
Valine	2.53 ± 0.41	1.82 ± 0.59	0.57 ± 0.06	0.30 ± 0.02	< 0.001	< 0.001	< 0.001
Methionine	3.13 ± 0.78	2.00 ± 0.29	4.64 ± 0.91	2.87 ± 0.04	< 0.001	< 0.001	< 0.001
Isoleucine	4.13 ± 0.49	2.76 ± 0.83	2.01 ± 0.06	1.49 ± 0.03	< 0.001	< 0.001	< 0.001
Leucine	6.48 ± 0.67	4.85 ± 1.62	3.05 ± 0.05	2.41 ± 0.01	< 0.001	< 0.001	< 0.001
Phenylalanine	5.50 ± 0.69	4.21 ± 0.99	3.31 ± 0.00	2.46 ± 0.01	< 0.001	< 0.001	< 0.001

> DH – higher degree of hydrolysis; < DH – lesser degree of hydrolysis; NA – Not Applied.

protein cleavage for the production of functional peptides (Centenaro, Prentice-Hernández, & Sallas-Mellado, 2009; Liu et al., 2014; Toldrá et al., 2016). This enzyme is described by acting on the cleavage of peptide bonds containing hydrophobic residues at the carboxyl side of the protein (Adler-Nissen, 1986), although their specificity has not been fully characterized.

Protein hydrolysates have different applications according to their degree of hydrolysis, the size of the formed peptides and the amino acid sequence. Hydrolysates with a low degree of hydrolysis exhibit technologically potential functional characteristics such as antioxidant activity, improved solubility, and foaming, among others. Those with a large degree of hydrolysis are mostly used as nutritional supplements in special medical diets or flavors (Vioque, Clemente, Pedroche, Yust, & Millán, 2001). In this study, we chose to select the hydrolysates which showed the highest and lowest degree of hydrolysis and subsequent physical-chemical and technological features.

3.2. Characterization of goat viscera protein hydrolysates

The protein hydrolysates goats of greater and lesser degree of

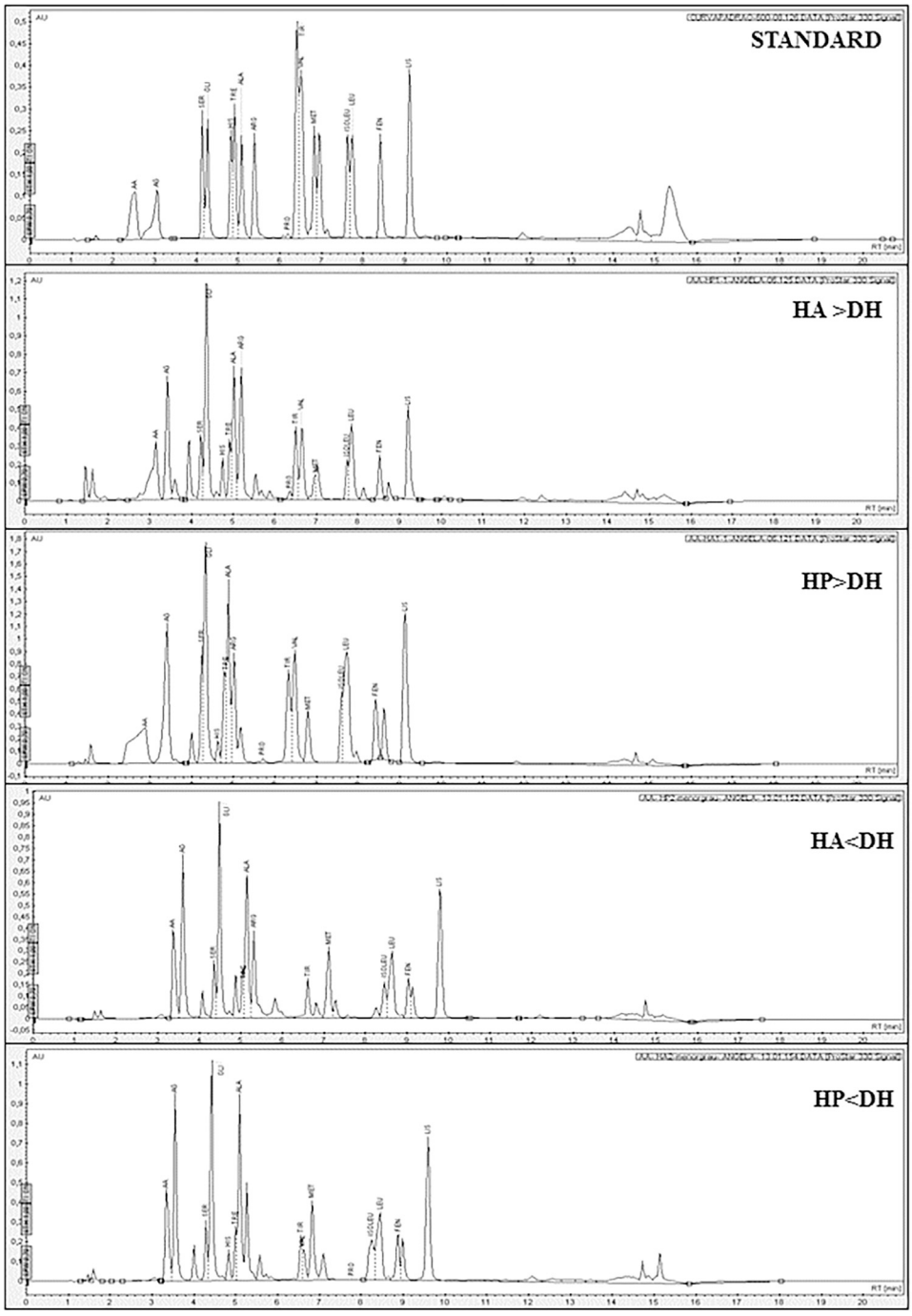

Fig. 2. Total amino acids chromatograms of hydrolysates goat viscera. Standard curve; (HA > DH) – higher degree of hydrolysis with Alcalase; (HP > DH) higher degree of hydrolysis with Brauzyn; (HA < DH) lower degree of hydrolysis with Alcalase; (HP < DH) lower degree of hydrolysis with Brauzyn.

hydrolysis showed significant differences ($P < 0.001$) in the chemical composition parameters and soluble protein due to the hydrolysis process conditions. The high total protein (range 67.04–80.59 g/100 g sample) and soluble content (between 25.84 and 32.72 mg/ml sample) present in the protein hydrolysates result in protein solubility during the hydrolysis process. All hydrophilic material and low molecular size was solubilized and is removed from undigested insoluble fraction (Klomklao et al., 2013), and was precipitated in the centrifugation and filtration steps. Studies by Klomklao et al. (2013), Witono, Taruna, Windrati, Azkiyah, and Norma (2016), Morales-Medina et al. (2016) reported approximate values found in this study, ranging from 64.65 to 89.02 g/100 g for protein content.

Lipid levels were satisfactory, with an average of 16.27–24.53 g/100 g; this is considered high, but justified since the raw material used in the experiment was goat viscera which has the particularity to store larger amount fats in the visceral cavity. According to Madruga, Dantas, Queiroz, Brasil, and Ishihara (2013), fat derived goat products are precursors of volatile compounds; therefore, they have potential technological application as flavorings and functional food ingredients. Martinez-Alvarez et al. (2015) emphasize that these properties make the protein hydrolysates of interest to manufacture products for human or animal consumption.

The ash content of the protein hydrolysates proved to be higher than that reported for the original feedstock (mixture of goat viscera), which was on average 3.20 g/100 g (dry basis), this increase is probably the result of the added hydroxide sodium in an enzymatic process for maintaining static pH (Dieterich et al., 2014; Morales-Medina et al., 2016). This hypothesis could be confirmed by comparing the results of

Table 3
Free amino acid profile (g amino acids (AA)/100 g samples) of protein hydrolysates goat viscera ($p < 0.01$) on a dry basis.

Amino acid	> DH		< DH		p	p	p
	Alcalase®	Brauzyn®	Alcalase®	Brauzyn®	Enzymes	DH	Enzymes*DH
Aspartic acid	1.82 ± 0.81	0.47 ± 0.50	0.65 ± 0.08	0.63 ± 0.50	< 0.001	< 0.001	< 0.001
Glutamic acid	3.13 ± 0.79	1.44 ± 0.20	2.02 ± 0.79	1.51 ± 0.20	< 0.001	< 0.001	< 0.001
Histidine	0.36 ± 0.62	0.24 ± 0.42	0.16 ± 0.06	0.32 ± 0.04	< 0.001	< 0.001	< 0.001
Arginine	0.47 ± 0.06	0.07 ± 0.18	0.06 ± 0.06	0.03 ± 0.18	< 0.001	< 0.001	< 0.001
Lysine	1.65 ± 0.32	0.60 ± 0.03	0.95 ± 0.27	0.51 ± 0.36	< 0.001	< 0.001	< 0.001
Hydrophilic							
Serine	0.98 ± 0.05	0.47 ± 0.04	0.25 ± 0.05	0.28 ± 0.47	< 0.001	< 0.001	< 0.001
Glycine	0.76 ± 1.12	0.76 ± 0.27	0.8 ± 0.12	0.94 ± 0.27	< 0.001	< 0.001	< 0.001
Threonine	1.45 ± 0.08	0.33 ± 0.72	0.21 ± 0.06	0.25 ± 0.07	< 0.001	< 0.001	< 0.001
Tyrosine	0.92 ± 0.33	0.27 ± 0.08	0.57 ± 0.31	0.26 ± 0.07	< 0.001	< 0.001	< 0.001
Hydrophobic							
Alanine	2.10 ± 1.77	0.94 ± 1.86	1.66 ± 0.17	0.88 ± 0.18	< 0.001	< 0.001	< 0.001
Proline	Traces	Traces	Traces	Traces	NA	NA	NA
Valine	1.81 ± 2.73	0.52 ± 0.19	0.94 ± 0.22	0.57 ± 0.19	< 0.001	< 0.001	< 0.001
Methionine	0.89 ± 0.22	0.25 ± 0.19	0.50 ± 0.22	0.25 ± 0.19	< 0.001	< 0.001	< 0.001
Isoleucine	1.72 ± 0.82	0.47 ± 0.04	0.59 ± 0.08	0.33 ± 0.04	< 0.001	< 0.001	< 0.001
Leucine	3.03 ± 0.77	0.83 ± 0.15	1.69 ± 0.77	0.93 ± 0.15	< 0.001	< 0.001	< 0.001
Phenylalanine	1.91 ± 0.38	0.82 ± 0.14	0.81 ± 0.25	0.43 ± 0.04	< 0.001	< 0.001	< 0.001

> DH – higher degree of hydrolysis; < DH – lesser degree of hydrolysis; NA – Not Applied.

the two enzymes since hydrolysates prepared with the Alcalase® enzyme (more alkaline nature) showed a higher concentration of mineral residue in relation to hydrolysates prepared with Brauzyn® (Zavareze, Silva, Sallas-Mellado, & Prentice-Hernández, 2009). High ash content was also reported by Zavareze et al. (2009), Dieterich et al. (2014) and Morales-Medina et al. (2016), which corroborate the values obtained in this study.

The composition of total amino acids are shown in Table 2 and Fig. 2, and free amino acids are shown in Table 3 and Fig. 3. Goat protein hydrolysates are excellent sources of amino acids, with well-balanced essential amino acids in adequate proportions. These data confirm the studies by Dieterich et al. (2014) and Klomklao et al. (2013) in evaluating the profile of amino acids in protein hydrolysates derived from animal agribusiness and fish viscera extract by-products, respectively.

In the total amino acids profile, the values of glycine, glutamic acid, lysine and aspartic acid were the highest 11.97–14.32%, 9.43–13.82%, and 10.70–11.86% 8.42–11.22%, respectively) which together account for over 40% of the total content of amino acids present in these hydrolysates. Data presented by Morales-Medina et al. (2016) also showed higher values for aspartic acid and glutamic acid when they studied the functional and antioxidant properties of sardines (S. pilchardus) and mackerel (T. mediterrraneus) protein hydrolysates. Witono et al. (2016) mention that the high glutamic acid content makes the samples a great alternative for use as flavoring/flavor enhancer.

The presence of hydrophobic amino acids such as leucine, methionine, proline and alanine also express an important performance in the functional properties of food proteins (Witono et al., 2016). These authors state that the hydrophobic amino acids present in protein hydrolysates can exhibit excellent antioxidant properties and may be incorporated into other food products as a supplement.

According to Table 3, we can see that the degree of hydrolysis and enzyme used resulted in the release of different free amino acids, showing that both are very influential variables in this parameter.

The specificity of the enzymes used in this experiment causes different breaking of peptide bonds along the protein chain, according to their amino acid sequence and aerodynamic forming exposure with different functional groups. In this way, the release of different amino acids occurs, as well as low molecular weight of peptides that are qualitatively observed in the hydrophilicity profile (Fig. 4).

The hydrophobicity profile (A) and the sum of peak areas of hydrophilic and hydrophobic peptides (B) of goat protein hydrolysates and mixture of goat viscera are shown in Fig. 4. From this profile, the majority presence of hydrophilic nature of peptides can be identified, having technological application of improved solubility, water holding capacity and those of hydrophobic character; also generally exhibiting greater antioxidant activity as a result of their greater solubility in lipids (Qian, Jung, & Kim, 2008).

The formed peptides were eluted for a total time of 60 min; however, the chromatographic profile shows that the peptides have a hydrophilic character, being eluted in the initial 30 min of the run, followed by hydrophobic character. This behavior leads to the conclusion that the protein hydrolysates under study presented a predominantly hydrophilic nature of peptides.

Compared with the mixture of viscera used as the substrate, there was a significant increase in the hydrophilicity peak areas in the evaluated samples. This difference results from the enzymatic hydrolysis process, which is the cleavage of peptide bonds between specific amino acids at the enzyme binding site and thereby processed into peptides of different sizes and free amino acids (Zavareze et al., 2009).

3.3. Functional properties of protein hydrolysates

Among the features of desirable technological ingredients, solubility, emulsifying capacity, and oil retention capacity (He et al., 2013) are of greatest importance in food formulation. Solubility is one of the main functional properties in the study of proteins in food (Witono et al., 2016), requiring that its value is high to achieve great features.

The solubility of protein hydrolysates with Alcalase® and Brauzyn® enzymes (pH 7), are shown in Fig. 5A. There was a significant difference ($P < 0.0001$) in the interaction between the enzyme and the degree of hydrolysis. The solubility of the samples was higher when using the Alcalase® enzyme (53.62% and 58.97% for < DH and > DH, respectively) compared with the values found for the hydrolysates prepared with the Brauzyn® enzyme 32.65–38.63% for the hydrolysates < DH and > DH, respectively). The increased hydrophilicity of the resulting hydrolysate is from ionizable amino and carboxyl groups of the amino acids that were exposed during the hydrolysis of the protein, thus improving the product's solubility (Schmidt and Salas-Mellado, 2009).

The solubility values of goat hydrolysates were consistent with the values mentioned by Liu et al. (2014) and Zavareze et al. (2009), which evaluated the solubility of protein hydrolysates obtained from fish by-products, with reported values of 80% and 40%, respectively.

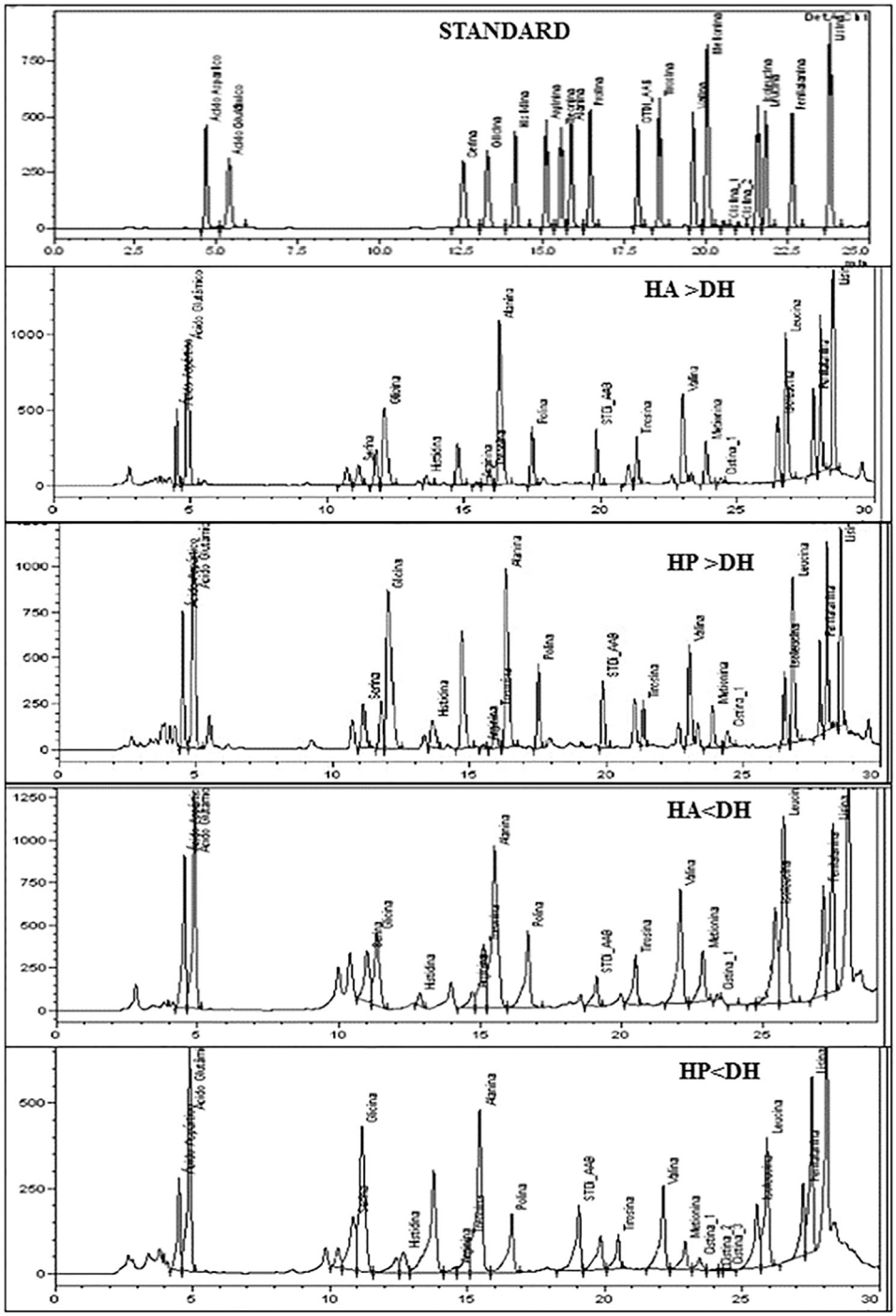

Fig. 3. Free amino acids chromatograms of hydrolysates goat viscera. Standard curve; (HA > DH) – higher degree of hydrolysis with Alcalase; (HP > DH) higher degree of hydrolysis with Brauzyn; (HA < DH) lower degree of hydrolysis with Alcalase; (HP < DH) lower degree of hydrolysis with Brauzyn. > DH – higher degree of hydrolysis; < DH – lower degree of hydrolysis.

Oil retention capacity (ORC) showed no difference ($P < 0.001$) for the different goat hydrolysates. According to Zavareze et al. (2009) this property is an important functional feature of great interest in industry, mainly for industrial processors of meat products. The ORC values obtained in this study ranged from 5.94 to 6.92 ml oil/g protein and corroborated with the data mentioned by He et al. (2013), who obtained values of 4.3–7.8 ml oil/g protein to assess protein hydrolysates prepared from fish byproducts. Halim, Yusof, and Sarbon (2016) report that the absorption of fat results in oil entrapment, and this ability of the peptides have influence on the product taste, causing wide and varied applicability in the food industry.

The emulsifying properties (defined as the ability of the material to form emulsion) and emulsion stability (defined as the ability to maintain the formed emulsion) (Witono et al., 2016) showed no significant difference in goat protein hydrolysates. The evaluated samples had values ranging from 63.25 to 64.83 ml oil/g protein for emulsifying capacity (EC) and 95.31–97.19% for emulsion stability (ES). These results were higher than those reported by Zavareze et al. (2009), who found values of 48.2 ml oil/g protein for the EC and 17.9% for ES of fish protein hydrolysates when using the Alcalase® enzyme.

Determination of the total antioxidant activity by free radical capture DPPH• and the ABTS•+ method is shown in Fig. 5B. This was evaluated in four samples of goat viscera protein hydrolysates and only one sample (> GH Alcalase ®) showed reduced value ($P < 0.0001$) activity for the capture of DPPH• in relation to other samples.

The results shown in Fig. 5B show that the values of the percentage

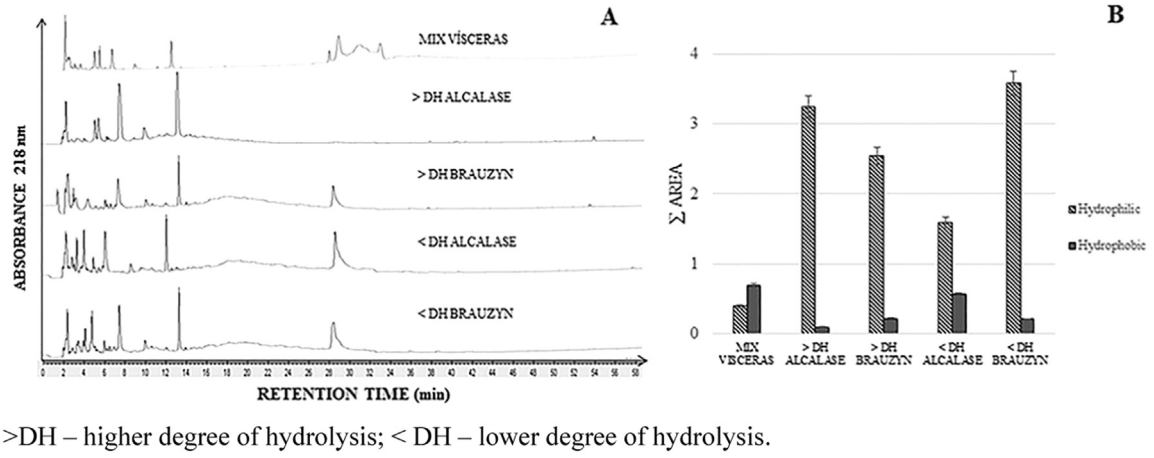

Fig. 4. Reversed-phase HPLC of the peptides of hydrolysates goat viscera. (A) HPLC chromatograms of analysis of hydrophobicity of peptides and (B) the sum of the peak areas.

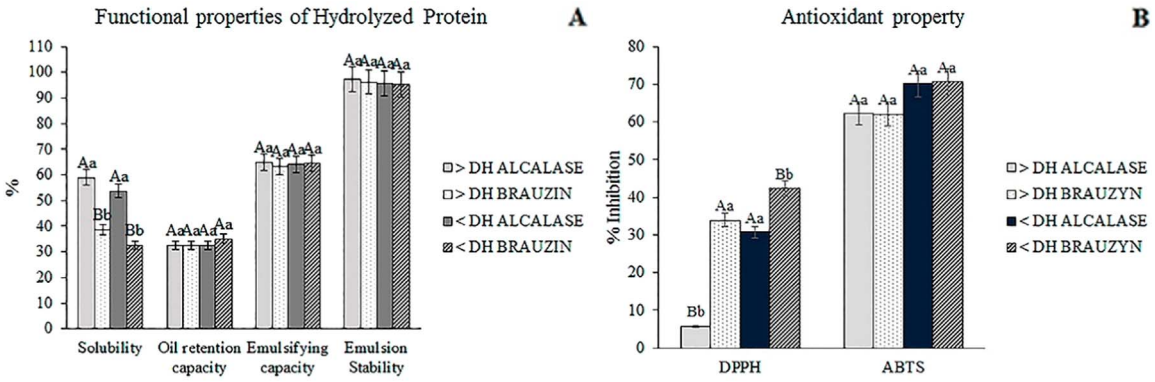

Fig. 5. Functional properties (A) and antioxidant (B) protein hydrolysates of goat viscera. Triplicate experiments. Different lowercase letters indicate significant difference between the enzyme ($p < 0.01$) according to the Tukey test. Different capital letters indicate a significant difference between the degree of hydrolysis ($p < 0.01$) according to the Tukey test.

free radical ABTS•+ reduction were more significant than for the free radical DPPH• for the four goat protein hydrolysates. The values were in the range from 62.05% to 70.65% for inhibiting the ABTS•+ and 5.76–42.24% for inhibition by DPPH•.

In general, these methods determine a substrate's ability to transfer electrons or hydrogen atoms which can react with free radicals to form more stable compounds (Morales-Medina et al., 2016). Protein hydrolysates that exhibit this functional property are very desirable because they can be applied to food products in order to extend their shelf life (He et al., 2013). The antioxidant activity evaluated by both systems (ABTS•+ and DPPH•) gives ability to stabilize the lipid component in the food, as well as extend its conservation (Herpandi, Rosma, & Nadiah, 2011).

The free amino acids in goat hydrolysates present acidic and basic characters, such as aspartic acid and histidine, respectively, by having carboxylic groups and amino groups in side chains, and therefore are chelators of metal ions and hydrogen donors (Qian et al., 2008). In addition, the amino acids tyrosine, methionine, tryptophan, cysteine and lysine are examples of amino acids that have antioxidant characteristics (Wang and Mejia, 2005). Moreover, others having the aromatic group in its chain such as phenylalanine and tyrosine, can contribute to donate electrons, and thus improve the capturing property of the radical (Rajapakse, Mendis, Jung, Je, & Kim, 2005). Thus, the presence of these amino acids is likely to influence the antioxidant capacity of the goat viscera protein hydrolysates.

It is worth mentioning that to improve upon on the physicochemical results (fat content, glutamic acid, free amino acids) and technological functionality (high CRO), we suggest other studies focusing on the flavoring potential of goat viscera protein hydrolysates.

4. Conclusion

Goat viscera protein hydrolysates showed excellent nutritional quality, with high protein content and a balanced amino acid profile of essential amino acids. They showed significant technological functional properties such as solubility, oil retention capacity, emulsifying property and emulsion stability, in addition to antioxidant activity, showing their potential for application as a functional ingredient in various products of the food industry.

Conflict of interest

None declared.

Acknowledgments

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