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Potential use of goat viscera to obtain protein hydrolysates

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ABSTRACT

In recent years, the amount of by-products from goat slaughtering has increased substantially and nutritionally valuable have been discarded. This project aimed to research the potential use of goat viscera to obtain products, such as protein hydrolysates, that can be incorporated into other foods and improving their nutritional and technological quality. The results obtained showed that goat viscera exhibited remarkable nutritional value, with a high protein content (~18 g/100 g of sample) and excellent total amino acid profile. The predominant essential amino acids were lysine, phenylalanine + tyrosine, leucine and methionine, with chemical score >3.3, being considered a protein source of high biological value. Fat content showed greater variation from 2.26 (liver) to 6.63 (heart) g/100 g, lipid contents. Twenty-two fatty acids were identified, where the predominant ones were the saturated fatty acids (SFA -0.88 to 3.29), followed in proportion by monounsaturated (MUFA - 0.60 to 1.78) and polyunsaturated (PUFA - 0.07–0.33 g fatty acid/100 g of sample), including C18:2n6t. It was concluded that goat viscera are ingredients of potential use in the production of new foods, such as protein hydrolysates, which have broad technological applicability in addition to increasing the economic value of slaughtering by-products.

1. Introduction

Research on new sources of functional ingredients and/or novel products has been the focus of studies that aim to convert the by-products of slaughterhouses as well as meat and fishery processing industries (Mora, Reig, & Toldrá, 2014; Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). Among the functional ingredients obtained from the wastes of these industries, protein hydrolysates and bioactive peptides are the most commonly exploited (Dieterich et al., 2014; He, Franco, & Zhang, 2013; Klomklao, Kishimura, & Benjakul, 2013; Morales-Medina, Tamm, Guadix, Guadix, & Drusch, 2016).

It is a fact that the industry benefits from science and innovation in adding value to animal slaughtering by-products, thus significantly improving their profitability. Generally, the current amount of animal slaughtering by-products generated by processing industries is approximately 24.5 million tons per year (Martinez-Alvarez, Chamorro, & Brenes, 2015). Regardless of the final destination of these by-products, it is necessary to apply more efficient tools to value their nutritional potential and identify compounds with functional properties as well as novel technological applications (Toldrá, Aristoy, Mora, & Reig, 2012).

The non-carcass components of goat viscera such as the heart, lungs, liver, kidneys, intestines and stomach as well as the brain and blood account for 15%–20% of the live weight of the animal (Costa, Madruga, Santos, & Medeiros, 2005; Santos, Costa, Medeiros, Madruga, & Queiroga, 2007). Such percentages would have great economic impact for slaughterhouses if part of these by-products were utilized as a raw material to produce new ingredients or to obtain a processed product.

Generally, goat viscera are used solely in traditional dishes, including *buchada* or *sarapatel* in Brazil (Brasil et al., 2014; Queiroz et al., 2013), *morcilla de burgos* in Spain (Santos, González-Fernández, Jaime, & Rovira, 2003) and *cavourmas* in Greece (Arvanitoyannis, Bloukas, Pappa, & Psomiadou, 2000), despite being excellent sources of proteins, fats and minerals (Anderson, 1988; Honikel, 2011; Nollet & Toldrá, 2011).

Previous studies evaluated the potential use of goat

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slaughterhouse by-products in the production and characterization of processed meat products such as bologna (Guerra et al., 2011), pates (Amaral et al., 2015; Dalmás, Bezerra, Morganob, Milanib, & Madruga, 2011), blood sausages (Silva et al., 2013) and *buchada* (Costa et al., 2005; Madruga, Rezer, Pedrosa, & Melo, 2003; Madruga et al., 2007; Queiroz et al., 2013). However, the potential use of goat viscera and slaughtering waste in obtaining new ingredients has not been studied. Kirton, Mercer, Duganzich, and Uljee (1995) and Rosa, Pires, Silva, and Motta (2002) proposed studies that focus on the use of goat viscera and other by-products as ingredients and flavoring in processed meat foods.

Considering the limited use of goat viscera, the lack of information on their chemical quality and the need for novel technological alternatives for their use, the present study has the objective of analyzing the goat viscera composition (heart, liver and lungs) and demonstrating their potential use as new products and/or ingredients that can be incorporated into processed meats, thus increasing their nutritional and/or technological quality.

2. Materials and methods

2.1. Raw material

Goat viscera (liver, lungs and heart) used in this study were obtained from the Central Public Market of the city of João Pessoa (Paraíba State, Brazil). Viscera were collected, transferred to polyethylene bags and transported under temperature-controlled conditions (2 °C ± 1 °C) to the laboratory. Samples were rinsed with chlorinated water to remove dirt, weighed, ground using a food processor (CAF Máquinas, model 5, São Paulo, Brazil), homogenized and stored in polyethylene bags in commercial freezers (−10 °C ± 1 °C) for up to 45 days until analysis.

In the present study, the potential use of goat viscera in technological applications was investigated, where goat viscera were analyzed both individually and as a 1:1:1 mixture of liver, lungs and heart (in weight). Chemical characterization was performed to determine the composition percentages, total amino acid profile, hydrophobicity profile, electrophoretic profile and fatty acid profile.

2.2. Analytical methods

2.2.1. Percentages

The moisture, ash and protein contents were determined using methods 926.07B, 923.03 and 930.25, respectively, according to the Association of Official Analytical Chemists (AOAC) (2010). Ether extracts were determined as described by Folch, Less, and Stanley (1957).

2.2.2. Fatty acid profile

Direct transesterification of fatty acids from goat viscera was performed as described by O'Fallon, Busboom, Melson, and Gaskins (2007). Ester identification and the quantification of fatty acid esters were performed using a gas chromatograph (HP Hewlett Packard 6890 Series GC System) equipped with a polar fused-silica capillary column (Supelco, USA) of 100 m by 0.25 mm and with a 0.2 μm film thickness. Fatty acids were identified by comparing the retention times of methyl esters from the samples to standards from a Supelco ME19-Kit (*Fatty Acid Methyl Esters* C4-C24). Fatty acid concentrations are presented as g fatty acid/100 g sample.

2.2.3. Total amino acids

Samples were previously hydrolyzed in 6 N double distilled hydrochloric acid (110°C/24 h), followed by precolumn derivatization with phenyl isothiocyanate (PITC) according to White, Hart, and Fry (1986). Identification was performed using a high-resolution liquid chromatograph (Varian, Waters 2690, California, USA) equipped with a reversed-phase C18 column (PICO-TAG, 3.9 × 150 mm) with detection at 254 nm. Chromatographic separation was performed at a 1 mL/min constant flow rate and 35 °C, with a 21 min run length. Quantification was performed using external standards (Sigma Aldrich Chemie GmbH, Steinheim, Germany).

Chemical scores provide a comparison between the essential amino acid content from a test protein to the corresponding amino acid from a standard protein. The reference standard most utilized is that by the Food and Agriculture Organization/World Health Organization (FAO/WHO) (2007).

2.2.4. Hydrophobicity profile

This analysis was performed according to Bezerra et al. (2016) with modifications. A total of 1 g of each sample was diluted in 10 mL of ultrapure water (MilliQ), mixed in a mini Turrax mixer for 3 min, centrifuged and filtered through a 0.45 μm cellulose membrane. Analyses were performed using a high-resolution liquid chromatograph (Varian, Waters 2690, California, USA) equipped with a reversed-phase C18 column (Nova-Pak 4.6 × 250 mm cartridge) and 4 μm beads (Waters, Ireland). The injection volume was 20 μL. The mobile phase buffer A was composed of 98.925% water, 0.075% trifluoroacetic acid (TFA) and 1.000% acetonitrile (mL/mL/mL). After equilibrium was achieved inside the column with 100% buffer A for 30 min, the peptide samples were eluted by a linear gradient from 0 to 100% of buffer B. The isocratic flow rate was 0.5 mL/min with detection at 218 nm.

2.2.5. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS) and β-mercaptoethanol

This procedure was performed to determine the protein fractions of the viscera, as described by Laemmli (1970). The stacking gel was prepared at 4% polyacrylamide in 0.5 mol/L Tris-HCl buffer at a pH of 6.8 with 1% SDS. The separating gel was prepared at 15% polyacrylamide in 3 mol/L Tris-HCl buffer at a pH of 8.8 with 1% SDS. Samples were dissolved (10 mg protein/mL) in a reducing buffer at a pH of 6.8 (0.5 mol/L Tris–HCl, 10% SDS, 10% glycerol, 5% β-mercaptoethanol and 0.1% bromophenol blue) and kept at 90 °C for 15 min. Aliquots of 10 μL of each sample were transferred to the wells. The run was performed at constant current (25 mA) and the run the gels for approximately 4 h. At the end of the run, the gel was removed from the plate, fixed in 12.5% TCA for 1 h and stained with 0.005% Coomassie brilliant blue R-250. The removal of excess dye was performed with a bleaching solution of methanol/acetic acid/water 1:3.5:8 (mL/mL/mL). The molecular weight standard, GE marker, was used with molecular weights of 12–225 kDa.

2.3. Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) with a completely randomized design (CRD), and the means were compared using the Tukey test at the 5% significance level with the SAS Institute software (2014).

3. Results and discussion

3.1. Chemical quality and fatty acid profile

The results in Table 1 show that individual and mixed goat viscera display high protein (16.76–22.63 g/100 g) and moisture

Table 1
Averages of chemical quality (g/100 g sample) and fatty acid profile (g fatty acid/100 g sample) of goat viscera and their mixture.

Parameters	Mixture of viscera	Heart	Liver	Lung
Moisture	71.22^{ab} ± 0.86	70.94^{b} ± 3.68	63.47^{c} ± 3.29	77.06^{a} ± 1.10
Ashes	0.92^{bc} ± 1.37	0.74^{c} ± 0.23	1.23^{b} ± 0.13	0.68^{c} ± 0.19
Protein	18.55^{b} ± 0.35	16.76^{b} ± 1.38	22.63^{a} ± 3.85	17.19^{b} ± 0.94
Fat	4.01^{b} ± 0.91	6.36^{a} ± 2.81	5.98^{a} ± 1.18	2.26^{b} ± 0.31
SFA	3.27 ^{a} ±0.01	3.29^{a} ± 0.05	2.23^{b} ± 0.03	0.88^{c} ± 0.00
C12:0	0.04^{bc} ± 0.00	0.04^{a} ± 0.01	0.04^{bc} ± 0.00	0.03^{c} ± 0.00
C14:0	0.15^{b} ± 0.01	0.23^{a} ± 0.01	0.07^{c} ± 0.06	0.03^{c} ± 0.01
C15:0	0.04^{b} ± 0.01	0.09^{a} ± 0.01	0.02^{c} ± 0.01	0.03^{bc} ± 0.00
C16:0	1.32^{a} ± 0.01	1.10 ^{a} ±0.71	0.97^{ab} ± 0.21	0.43^{b} ± 0.04
C17:0	0.02^{ab} ± 0.01	0.04^{a} ± 0.03	0.01^{b} ± 0.01	0.03^{ab} ± 0.00
C18:0	1.68^{a} ± 0.08	1.78^{a} ± 0.87	1.10^{a} ± 0.42	0.32^{b} ± 0.04
C22:0	0.02^{a} ± 0.00	0.01^{b} ± 0.00	0.02^{a} ± 0.01	0.01^{b} ± 0.00
MUFA	1.78^{a} ± 0.00	1.77^{a} ± 0.08	1.40^{b} ± 0.03	0.60^{c} ± 0.00
C14:1	0.05^{ab} ± 0.00	0.08^{a} ± 0.02	0.03^{bc} ± 0.02	0.01^{c} ± 0.00
C16:1	0.11^{b} ± 0.01	0.15^{a} ± 0.05	0.07^{b} ± 0.03	0.02^{c} ± 0.00
C17:1	0.03^{b} ± 0.00	0.04^{a} ± 0.01	0.02^{b} ± 0.00	0.01^{c} ± 0.00
C18:1n9t	1.34^{a} ± 0.08	1.24^{a} ± 0.69	0.95^{ab} ± 0.39	0.40^{b} ± 0.04
C18:1n9c	0.01^{a} ± 0.00	0.11^{a} ± 0.01	0.01^{ab} ± 0.00	0.00^{b} ± 0.00
C20:1	0.04^{b} ± 0.03	0.05^{ab} ± 0.02	0.07^{a} ± 0.01	0.01^{c} ± 0.00
C22:1n9	0.19^{ab} ± 0.01	0.10 ^{c} ±0.05	0.24^{a} ± 0.05	0.14^{bc} ± 0.00
C24:1	0.01^{b} ± 0.00	0.00^{d} ± 0.00	0.01^{a} ± 0.00	0.01^{c} ± 0.00
PUFA	0.50^{b} ± 0.00	0.53^{b} ± 0.00	0.55^{b} ± 0.00	0.13^{a} ± 0.00
C18:2n6t	0.33^{ab} ± 0.04	0.43 ^{a} ±0.05	0.32^{b} ± 0.10	0.07^{c} ± 0.00
C18:2n6c	0.02^{a} ± 0.00	0.02^{a} ± 0.00	0.01^{b} ± 0.00	0.01^{a} ± 0.00
C20:2	0.02^{b} ± 0.01	0.02^{b} ± 0.00	0.03^{a} ± 0.01	0.01^{b} ± 0.00
C18:3n3	0.01 ^{ab} ± 0.00	0.00^{b} ± 0.00	0.01^{a} ± 0.00	0.00^{c} ± 0.00
C20:4n6	0.01^{ab} ± 0.01	0.01^{a} ± 0.01	0.00^{ab} ± 0.00	0.00^{b} ± 0.00
C22:2	0.04^{b} ± 0.00	0.03^{bc} ± 0.00	0.06^{a} ± 0.01	0.02^{c} ± 0.00
C22:6n3	0.07^{b} ± 0.01	0.02^{c} ± 0.01	0.12^{a} ± 0.04	0.02^{c} ± 0.00

The different letters on the same line are significantly different (Tukey's test. $P < 0.05$).

(63.47–77.06 g/100 g) contents but lower fat concentrations (2.26–6.36 g/100 g). Among the viscera, lungs had the highest protein content (~75 g/100 g dry weight, d. w.). The high protein content of the mixture (~65 g/100 g d. w.) showed that the viscera are significant nutrient sources, indicating their potential use for the nutritional enrichment of other foods (Honikel, 2011; Nollet & Toldrá, 2011).

The fat content showed greater variation from 2.26 to 6.63 g/100 g, with the heart and liver displaying the highest lipid contents. According to Honikel (2011), the fat content in these by-products is generally low (<5%) and comparable to that of the meat. It is well known that goats can store high amounts of fat in the visceral cavity, which explains the fat percentages obtained. Individually, the viscera showed variable percentage values ($P < 0.05$), and as expected, the mixture had intermediate values. The composition of goat viscera was in accordance with the values for goats and sheep reported by Honikel (2011), Madruga et al. (2003) and Anderson (1988). It is noteworthy that the total protein and lipid contents in the raw material are generally relevant in the decision making relating to the use and recovery of these by-products (Mamelona, Santi-Louis, & Pelletier, 2010).

Regarding the chemical quality, the saturated fats, which are rich in saturated fatty acids, found in goat viscera represent 0.88–3.29 g fatty acids/100 g of sample, followed by monounsaturated fatty acids (0.60–1.78 g fatty acid/100 g of sample) and polyunsaturated fatty acids (0.13–0.55 g fatty acids/100 g of sample). In a detailed analysis (Table 1), 22 fatty acids were identified in the lipid profiles of both the individual viscera and the mixture, of which seven were saturated, eight were monounsaturated, and seven were polyunsaturated.

The heart had the highest values of fatty acids, which is explained by it having the highest fat content. According to Prates, Alfaia, Alves, and Bessa (2011), the fatty acid composition of edible by-products depends on the fatty acid content of the tissue in addition to genetic as well as nutritional characteristics of the animal.

Among the identified fatty acids, stearic (C18:0) and palmitic (C16:0) acids are highlighted, with values varying from 0.32 to 1.78 g fatty acid/100 g of sample and 0.43–1.32 g fatty acid/100 g of sample, respectively. According to Branskalieva, Sahlu, and Goetsch (2000), these fatty acids are among the main acids found in goat meat. The highest concentrations of unsaturated fatty acids were found for C18:1n9t (0.40–1.34 g fatty acid/100 g of sample) and C18:2n6t (0.07–0.33 g fatty acid/100 g of sample).

Madruga, Dantas, Queiroz, Brasil, and Ishihara (2013) emphasize that fatty acids derived from goat products are precursors of volatile compounds and that the use of goat viscera to obtain protein

Table 2
Total amino acid profile (mg/g protein) of goat viscera and their mixture.

Amino acids	Mixture of viscera	Heart	Liver	Lung	Chemical score (Min –Max)
Essential amino acids					
Histidine	0.97^{b} ± 0.05	1.23^{a} ± 0.02	1.19^{a} ± 0.04	1.01^{b} ± 0.03	6.5–3.2
Threonine	2.22^{b} ± 0.07	2.50^{a} ± 0.19	2.56^{a} ± 0.05	2.04^{b} ± 0.04	8.9–11.1
Valine	1.29^{b} ± 0.11	1.51^{a} ± 0.06	1.63^{a} ± 0.04	1.32^{b} ± 0.06	3.3–4.2
Methionine	3.74^{b} ± 0.09	3.55^{b} ± 0.15	4.18^{a} ± 0.17	3.49^{b} ± 0.11	15.9–19
Isoleucine	1.86^{c} ± 0.11	2.26^{b} ± 0.04	2.72^{a} ± 0.06	2.01^{c} ± 0.07	6.2–9.1
Leucine	4.87^{bc} ± 0.03	4.98^{b} ± 0.10	5.66^{a} ± 0.13	4.75^{c} ± 0.06	8.1–9.6
Phenylalanine + Tyrosine	6.85^{bc} ± 0.09	7.57^{b} ± 0.18	8.48^{a} ± 0.05	7.14^{c} ± 0.14	18.1–22.3
Lisine	7.48^{c} ± 0.22	9.27^{a} ± 0.18	9.17^{a} ± 0.15	8.23^{b} ± 0.19	16.6–20.6
Non-Essential Amino Acids					
Aspartic acid	2.17^{c} ± 0.05	2.66^{a} ± 0.14	2.71^{a} ± 0.05	2.38^{b} ± 0.07	NA*
Glutamic acid	3.35^{b} ± 0.14	3.94^{a} ± 0.16	3.31^{b} ± 0.08	3.33^{b} ± 0.10	NA
Serine	1.49^{ab} ± 0.10	1.65^{a} ± 0.09	1.56^{a} ± 0.07	1.34^{b} ± 0.05	NA
Glycine	2.98^{b} ± 0.10	2.91^{b} ± 0.11	3.71^{a} ± 0.02	3.59^{a} ± 0.03	NA
Alanine	4.55^{ab} ± 0.26	4.42^{ab} ± 0.12	4.70^{a} ± 0.22	4.18 ^{b} ± 0.13	NA
Arginine	2.12^{c} ± 0.11	2.25^{bc} ± 0.07	2.80^{a} ± 0.06	2.39 ^{b} ± 0.06	NA
Total amino acids	45.94^{b} ± 0.08	50.70^{a} ± 0.15	54.38^{b} ± 0.05	47.20^{a} ± 0.06	NA

The different letters on the same line are significantly different (Tukey's test. $P < 0.05$).
*NA- Not applicable. Reference FAO/WHO (2007).

hydrolysates increases their potential applicability in food technology as flavorings and functional ingredients. According to Martinez-Alvarez et al. (2015), such properties increase the interest in utilizing these protein hydrolysates in the production of animal or human food.

3.2. Total amino acid profile

Table 2 shows the values of amino acids identified in individual goat viscera and their mixture. All essential amino acids were detected in non-limiting amounts given that their mean chemical

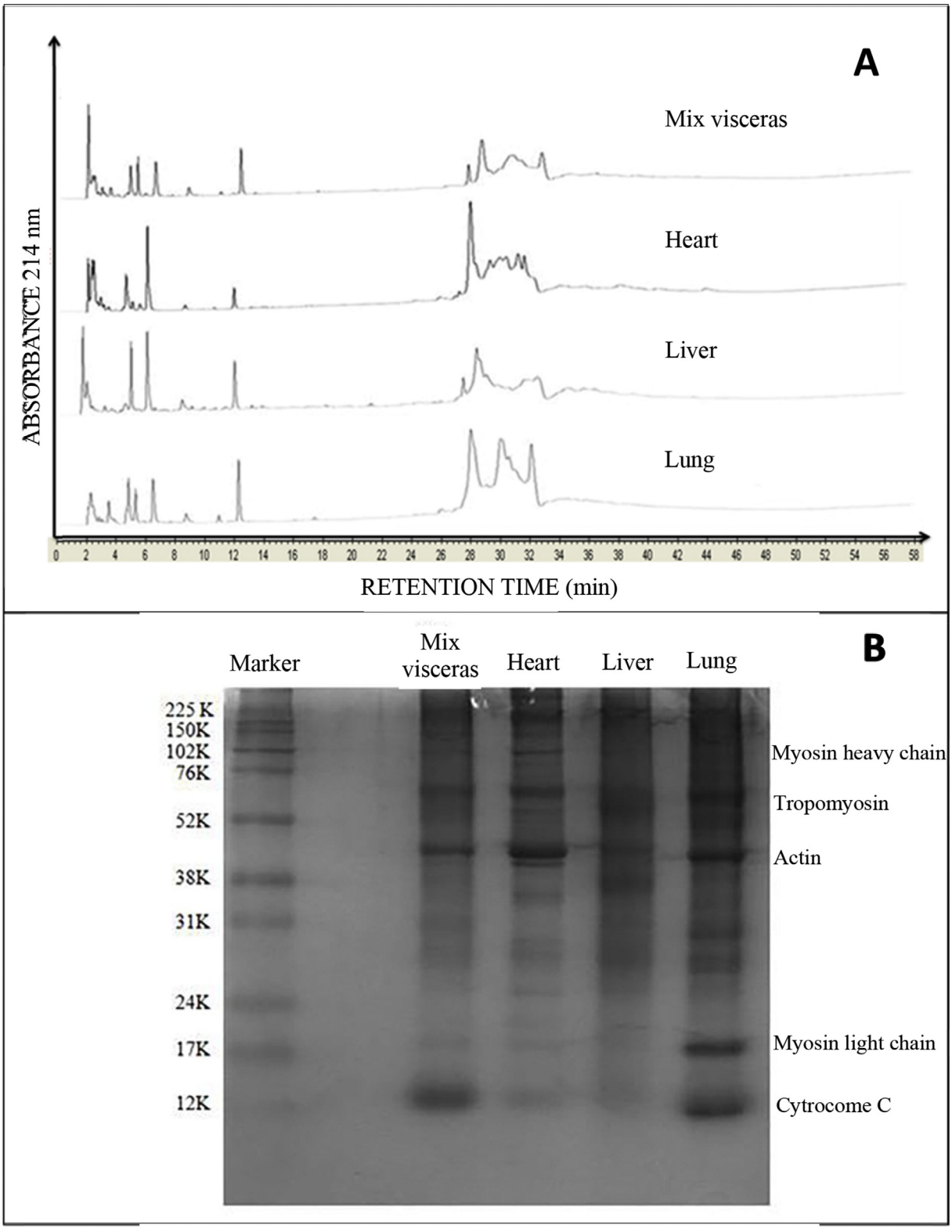

Fig. 1. Profile of hydrophobicity (A) and electrophoretic profile of the protein fractions (B) of goat viscera and their mixture.

score varied from 3.2 to 22.3. According to Pires, Oliveira, Rosa, and Costa (2006), essential amino acids with a chemical score greater than 1.0 can be characterized as excellent protein sources and as having a high biological value capable of nourishing the organism, as they represent values exceeding the daily recommended amounts for adults according to FAO (2007).

At the same time, the amino acid profile found in this study for goat viscera is in accordance with the statements of Aristoy and Toldrá (2011) that edible meat by-products are an excellent nutrient source, exhibit a balanced amino acid profile similar to that found in the meat and include all essential amino acids.

A few authors have studied the amino acid profile in goat viscera-based products, e.g., goat *sarapatel* (Brasil et al., 2014) and goat *buchada* (Queiroz et al., 2013), and they revealed results similar to those found in the present study. This similarity confirms that these by-products are an excellent alternative for nutritional enrichment and a potential source for novel products.

3.3. Hydrophobicity and electrophoretic profiles

The water soluble proteins found in individual samples of viscera and their mixture were eluted for a total of 60 min by reversed-phase (RP) high-resolution liquid chromatograph (HPLC). The hydrophilic peptides were eluted within the first 30 min, followed by the hydrophobic peptides (Fig. 1A). Considering that the chromatograms for the individual viscera and the mixture displayed similar profiles with peaks throughout the run, it can be noted these goat viscera samples exhibited both hydrophilic and hydrophobic peptides. According to Adler-Nissen (1986), hydrophobic peptides pose a disadvantage in producing protein hydrolysates due to their bitter taste. However, this analysis is fundamental for the selection of the enzyme to be used in the proteolysis process. In this sense, the enzyme Alcalase® is listed as an excellent option to minimize bitterness. Alcalase® is a type of serine protease, very efficient to cleave peptide bonds, particularly those with a hydrophobic side chain at the C-terminal position (Adler-Nissen, 1986).

Fig. 1A shows that the sum of peak areas from the chromatograms of goat viscera varied from 0.59 to 1.01 to hydrophilic and hydrophobic content. The heart and lungs samples presented the lowest and highest values, respectively, within the method settings. The mixture and the liver samples had intermediate values of 0.69 and 0.89, and they were significantly different from one another at a 5% significance level. In the study of Costa et al. (2014), peptides resulting from hydrolysates can display antioxidant and antimicrobial activities. These results reinforce the potential of goat viscera as raw materials for products with biological activity, such as protein hydrolysates.

Fig. 1B shows the result of the SDS-PAGE electrophoresis analysis, where the proteins are separated according to their molecular weight (MW) and identified by comparison to a standard composed of a mixture of known MW proteins (Simpson, 2011, p. 926; Lovric, 2011, p. 283; Nelson &, Cox, 2011, p. 1273). The viscera proteins show several protein bands of molecular weight between 12 and 225 kDa, where characteristic meaty proteins were identified, such as strong chain myosin with MW > 100 KDa, tropomyosin with MW of 60 KDa, actin with 45 KDa, light chain myosin with 17 KDa and cytocrome C with 12.5 KDa (Claeys, Uytterhaegen, Buts, & Demeyer, 1995). The protein bands of the viscera mix sample showed all bands of the individual samples (liver, heart and lung), as the cytochrome C band present in the lung sample. In this way the adequate proportion of the mixture (1: 1: 1) was demonstrated in relation to the protein diversity presented individually. Studies by Mora et al. (2014) mention that meat by-products are an excellent substrate for proteolysis because they are rich in diversified proteins. In this context, the potential of the goat viscera mixture to generate hydrolyzed peptides containing bioactive and functional peptides through the enzymatic hydrolysis process was based.

4. Conclusion

Goat viscera are raw materials with the potential to be used in the production of novel food products or functional ingredients due to their nutritional quality, their protein profile, particularly the amino acid composition, hydrophobicity and electrophoretic profiles. These observations confirm that these proteins can be cleaved by enzymatic hydrolysis to produce protein hydrolysates with broad technological applications in addition to adding value to goat slaughtering by-products, as well as showing their potential for application as a functional ingredient in various food industry products.

Conflict of interest

Nothing to declare.

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