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Effects of thermal processing on the flavor molecules of goat by-product hydrolysates

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ABSTRACT

The aim of this study was to obtain flavor molecules from goat by-product hydrolysates, emphasizing the thermal action during processing. A mixture of by-products submitted or not to the inactivation of endogenous enzymes was used, followed by hydrolysis with the proteolytic enzyme Alcalase® (*Bacillus licheniformis*), and autoclaving after hydrolysis. The production of hydrolysates provided both quantitative and qualitative data on the precursors involved in the aromatic formation of protein hydrolysates. The inactivation process of endogenous enzymes resulted in hydrolysates with a higher degree of hydrolysis and greater protein content. The autoclaving process produced a significant increase in the concentration of free amino acids and maltose and a reduction in the glucose content. Application of the two heat treatments resulted in the production of goat by-product protein hydrolysates with different volatile profiles. The goat by-product protein hydrolysate without heat treatment but with autoclaving (HCA), showing a higher concentration of flavor precursors and the formation of heterocyclic volatiles, is expected to impact the aroma quality of goat hydrolysates.

1. Introduction

When goats are slaughtered, by-products are among the edible components that are not part of the carcass (blood, brain, intestines, liver, kidneys, stomach, heart and lungs), and they represent approximately 15–20% of the live weight of the animal (Queiroz, Araújo, Pacheco, & Madruga, 2017a). Some of these internal organs are considered excellent sources of protein, minerals (mainly iron and phosphorus), and vitamins (Queiroz et al., 2013).

A growing effort has been made to use and transform animal by-products into new products and functional ingredients with added value (Mora, Reig, & Toldrá, 2014). In studies on the proper use of goat by-products, it was observed that these by-products are interesting resources for the emergence of new products (Queiroz et al., 2013, 2017a). In this context, the conversion of goat by-products into a flavoring agents may be a commercially viable proposal and innovative use for goat by-products. Protein hydrolysates derived from meat systems are reagents commonly used to produce flavoring compounds (Zou, Kang, Yang, Song, & Liu, 2019); however, knowledge related to goat by-product hydrolysates is very restricted.

Traditionally, there are a variety of technological applications aimed at the use of meat by-products for the production of new molecules (Araújo et al., 2018; Queiroz et al., 2017a; Xing, Liu, Cao, Zhang, & Guanghong, 2019), such as enzymatic and thermal hydrolysis, solvent extraction, microbial fermentation and autolysis (Toldrá, Reig, Aristoy, & Mora, 2017).

Enzymatic hydrolysis is the most widely used method in the food and pharmaceutical industries due to its sustainability, as it does not use organic solvents or products that present toxicity, in addition to its quick and controllable applications. It is also used to modify the physical, chemical and functional properties of foods and to improve their intestinal absorption characteristics without affecting their nutritional value (Benhabiles et al., 2012; Ngo, Vo, Ngo, Wijesekara, & Kim, 2012). A new approach has been created for the application of enzymatic hydrolysis to obtain flavoring compounds, thickening agents, emulsifiers, foaming agents and stabilizers (Zhan, Tian, Zhang, & Wang, 2013).

Studies focusing on the use of goat by-products carried out by our research group have shown that goat by-product protein hydrolysates

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have functional technological properties such as solubility, oil retention capacity, and emulsifying properties (Queiroz & Bezerra, 2017b). During protein hydrolysis, peptides, free amino acids, free fatty acids, and volatile compounds are formed, which interact with each other to give unique flavor characteristics (Mora, Toldrá-Reig, Reig, & Toldrá, 2019). Sometimes thermal processing must be used to ensure the creation of flavor compounds, mainly through the Maillard reaction. To reach this goal, high temperatures are usually employed. A process that is often carried out is autoclaving, which not only leads to the formation of aromatic compounds but also ensures the safety of the material. In the construction of flavor formulations, thermal processing must be used to promote the formation of flavor, through the Maillard reaction. For this, high temperatures are employed, a process that can be carried out by autoclaving, allowing the formation of aromatic compounds in addition to ensuring the safety of the food under study (Peinado, Koutsidis, & Ames, 2016; Song et al., 2017; Zou et al., 2019).

In this context, further studies focusing on the potential of using goat hydrolysates as flavoring agents were carried out. For this, the present work aimed to obtain aromatic molecules from goat internal organ (liver, heart and lung) hydrolysates, emphasizing the effects of inactivation of endogenous enzymes and the use of autoclaving after hydrolysis.

2. Materials and methods

2.1. Materials

Goat by-products (liver, heart and lung) were provided by a local supplier located at the Central Public Market in the city of João Pessoa (Paraíba, Brazil). For the study, a mixture of the liver, heart and lung (at a 1:1:1 ratio) was used. The proteolytic enzyme used to obtain the protein hydrolysate was Alcalase® (*Bacillus licheniformis*), supplied by Novozymes Latino Americana Ltda (Paraná, Brazil), based on previous studies carried out by Queiroz and Bezerra (2017b).

2.2. Experimental design and production of goat by-product hydrolysates

The sanitized and refrigerated by-products were weighed, crushed in a meat processor (model CAF, São Paulo, Brazil), homogenized and frozen (−15 ± 1 °C). Samples of the by-product mixture (30 g) were used to obtain the hydrolysates. Initially, studies on the thermal inactivation of endogenous enzymes were carried out, where the by-product mixture was submitted to heat treatment at 90 °C for 15 min; a control by-product mixture was prepared without thermal inactivation. Inactivated and control goat by-product mixtures were then submitted to the enzymatic hydrolysis process with Alcalase®, according to methodology reported by Queiroz and Bezerra (2017b) and the hydrolysis degree (HD) was defined according to the procedure by Adler-Nissen (1986).

Two by-product hydrolysates were obtained at the end of the process: HC (goat by-product protein hydrolysate without previous thermal treatment or control) and HT (goat by-product protein hydrolysate after heat treatment (90 °C for 15 min with agitation). Then, both hydrolysates were autoclaved for 1 h at 121 °C, resulting in two new products: HCA: goat by-product protein hydrolysate without heat treatment and with autoclaving, and HTA: goat by-product protein hydrolysate with both heat treatment and autoclaving (Fig. 1). The four hydrolysates were then characterized in terms of total protein and lipid contents, fatty acids, free and total amino acids, sugars and volatiles.

2.3. Methods

Proximate composition: Moisture, ash and protein analyses were performed according to the methodology described in AOAC items 950.46A, 920.153 and 928.08, respectively (2010). Lipids were measured according to the procedures of Folch, Less, and Stanley (1957). Carbohydrates were calculated by the difference between 100 and the sum of the percentages of water, protein, fat and ash.

Total amino acids (TAAs): Total amino acids were quantified according to the methodology described by White, Hart, and Fry (1986), in which amino acids were determined in samples previously hydrolyzed at 110 °C/22 h, with 6 mol/L double-distilled hydrochloric acid under vacuum, followed by precolumn derivation of free amino acids with phenylisothiocyanate (PITC).

Free amino acids (FAAs): Free amino acids were extracted from lyophilized samples by shaking with 0.1 mol/L hydrochloric acid (g/mL) for 60 min followed by precolumn derivation of free amino acids with phenylisothiocyanate (PITC) according to White, Hart, and Fry (1986) and Hagen, Frost, and Augustin (1989).

Sugars: Sugar analysis was performed using an Agilent HPLC system, model 1260 Infinity LC (VARIAN, Waters, California, USA), coupled with an Agilent Hi-Plex Ca column (7.7 × 300 mm × 8 μm) at 85 °C with a refractive index detector (RID, VARIAN) and a manual sampler with a 20 μL handle. The flow rate applied was 0.6 mL/min, with a total analysis time of 30 min. The mobile phase used was ultrapure water. Standard solutions were injected to obtain the retention time for each compound ($R^2_{maltose} = 0.9988$; $R^2_{glucose} = 0.9910$; $R^2_{fructose} = 0.9997$; $R^2_{ribose} = 0.9997$). The results are expressed as g of sugars/100 g.

Fatty acids: Direct transesterification of the fatty acids present in the goat by-product mixture was performed as described by O'fallon, Busboom, Melson and Gaskins (2007). Identification and quantification of the fatty acid esters was performed using a gas chromatograph (HP Hewlett Packard 6890 Series GC System) coupled with a fused-silica polar capillary column (Supelco, USA) with dimensions of 100 m × 0.25 mm and a film thickness of 0.2 μm. Fatty acids were identified by comparing the retention times of the methyl esters of the samples with Supelco ME19 kit standards (Fatty Acid Methyl Esters C4-C24). Fatty acid concentrations are presented as g of fatty acid/100 g of hydrolysate.

Volatile profile: Volatile extraction was performed using a solid phase microextraction (SPME) technique with a SPME device (Supelco, Bellefonte, USA) methodology adapted from Breternitz, Bolini, and

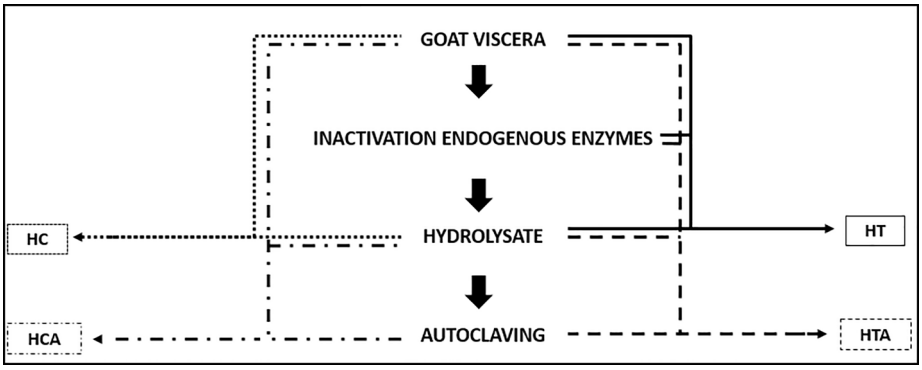


Fig. 1. General process of obtained flavoring molecules from goat by-product.

Hubinger (2017), using 7890B gas chromatograph (Agilent Technologies 5977B, Little falls, DE, USA) coupled to mass spectrometer. The fiber used was 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) and activated according to the manufacturer's conditions. After reaching equilibrium (50 °C for 10 min), the fiber was exposed to the headspace for 30 min of extraction. The following conditions were used: a VF-5MS (30 m × 0.25 mm × 0.25 μm) column; and an initial oven temperature of 40 °C for 2 min, that increased by 15 °C/min to 230 °C and remained for 10 min, for a total run time of 24.6 min. The injector temperature was fixed at 250 °C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min in the 1:20 split injection system. The temperature of the transfer line was 250 °C. The mass spectrometer was operated in electron impact mode (70 eV) and the mass scanning range was from 29 to 350 u.m.a at 3.33 scans/s. Compounds were identified by comparing their mass spectrum and LRI with those of an authentic compound run on a DB-5 column, or comparing their mass spectra with standard compounds present in the database provided by the NIST (National Institute of Standards and Technology, USA) equipment. The results are expressed in digital units (counts). The linear retention index (LRI) of each compound was calculated via the retention times of a homologous series of C6–C25 n-alkanes.

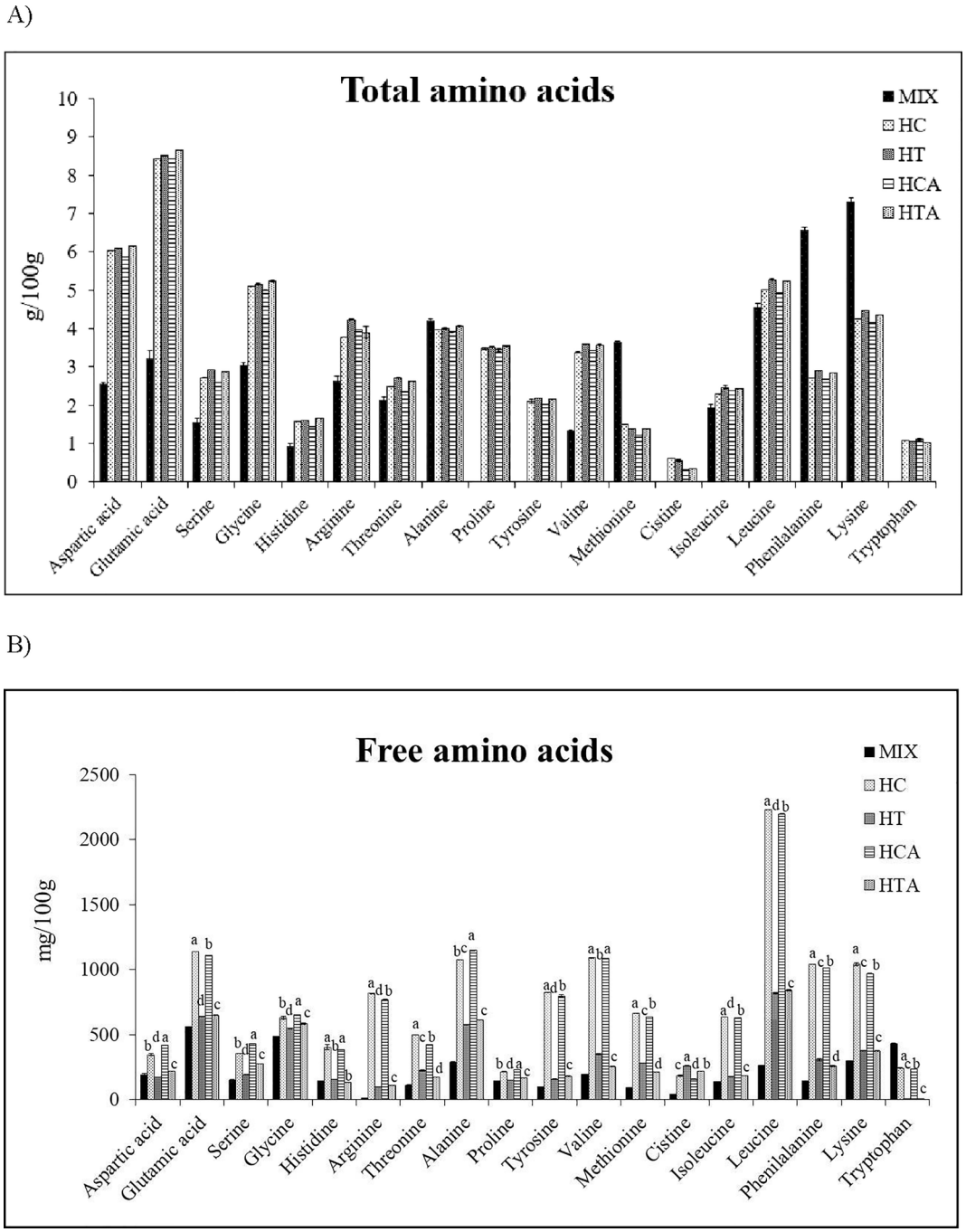
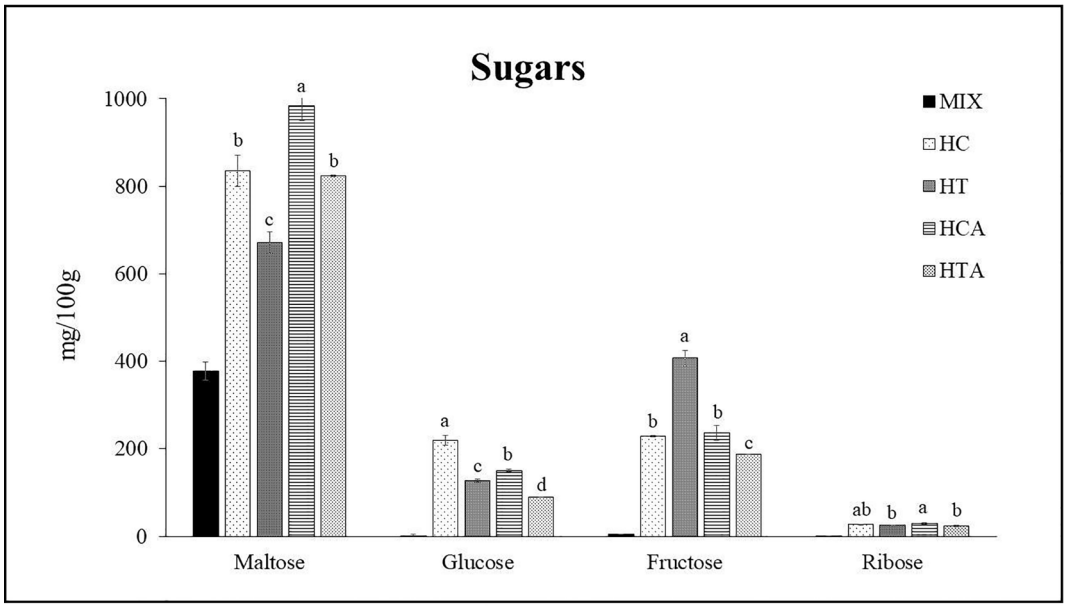


Fig. 2. Total amino acids (A), Free amino acids (B), sugars (C), Fatty acids (D) quantified in goat by-product mixture (Mix) and in goat by-product protein hydrolysates (HC: goat hydrolysate without previous thermal treatment or control, HT: goat hydrolysate after heat treatment, HCA: goat hydrolysate without heat treatment, and with autoclaving, HTA: goat hydrolysate with heat treatment and autoclaving). a,b,c,d different letters indicate significant difference at $p < 0.05$.

C)



D)

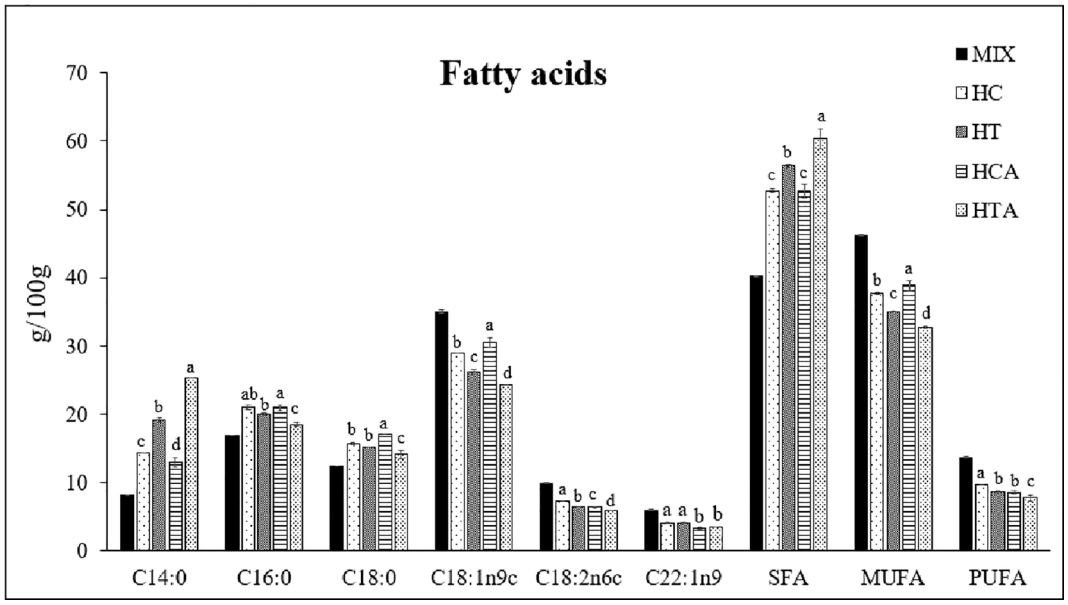


Fig. 2. (*continued*).

2.4. Statistical analysis

Statistical analysis of the goat by-product hydrolysate characterization results was performed using the fully randomized design (FRD) and Statistical Analysis System software (version 11.0) (SAS Institute, 2014). Responses were evaluated using analysis of variance (ANOVA) at the 5% significance level, with treatment averages compared to each other by the Tukey test.

Principal component analysis (PCA) was used to obtain a two-dimensional representation of the hydrolysate samples and the fatty acid, amino acid, sugar and volatile compound contents. Data were processed using XLStat software (version 2014.5.03, Addinsoft, New York, USA).

3. Results and discussion

3.1. Composition of the mixture of goat by-products

The goat mixture (liver, heart and lung) stood out for its high protein content (18.77%) and humidity (74.18%), with low concentrations of lipids (4.82%) and ash (1.20%); the total carbohydrate content was 1.03%. The high protein content revealed its potential for the production of hydrolysates as excellent sources of protein and the potential for the production of peptides, wich are molecules that are used as precursors for the production of flavorings via the Maillard reaction (MR) (Nollet & Toldra, 2011; Queiroz et al., 2017a). The values found are in agreement with the results reported by Queiroz et al. (2017a). The low fat content (<5%) found was expected and is a valuation criterion in the

use of these by-products (Honikel, 2011).

The quantified total amino acid profile revealed the presence of 18 amino acids, which together comprised 45.48 g/100 g of sample (Fig. 2A), and the amino acids lysine, phenylalanine, leucine, alanine and methionine were the most abundant. Eighteen free amino acids (Fig. 2B) were also identified in the sample, with a total concentration of 33.64 g/100 g, and higher amounts of the amino acids glycine, glutamic acid, tryptophan, alanine and lysine were obtained.

The total amino acid profile was similar to that reported by Queiroz et al. (2017a) in goat by-products, as well as the free amino acid profile in goat meat reported by Madruga, Elmore, Oruna-Concha, Balagiannis, and Mottram (2010), where the goat meat contained the highest amounts of the free amino acids glycine, alanine, glutamine, arginine and glutamic acid. Amino acids such as leucine, isoleucine, serine, threonine, valine and phenylalanine are important in the formation of the meaty flavor, supplying Strecker aldehydes and other aromatic compounds such as pyrazines (Madruga et al., 2010).

The total sugar concentration in the by-product mixture was 384.63 mg/100 g (Fig. 2C), and it was found that maltose, fructose, glucose and ribose were the sugars present in the samples. Maltose was present in the highest concentration (377.12 mg/100 g), while fructose, glucose and ribose were present in lower concentrations (4.91 mg/100 g, 1.97 mg/100 g, and 0.63 mg/100 g, respectively). These last three monosaccharides and one disaccharide (maltose) are generally reported in meat products, as they are generated by the glycolysis process or nucleotide degradation (Koutsidis et al., 2008; Madruga et al., 2010). Lower ribose concentrations have been reported in goat meat (Madruga et al., 2010) and bovine meat (Koutsidis et al., 2008).

Nineteen fatty acids were identified in the by-product mixture, mostly consisting of saturated fatty acids (40.2%) and monounsaturated fatty acids (46.2%), followed by polyunsaturated fatty acids (13.6%). The predominant fatty acids were oleic (35%), palmitic (16.8%), stearic (12.4%), linoleic (9.9%) and myristic (8.1%) acids, which were quantified at higher levels in the by-product mixture. The composition of fatty acids found in the by-products is conditioned by the content of fatty acids that compose the animal tissue, as well as by the diet and genetics of these animals. Branskalieva, Sahlu, and Goetsch (2000) found that stearic and palmitic acids are among the main fatty acids identified in goat meat. Queiroz et al. (2017a) highlight that fatty acids found in goat products are precursors of volatile compounds, therefore, goat by-products can be used as raw materials for the production of protein hydrolysates, which are a source of new functional ingredients.

3.2. Production of goat by-product protein hydrolysates

The protein hydrolysate obtained from the by-product mixture after inactivation by endogenous enzymes (HT) reached a hydrolysis degree of 22.75% under the test conditions, differing from the hydrolysate obtained without inactivation (HC), which presented a hydrolysis degree of 18.76% (Fig. 3). This behavior probably occurred by the inactivating endogenous enzymes competing for the same substrate, as well as their inhibition, paving the way for the action of the exogenous enzyme Bacillus licheniformis (Alcalase®) to act specifically without interference from other enzymes. The hydrolysis degree is related to the intensity of the breakdown of peptide bonds, and with this enzyme in particular, is unspecific with high hydrolytic capacity (Halim, Yusof, & Sarbon, 2016; Toldrá et al., 2017). Queiroz and Bezerra (2017b) confirmed that the highest level of hydrolysis (17, 63%) was prepared from the goat viscera protein hydrolysate using the Alcalase® enzyme.

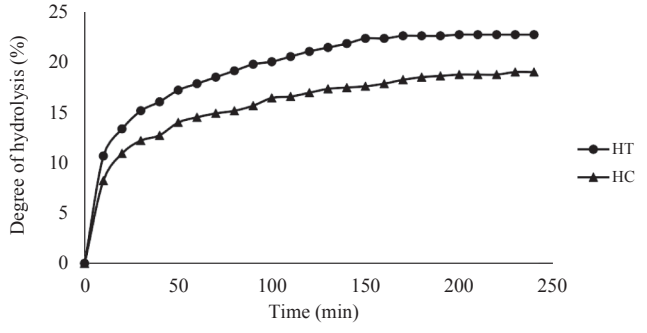


Fig. 3. Hydrolysis kinetics of the goat by-product mixture (1: 1: 1) with (HT) and without (HC) inactivation of endogenous enzymes using alkalase.

In addition, both curves showed common evolution, marked by the high hydrolysis rates during the initial minutes of the reaction, which decreased with hydrolysis time until the enzymatic reaction became stable after the first two hours. This is because hydrolysis products are responsible for enzyme inhibition and can act as agents capable of combating disturbances in the hydrolysis process (Abdelhedi et al., 2016).

Regarding the protein content found from the first two treatments (HC and HT) compared to the initial raw sample (goat by-product mixture), it was observed that hydrolysates had an increase of >60% of this component, which was expected. An increase in the percentage of lipids was also observed, being 61.20% in HC and 40.25% in HT compared to the percentage of lipids in the initial goat raw mixture.

After production of the hydrolysates, the autoclaved samples (HCA and HTA) showed a high protein content relative to the raw material (58.44% and 65.16%, respectively) and values similar to those of the first two hydrolysates (HT and HC).

3.3. Volatiles from goat by-product protein hydrolysates

Thirty-one volatile compounds were identified in the goat by-product protein hydrolysates (Table 1). The majority (25) of these compounds (6 aldehydes, 1 hydrocarbon, 7 ketones, 6 alcohols, 2 furans and 3 carboxylic acids) were formed via lipid degradation. The compounds formed via the Maillard reaction (8) included heterocyclic compounds containing oxygen, nitrogen and sulfur, such as pyrazines (5), a thiazole (1), and a thiophene (1) and nonheterocyclic compounds, such as a Strecker aldehydes (1). The profile of volatile compounds found corroborates studies carried out by Madruga et al. (2010), presenting compounds similar to those observed in cooked goat meat.

The main aromatic compounds identified in the headspace of the unautoclaved hydrolysates (HC and HT) were ketones, alcohols, aldehydes and pyrazines. The same classes of compounds are also in the majority in the autoclave-treated samples (HCA and HTA) but they are present in a different concentration order, with greater amounts of aldehydes followed by ketones, alcohols and pyrazines.

Carbonyl compounds (aldehydes and ketones) are important aromatic compounds (odor-active compounds) that contribute to the general odor attributed to a food. Both can be formed via the Maillard reaction or by lipid oxidation (Cardinal et al., 2020; Wei et al., 2020). Aldehydes, particularly those that are unsaturated are important intermediates and can participate in interactions between amino acids and carbonyl groups, for the subsequent formation of meat-specific volatile compounds (Song et al., 2017).

The contents of saturated aliphatic aldehydes, such as hexanal, octanal and nonanal, were higher than the contents of unsaturated aldehydes (2-nonenal; (*E*)-2-undecenal) after the treatments. This probably resulted from the fact that unsaturated aldehydes are more reactive than saturated aldehydes, and more likely to be involved in the Maillard reaction (Wei et al., 2020). In addition, it is believed that the formation of aliphatic aldehydes occurs via lipid oxidation, as reported in fish meat by Cardinal et al. (2020).

Aliphatic alcohols are known to contribute to meat flavor through unsaturated alcohols due to their lower threshold values. 1-Octen-3-ol, for example, which was present in all treatments, had its content reduced as the thermal treatments were applied. Its formation appears to be associated with fatty acid degradation (Sun et al., 2020) and it is

Table 1
Profile of volatile compounds (area units × 10^5) in goat by-product protein hydrolysates.

Code	Compound	LRI[a]	ID[b]	Goat hydrolysates[c]				Flavor description[d]
				HC	HT	HCA	HTA	
Acids								
	Hexadecanoic acid	1966	A	nd[e]	nd	nd	174.83	Waxy
	Z-11-Octadecenoic acid	2139	B	nd	nd	0.17	158.76	
	Octadecanoic acid	2172	A	nd	nd	0.58	85.40	
Alcohol								
	3-Methyl-3-buten-1-ol	729	A	3.24	3.04	0.64	0.87	Buttery, fatty, sweaty
	1-Pentanol	778	A	7.47	12.28	2.90	6.76	Pungent, fermented, bready, alcoholic, balsamic, sharp
	1-Octen-3-ol	982	A	48.81	35.27	10.95	9.86	Mushroom
	2-Ethyl-1-hexanol	1031	A	nd	nd	12.99	11.17	Mushroom, cucumber, cooked vegetable
	2-Octen-1-ol	1070	A	9.86	16.16	2.02	15.22	Fatty
	2,6-Dimethylcyclohexanol	1117	B	36.47	42.15	16.69	74.19	
Streacker Aldehydes								
	3-Methylbutanal	650	A	nd	nd	8.22	35.89	Malty, dark chocolate, toffee
Aldehydes								
	Hexanal	802	A	39.93	23.45	2.83	4.64	Green, fatty, leafy, vegetative
	Heptanal	903	A	11.76	5.63	4.35	3.24	Fatty, oily, citrus, fruit, green
	Benzaldehyde	966	A	142.88	97.63	95.09	80.10	Roasted pepper, nutty
	Nonanal	1107	A	31.79	18.24	30.14	56.21	Fatty, floral, citrus, green
	2-Nonenal	1164	A	5.99	1.03	7.83	3.38	
	(*E*)-2-Undecenal	1376	A	nd	nd	4.43	5.47	Geranium, green, waxy
Aromatic								
	Methyl benzene	769	A	nd	11.65	nd	nd	Fruity, sweet
Ester								
	Methyl arachidonate	2274	A	nd	nd	nd	4.77	
Furan								
	2-Pentylfuran	994	A	18.90	15.05	26.39	33.74	Fruity, green, sweet, pungent
	5,6,7,7a-Tetrahydro-4,4-7a-trimethyl-2-(4H)-benzofuranone	1557	B	4.23	3.68	4.69	1.46	
Hydrocarbons								
	p-Xylene	872	A	nd	nd	3.64	9.11	
Ketones								
	3-Hydroxy-2-butanone	709	A	11.05	7.31	3.70	13.27	Buttery, fatty, sweaty
	2-Heptanone	892	A	15.38	10.75	18.10	18.55	Soapy, fruity, blue cheese, green
	2,3-Octanedione	982	A	184.38	144.89	18.94	23.35	
	2-Nonanone	1094	A	8.02	2.10	20.61	65.67	Fatty, fruity, musty, blue cheese
	3,5,5-Trimethyl-2-cyclohexenone	1130	B	14.04	5.16	9.05	2.42	
	2-Decanone	1195	A	17.28	7.06	33.04	21.74	Fatty, cucumber, paper
	β-Ionone	1499	B	8.00	5.62	6.94	3.55	
Pyrazines								
	2,5-Dimethylpyrazine	914	A	171.94	11.63	381.38	463.41	Cocoa, roasted nut, roast Beef, medicine
	2-Ethyl-5-methylpyrazine	1005	A	13.41	9.07	224.83	101.37	Fruity
	3,5-Dimethyl-2-ethylpyrazine	1084	A	nd	nd	374.45	142.47	
	2,3-Dimethyl-5-ethylpyrazine	1089	A	3.67	3.88	10.25	36.84	
	2,5-Dimethyl-3-isoamylpyrazine	1322	A	12.00	11.50	13.09	14.29	
Terpenes								
	α-Copaene	1370	B	2.57	1.40	1.06	1.23	Woody
	Caryophyllene	1442	B	3.80	3.12	2.96	1.62	Spicy
Thiazole								
	2-Acetylthiazole	1024	A	nd	nd	117.44	80.33	Roasty, nutty, popcorn, meat
Thiophene								
	2-Acetylthiophene	1097	A	6.38	nd	14.86	nd	

[a] Linear retention indices.
[b] A, mass spectrum and LRI agree with those of an authentic compound ran on DB-5 column; B, tentative identification where mass spectrum agrees with reference spectrum in the NIST/EPA/NIH mass spectral database.
[c] HC (goat hydrolysate without previous thermal treatment or control), HT (goat hydrolysate after heat treatment), HCA (goat hydrolysate without heat treatment, and with autoclaving), HTA (goat hydrolysate with heat treatment and autoclaving).
[d] Aroma description obtained from database available on the web at https://www.flavornet.org/flavornet.html.
[e] nd, not detected.

considered to be an important contributor to the overall meat aroma, as it provides a striking mushroom aroma with a lower threshold (Peinado et al., 2016; Ramalingam, Song, & Hwang, 2019).

The aromatic compound profile of the four hydrolysates demonstrated that the unautoclaved hydrolysates (HC and HT) had a lower number of volatile compounds (26 compounds each) and lower peak area counts (827.82 and 508.75 counts, respectively). Regarding the autoclaved treatments, a higher number of compounds was found:

34 compounds in HCA and 35 in HTA; additionally, the peak counts were higher (1485.25 and 1765.18 counts, respectively).

Changes in the volatile compounds after the autoclaving process mainly showed a decrease in alcohol and aldehyde compound contents and an increase in pyrazines and benzaldehyde. The production of benzaldehyde can occur via the Maillard reaction or through the oxidation or photochemical degradation of toluene or other hydrocarbons. Pyrazines are also generated via the Maillard reaction and usually formed at high temperatures. Pyrazines occurred mainly when the hydrolysates were heated, and represent an important component of the aroma of goat hydrolysates; they were also reported to contribute to the aroma of roasted meat (Cardinal et al., 2020; Wei et al., 2020).

The autoclaving process (samples HCA and HTA) also favored the formation of six new compounds (3-methylbutanal, *p*-xylene, 2-acetylthiazole, 2-ethyl-1-hexanol, 3,5-dimethyl-2-ethylpyrazine and 2-undecenal), which are related to lipid degradation and products from the Maillard reaction. Similar results were observed in the studies carried out by Song et al. (2017) in swine lard hydrolysates, in which the hydrolysis process favored an increase in these volatile compounds.

The 2,5-dimethylpyrazine content was 2.21 times higher in HCA and 39 times higher in HTA compared to the corresponding unautoclaved samples, and the 2-pentylfuran content was 1.4 times higher in HCA and 2.24 times higher in HTA, respectively. The compound 2,5-dimethylpyrazine, which considerably contributes to the aroma of roasted meat and nuts, is believed to have been formed through the Maillard reaction, and therefore a higher amount of this compound would enhance the Maillard notes (Cardinal et al., 2020). In turn, 2-pentylfuran, contributing a grassy and pungent aroma, has a low threshold value in water, with little aromatic contribution to the hydrolysate produced.

3.4. Total amino acids (TAA) and free amino acids (FAAs) of the goat by-product hydrolysates

The total amino acid content for all samples (HC, HT, HCA, HTA) is shown in Fig. 2A. For all samples eighteen amino acids were identified and quantified, and the concentrations of total amino acids did not vary among the hydrolysates. The TAA percentage was 60.55 g/100 g for HC, 62.65 g/100 g for HT, 59.22 g/100 g for HCA, and 62.04 g/100 g for HTA. Glutamic and aspartic acids, glycine, leucine and lysine were the most abundant, together representing more than 45% of the total amino acids found. Similar results were observed in the goat by-product hydrolysates studied by Queiroz and Bezerra (2017b). That same study indicated that the high glutamic acid content enables the use of these hydrolysates as flavorings or flavor enhancers.

The content of free amino acids in the hydrolysate samples is shown in Fig. 2B. There was a significant difference ($p < 0.05$) in the concentrations of free amino acids in the hydrolysates, indicating that the application of temperature was able to change the amount of free amino acids in the hydrolysates. Their concentrations varied: 13.42 mg/100 g in HC, 5.49 g/100 g in HT, 13.28 g/100 g in HCA and 5.45 g/100 g in HTA. The amino acids leucine, glutamic acid, valine, alanine, phenylalanine and lysine were present in higher amounts in the control treatments (HC and HCA), representing more than 56% of the total free amino acids. In treatments where there was inactivation of endogenous enzymes (HT and HTA), higher concentrations of leucine, glutamic acid, alanine and glycine were observed, representing more than 45% of the total free amino acids found. These results corroborate with the results obtained by Madruga, Dantas, Queiroz, Brasil, and Ishihara (2013), who identified glutamic acid, alanine, glycine, leucine and lysine as the main amino acids in goat meat.

The metabolism of amino acids is an important route of formation of aromatic compounds. Through the Ehrlich pathway and subsequent conversion to its alcohol or corresponding acid, amino acids such as leucine, isoleucine, valine and phenylalanine are transformed into 3-methylbutanal, 2-methylbutanol, 2-methylpropanol and 2-phenylethanol, respectively (Sun et al., 2020).

The amino acids leucine, valine and phenylalanine are fundamental in the formation of aroma, supplying Strecker aldehydes and other aromatic compounds, such as pyrazines (Madruga et al., 2010). Additionally, free amino acids obtained from enzymatic hydrolysis are important precursors in the formation of compounds that are characteristic of meat aroma and roasting, and are associated with the flavor of cooked meat (Madruga et al., 2010; Zhan et al., 2013).

It is also notable that free amino acids play important roles in the taste of foods, and may or may not contribute to a sweet, sour, bitter, or umami taste (Sun et al., 2020). Glycine and alanine showed considerably strong sweetness, demonstrating that the sweet taste of lobster and crab, for example, results from the presence of these amino acids. Hydrophobic amino acids, such as phenylalanine, tyrosine, tryptophan, leucine, valine and isoleucine are responsible for a bitter taste. Data reported by Ramalingam et al. (2019) and Nishimura and Kato (1988) showed that almost all peptides made up of these hydrophobic amino acids also give a bitter taste.

Glutamic and aspartic acids, when dissociated, cause sour *stimuli*, and in the presence of a sodium salt, cause an umami taste (Nishimura & Kato, 1988; Sun et al., 2020). Studies have suggested that glutamic acid plays an important role in the umami and savory flavors in meat (Solms, 1969). It has been found that the fraction of oligopeptides rich in glutamic acid can effectively mask the bitter taste. Thus, the addition of these peptides to drugs and beverages that have a bitter taste seems to be a useful alternative to reduce the bitter taste of these products (Nishimura & Kato, 1988).

3.5. Sugars from goat by-product protein hydrolysate

The concentrations of the sugars (maltose, glucose, fructose and ribose) after treatment are shown in Fig. 2C. Considerable variation found in their concentrations is due to the application or not of temperature in the processes to obtain the hydrolysates, with maltose at higher concentrations and ribose at lower concentrations in all by-product hydrolysates.

A significant increase ($p < 0.05$) in maltose concentration was observed in the autoclaved hydrolysates (HCA and HTA), thus demonstrating that autoclaving may have hydrolyzed glycogen, favoring maltose production. Glycogen is the main animal energy reserve compound and is mainly stored in the liver. The increase in the amount of carbohydrates in meat comes from the breakdown of glycogen and ATP, as well as from energy metabolism (Aaslyng & Meinert, 2017). In addition, heating meat affects the carbohydrates and modifies its flavor through the production of volatile compounds (Ramalingam et al., 2019).

On the other hand, the significant reduction ($p < 0.05$) in glucose after autoclaving treatment (31.1% in HCA and 29.8% in HTA) must have resulted from the involvement of glucose in the Maillard reaction. It is known that this is one of the most important reactions in the production of volatile compounds in cooked meat through the formation of certain pentose and hexose degradation products containing carbonyl groups, wich are the main reagents in the formation of relevant heterocyclic compounds (pyrazines, thiazoles and thiophenes), already identified in the volatiles of hydrolysates produced and in cooked goat meat (Karangwa et al., 2013; Madruga et al., 2010).

With a concentration approximately 30 times lower than that of maltose, ribose showed behavior contrary to what was expected, with the initial amount practically unchanged after heat treatment. It is known that among reducing sugars, ribose is the most reactive in the Maillard reaction, thus resulting in less free ribose, corroborating the results found by Madruga et al. (2010).

In turn, fructose has been described as one of the sugars found in higher percentages in goat meat, a fact that was observed in all hydrolysates produced. Its concentration remained unchanged for the control hydrolysate (HC) even after autoclaving. However, it was observed that its concentration was reduced by approximately 54% in the HTA sample.

The heat treatment applied may have favored the reaction of fructose with an amino acid present through the glycation process, generating other Maillard reaction products or advanced glycation end products (Frolov, Hoffmann, & Hoffmann, 2006; Jerić, Versluis, Horvat, & Heck, 2002).

It is also known that sugars have variable levels of sweetness. Sucrose, for example, has by convention a sweetness of 100; thus, all other sugars are compared to it. Fructose is the sweetest (172%), followed by maltose (65%) and glucose (31%) (McCaughey, 2008).

3.6. Fat and fatty acids from goat by-products protein hydrolysates

The cooking methods and conditions (time, temperature and heating rate) are able to modify the chemical composition and nutritional value of meat (Peinado et al., 2016). In meat products, the specific flavor of each species comes from lipids. For example, the tenderization and/or oxidation process of lipid components is responsible for providing the specific flavors of the species identified in meat products. Through their interaction with products from the Maillard reaction, lipid oxidation products generate new flavors associated with cooked meat (Khan, Jo, & Tariq, 2015).

Comparing the initial treatments (HC and HT) and autoclaved treatments (HCA and HTA), the same behavior was observed after the thermic process regarding the fatty acid profile: a significant increase ($p < 0.05$) in the total content of saturated fatty acids and a decrease in the content of mono and polyunsaturated fatty acids.

The metabolism of fatty acids represents an important route of aroma production (Wei et al., 2020). It is clear that unsaturated fatty acids, mainly oleic and linoleic acids, play an important role in the formation of flavoring products, and in this study, they their contents were higher in the control treatment. Hundreds of volatile compounds are derived from the degradation of lipids, such as aldehydes, ketones, alcohols, esters, carboxylic acids, and lactones, which impact the aroma of cooked meat, in addition to alkylfurans and aliphatic hydrocarbons that have a reduce importance in the meat flavor (Mottram, 1998; Sun et al., 2020).

The decomposition of linoleic acid, for example, forms compounds, such as hexanal, 1-octen-3-ol, and 2-pentylfuran, which were observed in the hydrolysates, while the decomposition of linoleic acid can generate, particularly, nonanal and octanal (Ramalingam et al., 2019; Wei et al., 2020; Xia, Mei, Yu, & Li, 2017). Considering the pleasant odors of hexanal, its moderate increase is desirable for maintaining the fresh flavor as long as it is in sufficiently low concentration. Probably, hexanal contributed to the aroma of the HC and HT hydrolysates.

3.7. Principal component analysis

Principal component analysis (PCA) was performed based on the average values of free and total amino acids, sugars and fatty acids in the four hydrolysates (Fig. 4). Through PCA, it was possible to explain 92.28% of the variability among the hydrolysate samples. The two main components (PC1 and PC2) showed variances of 61.70% and 30.58%, respectively.

Differences resulting from the applications of inactivation by endogenous enzymes and autoclaving were significant, with protein hydrolysate samples dispersed in different quadrants and most compounds located in the positive region of PC1.

Therefore, the hydrolysates that underwent inactivation by heat treatment by endogenous enzymes (samples HT and HTA) were better characterized by the content of total amino acids, while the control treatments (HC and HCA) were characterized by free amino acids (alanine, leucine, arginine, glutamic acid, lysine, phenylalanine and valine), as was the case with HCA; therefore, it is expected that this treatment will have a high impact on the formation of volatile compounds.

From the principal component analysis based on the values of volatile compounds (Fig. 5), it was possible to obtain a better representation of the differences among the four hydrolysates, since their distribution in the PCA graph revealed that the hydrolysates were divided into four different quadrants.

Approximately 83.79% of the variability among the hydrolysates could be explained, with the first principal component (PC1) explaining 52.59% of the variability and the second (PC2) explaining 31.20%. Differences due to autoclaving were significant, with HCA and HTA remaining on the positive axis of PC1 and HC and HT on the negative axis. Thus, it was observed that autoclaving had a significant impact on the formation of pyrazines and volatile compounds the meat aroma. In addition, important heterocyclic compounds, such as 2,5-dimethylpyrazine and 2-pentylfuran, strongly characterized the autoclave-treated

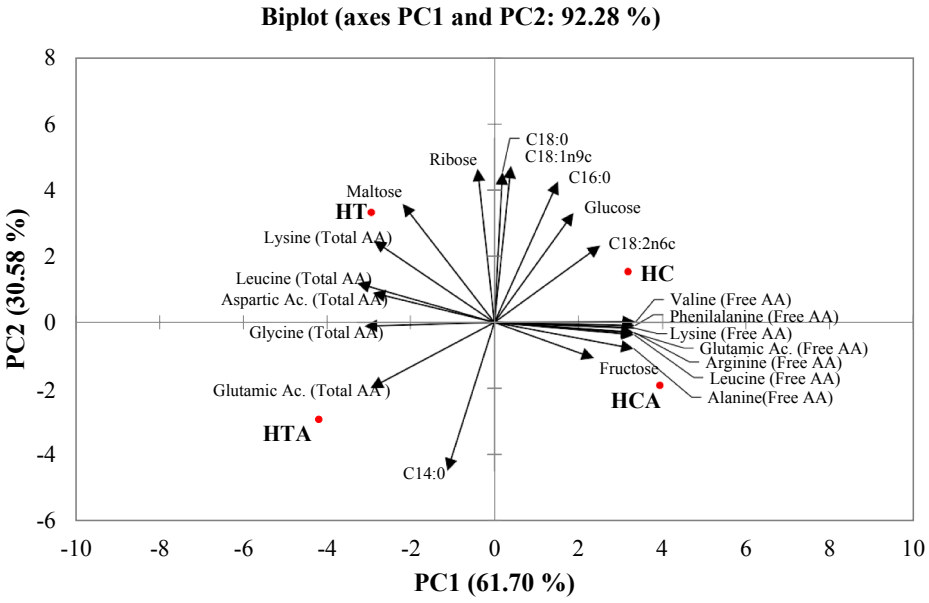


Fig. 4. Biplot for the different goat by-product hydrolysates generated with different heat treatments (HC: goat hydrolysate without previous thermal treatment or control, HT: goat hydrolysate after heat treatment, HCA: goat hydrolysate without heat treatment, and with autoclaving, HTA: goat hydrolysate with heat treatment and autoclaving), and total amino acids, free amino acids, sugars and fatty acids obtained by PCA (PC1: 61.70%, PC2: 30.58%).

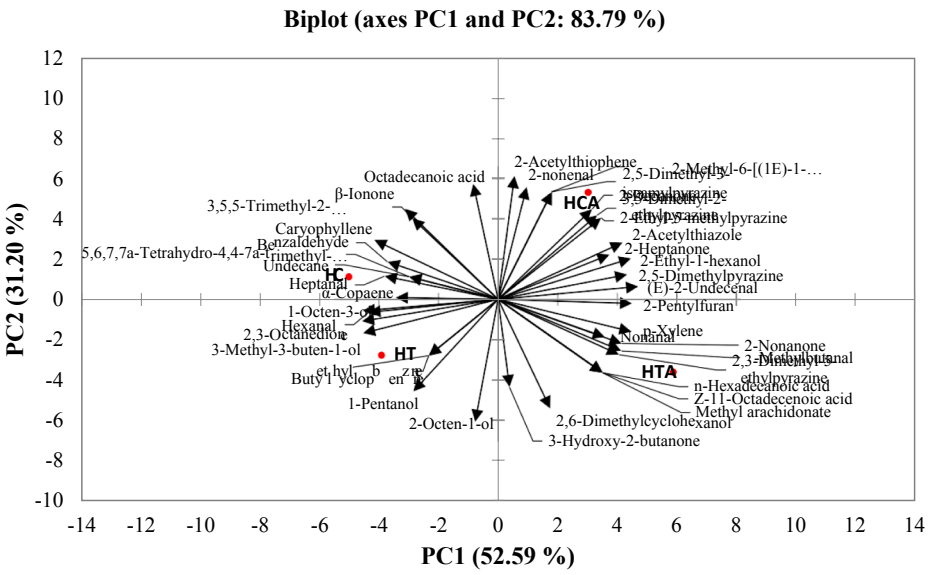


Fig. 5. Biplot for the different goat viscera hydrolysates generated with different heat treatments (HC: goat hydrolysate without previous thermal treatment or control, HT: goat hydrolysate after heat treatment, HCA: goat hydrolysate without heat treatment, and with autoclaving, HTA: goat hydrolysate with heat treatment and autoclaving), and volatile compounds obtained by PCA (PC1: 52.59%, PC2: 31.20%).

samples.

4. Conclusion

The thermal process of inactivating endogenous enzymes and autoclaving sterilization provided quantitative and qualitative data on the precursors involved in the aromatic formation of protein hydrolysates from goat by-products. The inactivation process of endogenous enzymes resulted in hydrolysates with a high degree of hydrolysis and high protein content. The autoclaving process provided a significant increase in the concentration of free amino acids, such as glutamic acid, alanine, arginine, valine, leucine, lysine and phenylalanine, along with a significant reduction in the glucose content and an increase in the maltose content. The fatty acids oleic and linoleic were observed in the products, which play an important role in the formation of the volatile compounds that are characteristic of the meat aroma. The application of two heat treatments resulted in the production of protein hydrolysates with different volatile profiles, demonstrating that the autoclaving process favored the production of pyrazines and other heterocyclic compounds. The HCA process, showing higher concentrations of flavor precursors and a greater formation of heterocyclic volatiles is expected to impact the aroma quality of goat hydrolysates. Further studies using different flavoring formulations and the application of goat protein hydrolysate (HCA) should be carried out.

CRediT authorship contribution statement

Ana Rita Ribeiro de Araújo Cordeiro: Conceptualization, Data curation, Writing - original draft, Investigation. **Lorena Lucena de Medeiros:** Validation, Software. **Taliana Kenia Alencar Bezerra:** Conceptualization, Methodology. **Maria Teresa Bertoldo Pacheco:** Visualization, Supervision, Methodology. **Mércia de Sousa Galvão:** Software, Methodology. **Marta Suely Madruga:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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