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Volatilomic evaluation of protein hydrolysates from free-range chicken bones treated with hot-pressure process

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ABSTRACT

The aim of this study was to develop flavoring agents that can be used in the food industry using protein hydrolysates from free-range chicken bones. The chemical composition of chicken bones was determined and showed high protein and lipid content of 26.27 g/100 g and 12.21 g/100 g, respectively. Enzyme hydrolysis was performed with Flavourzyme® (HF), Alcalase® (HA) and a mixture of both in a 1:1 ratio (HFA). Alcalase® has the highest degree of hydrolysis (20.59%), but the flavoring was then prepared with the Flavourzyme® hydrolysate because the volatiles are present in greater quantity and quality for meat flavor. Finally, glucose or xylose was added to the hydrolysates to obtain the flavorings GF and XF. It was found that XF had a higher browning intensity (Maillard reaction) with luminosity values (L*) ranging from 74.24 to 65.17, and the XF flavoring contained eight more aldehydes than the GF flavoring. Thus, the flavoring made with protein hydrolysate of free-range chicken bones added with xylose showed the aromatic potential of meat and can be used as an ingredient in the food industry.

1. Introduction

Chicken slaughter by-products, which consist of bones, head, skin, feathers, comb, wattles, meat trimmings, blood, fat tissue, feet and internal organs, can account for up to 37% of the animal's total live weight (Mora, Reig, & Toldrá, 2014). Bones from free-range chickens represent a large quantity and quality of protein content (Wang et al., 2016), and according to Bezerra et al. (2020), there is great interest on the part of the food and pharmaceutical industries in exploring the application of different conversion methods to utilize these by-products.

The use of industrial by-products of animal origin through the enzymatic hydrolysis of proteins to obtain flavor precursors has been widely described. Flavors produced by hydrolysates of bovine bones (Chiang, Eyres, Silcock, Hardacre, & Parker, 2019), goat viscera (Cordeiro et al., 2022), volatiles in protein hydrolysates of cod bones (Tan et al., 2018), formation of flavors and volatiles by protein hydrolysates of pig blood (Fu et al., 2019), and production of flavoring from seafood by enzymatic hydrolysis of fish by-products (Peinado, Koutsidis, & Ames, 2016).

Meat aromas and flavors desired by the food industry are mostly produced by the formation of aromatic compounds during the Maillard reaction (MR), oxidative and thermal degradation of lipids, and subsequent combination during formulation (Simon, Mumm, & Hall, 2019). The process of obtaining flavorings is characterized by the development of MR compounds through the application of heat and a reducing sugar that interacts with amino acids present in the protein hydrolysate (Sun et al., 2014). Further, autooxidation and lipid degradation increase with temperature, and the production of hydroperoxides occurs, leading to the formation of aroma-related metabolites by many pathways (Simon et al., 2019). At the end of these processes, their sensory attributes such as aroma, flavor, and color can be added to the final product.

In view of the above, the current study aimed to present an

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Table 1
Mineral content of bones from free-range chicken.

Element (mg/100g)	Average ± Standard Deviation
Calcium	11.565,00 ± 417,00
Copper	0,06 ± 0,006
Iron	9,07 ± 0,23
Phosphorus	4.554,00 ± 80,00
Magnesium	192,00 ± 3,00
Manganese	0,23 ± 0,002
Potassium	283,00 ± 4,00
Sodium	210,00 ± 3,00
Zinc	7,56 ± 0,006

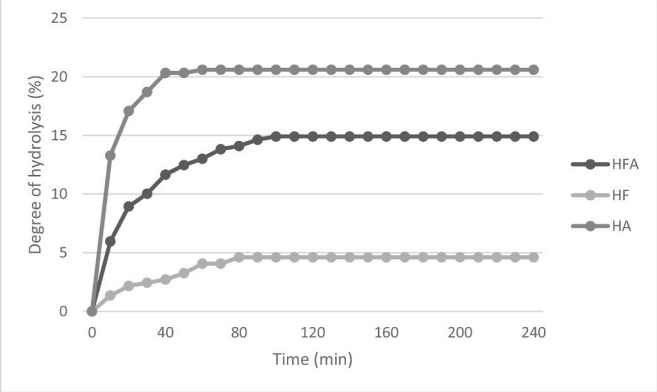

Fig. 1. Hydrolysis kinetics of the free-range chicken bones.

Table 2
Total and free amino acid profile of free-range chicken bones and protein hydrolysates.

Aminoacids (AA)	Total AA (g/100g sample) dry residue from free-range chicken bones[a]	Free AA (mg/100g of protein) Hydrolysates[b]		
		HF	HA	HFA
Aspartic Acid	0,940	8,98^{a}	nd	nd
Glutamic Acid	2105	29,73^{c}	54,84^{b}	75,94^{a}
Serine	1601	67,14^{a}	21,05^{b}	77,49^{a}
Glycine	0,608	106,95^{b}	43,70^{c}	274,19^{a}
Histidine	8334	85,31^{b}	46,28^{c}	126,73^{a}
Arginine	2229	402,93^{b}	109,75^{c}	751,98^{a}
Threonine	1005	148,91^{b}	83,39^{c}	299,62^{a}
Alanine	2034	205,40^{b}	56,53^{c}	236,12^{a}
Proline	2255	54,02^{a}	22,46^{b}	23,27^{b}
Tyrosine	0,628	222,83^{b}	54,15^{c}	257,55^{a}
Valine	1061	214,12^{b}	55,01^{c}	375,96^{a}
Methionine	0,438	99,61^{b}	16,47^{c}	152,60^{a}
Cystine	0,099	15,28^{b}	8,60^{b}	89,75^{a}
Isoleucine	0,455	139,39^{b}	30,33^{c}	206,09^{a}
Leucine	1208	438,29^{b}	37,73^{c}	492,84^{a}
Phenylalanine	1230	283,67^{a}	10,92^{b}	271,37^{a}
Lysine	1488	182,27^{b}	33,99^{c}	425,62^{a}
Total	27,718	2704,83	685,2	4137,12

Averages with different letters within the same row showed significant difference ($p < 0.05$), by Tukey's test.

[a] Mean values of total amino acids contained in the dry residue from free-range chicken bones.

[b] Values referring to the free amino acids contained in the hydrolysates.

alternative use for free-range chicken bones by obtaining protein hydrolysates and then producing a flavoring agent with potential industrial application.

Table 3
Sugar profile of free-range chicken bones and protein hydrolysates.

Sugars (mg/100g)	Dry residue from free-range chicken bones	Hydrolysates		
		HF	HA	HFA
Maltose	342,97 ± 3,84^{a}	132,32 ± 1,92^{c}	175,21 ± 2,21^{b}	135,08 ± 1,91^{c}
Glucose	221,10 ± 6,72^{a}	51,02 ± 0,52^{c}	35,63 ± 0,38^{d}	78,24 ± 0,70^{b}
Fructose	75,00 ± 1,80^{a}	73,02 ± 2,47^{a}	41,96 ± 0,315^{c}	63,98 ± 0,69^{b}
Ribose	4,18 ± 0,10^{ab}	3,56 ± 0,35^{bc}	3,14 ± 0,068^{c}	4,61 ± 0,33^{a}
Total	643,25	259,92	255,94	281,91

HF - Protein hydrolysate from free-range chicken bones prepared with Flavourzyme; HA - Protein hydrolysate from free-range chicken bones prepared with Alcalse; HFA - Protein hydrolysate from free-range chicken bones prepared with the mixture of Flavourzyme and Alcalase (1:1). Averages with different letters within the same row showed significant difference ($p < 0.05$), by Tukey's test.

2. Material and methods

2.1. Material

The slaughtered free-range chickens were purchased at a local market in the city of João Pessoa (Paraíba, Brazil), being reported to the Federal Inspection Service (FIS). To obtain the bones, deboning was started after removing the head, skin, feet, and internal organs. The bones were cleaned with a knife and the residues removed in distilled water. Subsequently, the bones were packaged, labeled, and stored at −20 °C until use, in accordance with Zhan, Tian, Zhang, and Wang (2013) with adaptations.

The proteolytic enzymes used to obtain the protein hydrolysates were Alcalase® (*Bacillus licheniformis),* and Flavourzyme® (*Aspergillus oryzae*), both supplied by Novozymes Latino Americana Ltda (Paraná, Brazil).

2.2. Obtaining dry residue from free-range chicken bones using high pressure and drying

The free-range chicken bones were submitted to a high pressure pretreatment, autoclaved (Phoenix, Araraquara, Brazil) at 121 °C for 4 h, and subsequently dried in an oven at 55 °C for 5 h; then in accordance with Zhan et al. (2013), grinding in a knife mill to obtain a dry bone residue.

The dry bone residue was characterized for determination of moisture, ash, and protein contents in accordance with the AOAC methodology (2010), as described in the respective numbered procedures: 39.1.03, 39.1.09, and 39.1.15. Lipids were measured using the Folch, Lees, and Sloane stanley (1957) methodology.

To quantify the minerals, an inductively coupled plasma optical emission spectrometer (ICP OES 5100 VDV, Agilent Technologies, Tokyo, Japan) equipped with a 27 MHz radiofrequency (RF) source, using the radial view of the optical detector, a peristaltic pump, a double-step cyclonic nebulization chamber, a 1.8 mm quartz torch, and a sea-spray nebulizer were used. As plasma gas, the system used liquid argon (Air Liquide, São Paulo, Brazil). The analytical curves for the minerals were prepared from dilutions of analytical standards at 10 mg/100 mL and 1000 mg/100 mL (Specsol - Quimlab, Jacareí, Brazil) in the ranges from 0.041 to 41.0 mg/100 mL for Ca and Na; 0.061–61.0 mg/100 mL for K; 0.062–62.0 mg/100 mL for P; 0.015–14.5 mg/100 mL for Mg, and 0.001–1.0 mg/100 mL for Cu, Fe, Mn, and Zn, with a correlation coefficient (r) greater than 0.9999.

Table 4
Volatile profile of dried free-range chicken bone residue and protein hydrolysates.

Nr	LRI	Compounds	Peak area (x10$^{5)}$				Aroma description
			DBR[a]	HF	HA	HFA	
Acids							
1	1373	Decanoic acid	nd	1,53^{ab}	1,97^{a}	1,40^{b}	Fatty
Alcohol							
2	765	1-Pentanol	2,33^{b}	4,57^{a}	2,53^{ab}	3,33^{ab}	Pungent, fermented, yeasty, chemical, alcoholic
3	868	1-Hexanol	9,03^{a}	1,39^{b}	1,32^{b}	1,17^{b}	Herbal, chemical, oily, fruity, alcoholic
4	955	2-Hepten-1-ol	nd	1,52^{a}	0,76^{b}	0,59^{bc}	Fatty, pungent, green
5	970	1-Heptanol	0,90^{c}	18,28^{b}	24,57^{a}	18,96^{b}	Green, fruity
6	980	1-Octen-3-ol	3,95^{c}	49,59^{a}	43,33^{ab}	31,93^{b}	Earthy, green, vegetable
7	1067	(E)-2-Octen-1-ol	0,31^{d}	11,38^{a}	9,13^{b}	6,79^{c}	Green, citric, vegetable
8	1071	1-Octanol	0,94^{c}	61,14^{ab}	71,28^{a}	53,65^{b}	Waxy, green, citric, floral
9	1080	1-Nonen-3-ol	nd	0,93^{a}	0,31^{b}	nd	Earthy, green, mushroom
10	1092	2-Methyl-3-octanol	nd	nd	18,77^{a}	nd	
11	1173	1-Nonanol	0,43^{c}	1,90^{b}	2,98^{a}	2,06^{ab}	Floral, fresh
12	1257	(Z)-4-Decen-1-ol	nd	0,72^{a}	0,43^{a}	nd	Waxy, fatty, fruity
13	1273	1-Decanol	nd	5,76^{a}	6,72^{a}	6,24^{a}	Fatty, Waxy, floral, sweet
14	1277	2-Butyl octanol	nd	1,46^{a}	2,16^{a}	1,65^{a}	
Aldehydes							
15	800	Hexanal	14,02^{c}	118,00^{a}	55,96^{b}	83,61^{b}	Green, grass, fatty
16	854	2-Hexenal	nd	1,42^{a}	0,86^{b}	1,02^{ab}	Green, fruity, vegetable
17	899	Z-4-Heptenal	nd	1,06^{a}	0,71^{b}	0,39^{c}	Green, oily, fatty, milky
18	900	Heptanal	0,75^{b}	17,07^{a}	13,04^{a}	18,40^{a}	Green, fresh, fatty
19	958	(Z)-Hept-2-enal	1,30^{c}	33,01^{a}	18,57^{b}	20,72^{b}	
20	962	Benzaldehyde	2,31^{c}	82,81^{a}	61,36^{b}	83,98^{a}	Fruity, almond, oily
21	1003	Octanal	13,30^{b}	81,07^{a}	76,86^{a}	71,04^{a}	Aldehydic, waxy, citric
22	1012	(E,E)-2,4-Heptadienal	nd	9,25^{a}	5,45^{b}	2,52^{c}	Fatty, green, oily
23	1045	Phenylacetaldehyde	nd	3,96^{a}	nd	nd	Green, floral, fermented, earthy
24	1049	(Z)-2-Octenal	nd	2,37^{a}	1,18^{b}	0,86^{c}	Fatty, fruity, nuts, green
25	1058	(E)-2-Octenal	1,38^{c}	64,78^{a}	39,31^{b}	50,62^{ab}	Fatty, fresh, green, herbal
26	1104	Nonanal	4,94^{b}	168,01^{a}	180,01^{a}	167,35^{a}	Aldehydic, waxy, citric, fresh
27	1115	(E,E)-2,4-Octadienal	0,23^{b}	1,72^{a}	1,70^{a}	1,55^{a}	Green, fatty
28	1148	(Z)-Non-2-enal	nd	0,88^{a}	0,76^{a}	0,67^{a}	Fatty, waxy
29	1162	(E)-2-Nonenal	0,31^{b}	34,07^{a}	27,02^{a}	33,74^{a}	Fatty, green, citric
30	1164	3-Ethylbenzaldehyde	nd	1,54^{a}	nd	0,77^{b}	
31	1206	Decanal	0,18^{b}	8,25^{a}	8,85^{a}	8,22^{a}	Aldehydic, sweet, citric, floral, waxy
32	1213	(E,Z)-2,4-Nonadienal	nd	38,87^{a}	33,07^{a}	31,35^{a}	Fatty, chicken soup-like
33	1216	(E,E)-2,4-Nonadienal	nd	0,30^{a}	nd	nd	Fatty, chicken fat-like
34	1223	β-Cyclocitral	nd	1,33^{b}	2,18^{a}	2,04^{ab}	Tropical, herbal, floral, fruity
35	1252	(Z)-2-Decenal	nd	1,17^{a}	1,07^{ab}	0,77^{b}	Fatty
36	1263	(E)-2-Decenal	nd	37,11^{a}	36,74^{a}	41,29^{a}	Fatty, waxy, earthy, mushroom, aldehydic
37	1300	(E,Z)-2,4-Decadienal	Nd	28,67^{a}	24,64^{a}	13,47^{b}	Sweet, fatty, chicken soup-like
38	1309	Undecanal	nd	0,39^{b}	1,20^{a}	1,36^{a}	Aldehydic, waxy, floral, green
39	1317	(E,E)-2,4-Decadienal	nd	163,45^{a}	78,64^{b}	41,88^{c}	Fatty, oily, citric, chicken fat-like
40	1355	(Z)-2-Undecenal	nd	2,35^{a}	1,99^{a}	0,57^{b}	
41	1367	(E)-2-Undecenal	nd	36,23^{a}	32,89^{a}	34,45^{a}	Fruity, fresh, citric, waxy
42	1378	2-Butyl-2-octenal	nd	0,37^{ab}	0,43^{a}	0,56^{a}	Green, aguado, metalic, oily
43	1409	Dodecanal	nd	10,79^{a}	8,62^{b}	6,78^{c}	Aldehydic, waxy, citric, floral
44	1448	(Z)-2-Dodecenal	nd	0,61^{b}	nd	1,65^{a}	
45	1468	(E)-2-Dodecenal	nd	0,33^{a}	nd	0,24^{a}	Herbal, citric, metalic
46	1512	Tridecanal	nd	0,18^{b}	0,18^{b}	0,49^{a}	Aldehydic, fresh, citric
47	1613	Tetradecanal	nd	0,71^{a}	0,55^{a}	0,67^{a}	Waxy, fatty
48	1817	Hexadecanal	nd	1,14^{a}	0,26^{b}	0,28^{b}	
Aromatics							
49	763	Toluene	0,37^{a}	nd	nd	nd	
50	1467	Octylbenzene	nd	0,66^{a}	0,26^{b}	0,23^{b}	
Ester							
51	1126	Methyl octanoate	nd	0,89^{b}	1,41^{a}	1,69^{a}	Waxy, green, sweet, aldehydic, herbal, vegetable
52	1180	Allyl heptanoate	nd	1,33^{c}	8,88^{a}	4,85^{b}	Fruity, sweet, waxy
53	1287	Pentyl hexanoate	nd	0,61^{b}	1,32^{a}	0,52^{b}	
54	1325	Methyl decanoate	nd	7,65^{a}	2,21^{b}	1,59^{bc}	Fermented, oily, fruity
55	1526	Methyl dodecanoate	nd	0,35^{a}	nd	nd	Waxy, mushroom
Furan							
56	993	2-pentyl-furan	0,74^{d}	47,28^{c}	167,87^{a}	77,67^{b}	Fruity, green, earthy, vegetable
Hydrocarbons							
57	1000	Decane	0,45^{c}	4,95^{a}	3,81^{b}	0,37^{c}	
58	1064	2-Methyldecane	nd	nd	10,67^{a}	nd	
59	1200	Dodecane	0,75^{a}	nd	nd	nd	
60	1300	Tridecane	nd	2,40^{a}	2,14^{ab}	1,74^{b}	
61	1348	5-Methyltridecane	nd	3,92^{ab}	4,11^{a}	5,02^{a}	
62	1371	3-Methyltridecane	nd	0,84^{a}	0,99^{a}	1,08^{a}	
63	1500	Pentadecane	nd	0,23^{b}	0,41^{a}	0,21^{b}	Waxy
64	1539	2,6,10-Trimethyltetradecane	nd	0,29^{a}	nd	0,19^{a}	
65	1600	Hexadecane	nd	0,41^{a}	nd	0,27^{a}	
Ketones							

(*continued on next page*)

Table 4 (*continued*)

Nr	LRI	Compounds	Peak area (x10$^{5)}$				Aroma description
			DBR[a]	HF	HA	HFA	
66	891	2-Heptanone	0,39^{c}	5,14^{b}	8,72^{a}	8,16^{a}	Cheesy, spicy, fruity, herbal, woody
67	984	2,5-Octanedione	5,29^{c}	66,17^{a}	28,81^{b}	37,14^{b}	
68	1031	3-Ethyl-2-methyl-1,3-hexadiene	0,37^{c}	18,18^{b}	24,82^{ab}	28,74^{a}	
69	1040	3-Octen-2-one	nd	25,98^{b}	35,50^{a}	21,58^{b}	Earthy, mushroom, sweet, oily
70	1091	3,5-Octadien-2-one	0,48^{c}	45,90^{a}	24,74^{b}	29,61^{b}	Fatty, fruity, mushroom
71	1142	3-Non-3-en-2-one	nd	1,52^{b}	2,95^{a}	1,94^{b}	Fruity, oily, spicy
72	1193	2-Decanone	0,27^{c}	12,44^{b}	23,24^{a}	14,45^{b}	Floral, citric, fatty
73	1283	3-Undecanone	nd	6,40^{a}	3,33^{b}	5,54^{a}	
74	1491	β-Ionone	nd	2,44^{a}	2,74^{a}	2,17^{a}	Floral, woody, sweet
Others							
75	1174	1,3,5-Undecatriene	nd	0,66^{b}	1,11^{a}	0,80^{b}	
76	1307	Dibutylformamide	nd	1,09^{a}	0,89^{a}	0,81^{a}	
77	1397	1-Tetradecyne	nd	0,27^{a}	0,23^{a}	0,33^{a}	
78	1519	2,4-Di-*tert*-butylphenol	nd	0,60^{a}	0,13^{b}	nd	
Pyrazines							
79	917	2,6-Dimethylpyrazine	nd	0,22^{b}	nd	53,81^{a}	Chocolate, nuts, roast, roasted meat
Pyridines							
80	1202	2-Pentylpyridine	nd	6,09^{a}	6,49^{a}	3,07^{ab}	Fatty, mushroom, herbal
Sulfur-containing							
81	1022	2-Acetylthiazole	0,77^{a}	0,59^{ab}	0,37^{bc}	0,24^{c}	Popcorn, nuts, peanut, hazelnut
Terpenes							
82	1025	*p*-Cymene	0,23^{c}	1,17^{ab}	0,79^{bc}	1,58^{a}	Terpenic, fresh, citric, woody, spicy
83	1030	Limonene	0,50^{a}	0,43^{a}	0,25^{a}	0,29^{a}	Citric, fresh
84	1453	Geranyl acetone	nd	0,63^{a}	0,56^{a}	0,43^{a}	Floral, fresh, fruity

HF - Country chicken bone protein hydrolysate prepared with Flavourzyme; HA - Country chicken bone protein hydrolysate prepared with Alcalase; HFA - Country chicken bone protein hydrolysate prepared with a mixture of Flavourzyme and Alcalase (1:1).
Means with different letters within the same row showed significant difference ($p < 0.05$), by Tukey test.
[a] DBR - Dry bone residue.

2.3. Preparation of the free-range chicken bone hydrolysate

Three protein hydrolysates were prepared using the dry residues of free-range chicken bones: HF – Protein hydrolysate from dry residues of free-range chicken bones using Flavourzyme®; HA – Protein hydrolysate from dry residue from free-range chicken bones using Alcalase®; and HFA – Protein hydrolysate from dry residue from free-range chicken bones using a mixture of Flavourzyme® and Alcalase® (1:1).

Enzymatic hydrolysis of the dry bone residues was performed using 10g of residue (crude sample) which was transferred to a jacketed beaker in a previously heated thermostated bath. 40 mL of ultrapure water was added to the system, with constant stirring and continuous pH checking. The proteolytic enzymes (Alkalase®, Flavourzyme, or a 1:1 mixture) were added, and the pH and temperature parameters were then established and maintained in accordance with the recommended optimal values, as provided by the enzyme manufacturers. To prepare the hydrolysate for the enzyme mixture (HFA), Alcalase® was initially used for 120 min at a pH of 8.0; afterwards the pH was adjusted for the use of Flavourzyme, to a value of 7.0 for 120 min. The hydrolysis was controlled for 240 min and enzymatic inactivation was performed using a temperature of 95 °C for 10 min in accordance with Zhan et al. (2013). The hydrolysates were centrifuged (Solab, Piracicaba, Brazil) at 12,000 g for 10 min.

After these processes, the protein hydrolysates were characterized for total and free amino acids and sugar profiles and evaluated for formation of aromatic compounds and/or precursors to the Maillard reaction or lipid oxidation. To elaborate the flavoring, the hydrolysate selected was used in a third stage (described in item 2.4). The degree of hydrolysis was determined by base consumption, using the methodology described by Adler-Nissen (1986).

2.4. Preparation of the flavoring product

The MR products were obtained using Flavourzyme® and mixing the chicken bone hydrolysates with either glucose (GF) or xylose (XF), at a 1:0.068 (protein/sugar w/w) ratio. The flavorings made with glucose (hexose) and xylose (pentose) were respectively called GF and XF. The mixtures were adjusted to pH 6.5 with 0.5 M HCl, and autoclaved at 113 °C for 10 min in accordance with Chiang et al. (2019).

2.5. Methods

2.5.1. Total and free amino acid profile

The total amino acids were hydrolyzed at 105 °C, and then derived with phenylisothiocyanate (PITC) in accordance with the methodologies proposed by White, Hart, and Fry (1986). Free amino acids were extracted using a milder process that involved orbital shaking for 60 min with 0.1 mol/L hydrochloric acid, and then derived with phenylisothiocyanate (PITC), in accordance with Hagen, Frost, and Augustin (1989).

Separation of the free and total amino acids derived (phenylthiocarbamoyl amino acid/PTC-aa) was performed in a High Performance Liquid Chromatograph (Shimadzu Corporation, Tokyo, Japan), with a reverse-phase column C18 - Luna - Phenomenex (250 mm × 4.6 mm, 5 μm; Phenomenex Inc., Torrence, CA, USA). The mobile phases consisted of an acetate buffer pH 6.4, and a 40% acetonitrile solution. Sample injection was performed automatically (50 μL) and detection took place at 254 nm. Chromatographic separation was performed at a constant flow rate of 1 mL/min at 35 °C. The chromatographic run time was 45 min, and the results were expressed in mg of amino acid per 100 g of sample. Quantification was performed by adding the internal α-aminobutyric acid standard and identified by comparison to a mixture of standards.

2.5.2. Sugar profile

The sugar profile was determined in accordance with Zeppa, Conterno, and Gerbi (2001). 2g of the sample were used in a 50 mL beaker and the weight was recorded. The material was transferred to a mini--Turrax® flask (IKA Works, Wilmington, USA), with the aid of 10 mL of ultra-pure water, and then homogenized for 10 min and transferred to a 15 mL falcon tube. Subsequently, centrifugation was performed at room temperature for 10 min, followed by filtering the supernatant through qualitative filter paper and then through a syringe filter with 0.45 μm diameter pores. The extract was used to determine sugar profile. When

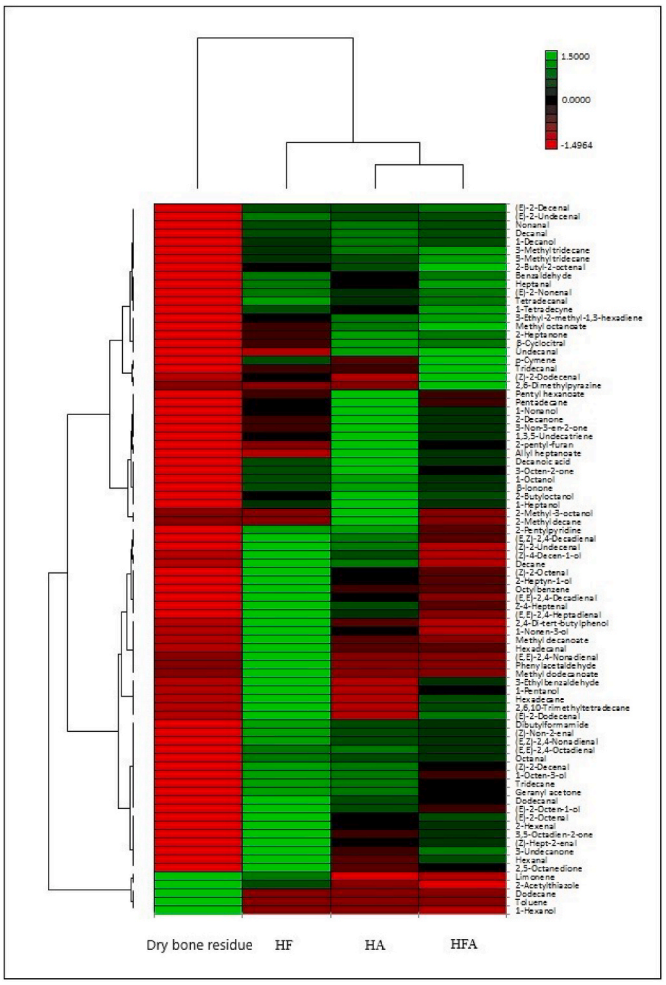

Fig. 2. Heat map of the volatile components of protein hydrolysates.

Table 5
Instrumental brightness (L*) values of the hydrolysates.

Heat treatment	FLAVORINGS	
	FG	FX
Before	72,81 ± 0,51	74,24 ± 0,24
After	69,45 ± 0,03	65,17 ± 0,11

necessary, the extract was stored at freezing temperature to increase its stability.

The total sugar content of the chicken bones and protein hydrolysate were determined by VARIAN High Performance Liquid Chromatography (Waters, California, USA), using a refractive index detector (Varian 356 - LC), equipped with an isocratic solvent system, a "Rheodyne" valve with a 20 μL handle; coupled with a Hi-plex Ca column (300 mm × 7.7 mm), and an oven temperature of 85 °C (Hi-plex Ca).

The processing software used was the GALAXIE Chromatography Data System. The chromatograms of the samples were compared with standards of the analyzed components, and quantification was performed by area composed from a calibration curve for each compound, in five concentrations, depending on the analyzed compound. Results were expressed in milligrams of compound per 100 g of sample.

2.5.3. Instrumental color

The determination of instrumental color parameters (L*, a* and b*) was performed using a digital colorimeter Model CR300 (Minolta, Osaka, Japan). The parameters black/white (L*), red/green (a*), and yellow/blue (b*) were determined in accordance with Commission Internationale de L'éclairage (CIE, 1986) specifications.

2.5.3.1. Volatile profile. The extraction of volatiles was performed using the headspace solid phase microextraction technique (HS - SPME) with SPME device (Supelco, Bellefonte, USA), a methodology adapted from Cordeiro et al. (2020). An aliquot of 2 ml of hydrolysate or 4 g of dry bone was transferred to a 20 ml glass vial and immediately sealed with a Teflon-coated septum cap. The volatiles were extracted at 50 °C reaching equilibrium for 10 min. The Divinylbenzene/Carboxene/Polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm fiber was then exposed to the headspace for 30 min of adsorption. The fiber used was conditioned in accordance with the manufacturer's specifications before the extraction procedure.

Chromatographic analyses were performed using a Gas Chromatograph 7890B (Agilent Technologies 5977B, Little falls, DE, USA) equipped with a VF-5MS column (30 m × 0.25 mm × 0.25 μm), coupled to a Mass Spectrometer. The following conditions from the methodology of Sun et al. (2014) were used: initial oven temperature of 40 °C for 3 min, which increased from 5 °C.min^{-1} to 120 °C and 10 °C.min^{-1} to 230 °C and remained for 5 min, for a total run time of 35 min. The injector temperature was set at 250 °C. Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1} in the split-less injection system. The transfer line temperature was 250 °C. The mass spectrometer was operated in electronic impact mode (70 eV) and the mass scan range was from 50 to 400 mz^{-1} at 4.44 scan.s^{-1}.

Compounds were identified using the NIST library database (2014) combined with a mass spectrum and linear retention index. The linear retention index (LRI) of each compound was calculated using the retention times of a homologous series of C6–C20 n-alkanes. Analyses were performed in triplicate and results were expressed as total chromatographic peak area.

2.6. Flavoring sensory analysis (GF and XF)

Sensory analysis - Quantitative Descriptive Analysis (QDA) was conducted in accordance with the methodology adopted by Zhan et al. (2013), to obtain the sensory attributes of the flavorings. Twelve trained panelists, aged between 22 and 40 years, were selected according to availability and motivation to participate on every day of the experiment.

The analysis was performed in a sensory laboratory at the Federal University of Paraíba. Four specific training sessions were performed: in the first session, the panelists discussed aroma and color characteristics for the sensory attributes. In the second and third sessions, they were trained to adopt a consensus on potential descriptors, evaluating color by the intensity of brown, as well as the following aroma attributes: artificial chicken broth, cooked chicken bone, seafood, roasted chicken, and rancidity. The samples were then evaluated in triplicate, using a 10-point range scale (0 for none and 10 for extremely strong).

The liquid samples were randomly coded in three-digit numbers to avoid the ordering effect, and placed in screw-top jars. The reference adopted for the appearance attribute was the color palette in shades of brown, from the weakest - B69E81, to the strongest - 90724F, and labeled as the attribute "brown intensity". For the aroma attributes, associated with the chicken flavors we used: Nissin brand chicken instant broth – 5g of the sachet diluted in 750 mL of water, (labeled as "artificial chicken broth"); chicken soup, using thigh bones and chicken thigh bones cooked in a pressure cooker, then crushed – 118.5g of bones to 500 mL of water, (labeled as "cooked chicken bone"); the sweet aroma inherent to seafood, (shrimp broth, cooked without heads, but with the shells) - i) a strong reference (labeled as "strong seafood") with 125g of shrimp in 190 mL of water, and ii) a weak reference (labeled as "weak seafood") using 95 mL of the previous broth, and adding 380 mL of

Table 6
Profile of volatile compounds in the flavorings.

Nr	LRI	Compounds	p-value	Peak area (x10^5)		Aroma description
				FG	FX	
Acids						
1	1373	Decanoic acid	<0,0001	0,24	1,14	Fatty
Alcohol						
2	765	1-Pentanol	0,0121	2,31	1,61	Pungent, fermented, bready, alcoholic
3	955	2-Heptyn-1-ol	0,9728	0,44	0,44	
4	970	1-Heptanol	0,2844	9,02	9,54	Green, fruity
5	980	1-Octen-3-ol	0,2794	14,66	13,27	Earthy, green, vegetative
6	1002	4-Ethylcyclohexanol	0,0207	0,43	0,67	
7	1032	2,4-Dimethylcyclohexanol	0,0011	1,34	0,85	
8	1067	(E)-2-Octen-1-ol	0,9627	2,75	2,76	Green, citrus, vegetable
9	1071	1-Octanol	0,2746	16,34	18,09	Waxy, green, citrus, floral
10	1080	1-Nonen-3-ol	0,0513	0,45	0,58	Earthy, green, mushroom
11	1173	1-Nonanol	0,0063	0,23	0,41	Floral, fresh
12	1273	1-Decanol	0,0809	0,87	1,42	Fatty, waxy, floral, sweet
13	1277	2-Butyl octanol	0,0013	0,43	1,10	
Aldehydes						
14	699	Pentanal	0,0170	2,76	5,20	
15	800	Hexanal	0,0085	87,63	75,45	Green, grass, fatty
16	854	2-Hexenal	0,3549	0,54	0,44	Green, fruity, vegetable
17	868	1-Hexanol	0,0010	1,00	0,76	
18	899	Z-4-Heptenal	<0,0001	0,27	0,07	Green, oily, fatty, milky
19	900	Heptanal	0,4835	11,43	11,97	Green, fresh, fatty
20	958	(Z)-Hept-2-enal	0,4120	6,09	5,54	
21	962	Benzaldehyde	0,0016	40,16	25,50	Fruity, almond, oily
22	1003	Octanal	0,0006	28,35	45,87	Aldehydic, waxy, citrus
23	1012	(E,E)-2,4-Heptadienal	0,0095	0,63	0,39	Fatty, green, oily
24	1045	Phenylacetaldehyde	0,0053	2,51	14,32	Green, floral, fermented, earthy
25	1049	(Z)-2-Octenal	0,0059	0,22	0,33	Fatty, fruity, nuts, green
26	1058	(E)-2-Octenal	0,4351	11,06	11,91	Fatty, fresh, green, herbal
27	1104	Nonanal	0,0047	23,93	49,78	Aldehydic, waxy, citrus, fresh
28	1115	(E,E)-2,4-Octadienal	0,0925	0,83	0,70	Green, fatty
29	1148	(Z)-Non-2-enal	0,0001	0,19	0,28	Fatty, waxy
30	1162	(E)-2-Nonenal	0,0132	5,05	7,88	Fatty, green, citrus
31	1164	3-Ethylbenzaldehyde	0,5374	0,54	0,59	
32	1206	Decanal	0,0106	1,32	2,60	Aldehydic, sweet, citrus, floral, waxy
33	1216	(E,E)-2,4-Nonadienal	0,0141	3,66	5,23	Fatty, green, waxy, chicken fat
34	1223	β-Cyclocitral		nd	0,49	Tropical, herbal, floral, fruity
35	1252	(Z)-2-Decenal	0,0043	0,25	0,44	Fatty
36	1263	(E)-2-Decenal	0,0012	4,41	11,13	Fatty, waxy, earthy, mushroom, aldehydic
37	1300	(E,Z)-2,4-Decadienal	0,0027	7,20	15,33	
38	1309	Undecanal	0,6128	0,52	0,56	Aldehydic, waxy, floral, green
39	1317	(E,E)-2,4-Decadienal	0,0006	38,20	85,89	Chicken fat, oily, citrus
40	1367	(E)-2-Undecenal	0,0019	3,81	8,32	Fruity, fresh, citrus, waxy
41	1378	2-Butyl-2-octenal	0,2300	0,13	0,17	Green, watery, metalic, oily
42	1409	Dodecanal	0,0013	1,15	2,71	Aldehydic, waxy, citrus, floral
43	1435	2,4-Undecadienal		nd	0,35	
44	1448	(Z)-2-Dodecenal		nd	0,23	
45	1476	4-Pentylbenzaldehyde		nd	0,18	
46	1512	Tridecanal		nd	0,24	Aldehydic, fresh, citrus
47	1613	Tetradecanal		nd	0,19	Waxy, fatty
48	1715	Pentadecanal		nd	0,15	
49	1817	Hexadecanal		nd	0,23	
Aromatics						
50	763	Toluene	0,1193	1,64	0,56	
Ester						
51	1126	Methyl octanoate	0,0133	0,23	0,40	Waxy, green, sweet, aldehydic, herbal, vegetable
52	1180	Allyl heptanoate	0,0021	0,85	1,27	Fruity, sweet, waxy
53	1325	Methyl decanoate	0,0009	1,33	0,24	Fermented, oily, fruity
Furan						
54	791	2-Propylfuran	0,0130	0,15	0,28	
55	993	2-Pentyl-furan	<0,0001	41,95	87,19	Fruity, green, earthy, vegetable
56	1226	3-Phenylfuran	0,2469	1,40	1,08	
Hydrocarbons						
57	1200	Dodecane	0,0374	0,11	0,29	
58	1300	Tridecane	<0,0001	0,56	1,26	
59	1348	5-Methyltridecane	<0,0001	1,90	5,05	
Ketones						
60	891	2-Heptanone	0,0025	3,73	5,06	Cheesy, spicy, fruity, herbal, woody
61	984	2,5-Octanedione	0,0818	4,83	6,38	
62	1031	3-Ethyl-2-methyl-1,3-hexadiene	0,8997	6,27	6,31	
63	1040	3-Octen-2-one	0,0480	11,86	13,31	Earthy, mushroom, sweet, oily
64	1091	3,5-Octadien-2-one	0,0022	12,47	18,37	Fatty, fruity, mushroom
65	1142	3-Non-3-en-2-one	0,0273	0,90	1,73	Fruity, oily, licorice, spicy

(*continued on next page*)

Table 6 (*continued*)

Nr	LRI	Compounds	p-value	Peak area (x10^5)		Aroma description
				FG	FX	
66	1193	2-Decanone	<0,0001	1,92	4,35	Floral, citrus, fatty
67	1283	3-Undecanone	0,0479	0,46	0,73	
68	1491	β-Ionone	0,0014	0,21	0,46	Floral, woody, sweet
Amide						
69	1307	Dibutylformamide	0,1044	0,29	0,35	
Phenol						
70	1139	2-Ethylphenol	0,0454	0,28	0,53	
Pyrazines						
71	917	2,6-Dimethylpyrazine	0,0124	0,26	0,12	Chocolate, nuts, roast, roast beef
Pyridines						
72	1202	2-Pentylpyridine	0,0012	0,30	1,02	Fatty, mushroom, herbal
Sulphur-containing						
73	1022	2-Acetylthiazole	0,3376	0,18	0,09	Popcorn, nuts, peanut, hazelnut
Terpenes						
74	1237	Pulegone	0,0004	0,23	0,53	
75	1483	α-Curcumene	0,1012	0,10	1,83	

FG – Flavoring with glucose added; FX – Flavoring with xylose added; p-values that presented significant difference ($p < 0.05$), by Sudent T test.

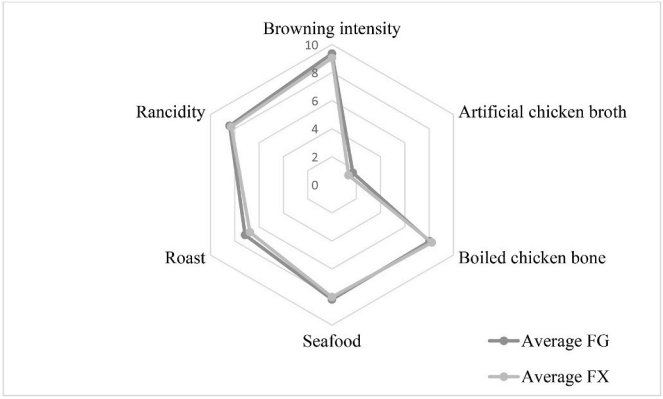

Fig. 3. Sensory evaluation of flavorings of free-range chicken bones.

water; roasted chicken, using grilled chicken breast fillet, without seasonings, (labeled as "roasted"); and finally, lipid oxidation, using slightly oxidized soybean oil for the weak reference and highly oxidized soybean oil for the strong reference, (labeled as "rancidity").

2.7. Statistical analysis

The data obtained from the hydrolysates and flavoring analyses were evaluated by analysis of variance (ANOVA) using the Statistical software Analysis System version 11.0 (SAS, 2014), based on significance levels of 5%, followed by the Tukey test (hydrolyzed) or Student 's T test (flavoring) to compare the means.

3. Results and discussion

3.1. Chemical characterization and mineral quantification of free-range chicken bone dry waste

The dry residue of the free-range chicken bones showed high protein and lipid content values; respectively 26.27 g/100g and 12.21 g/100g. Dong et al. (2014) mention a similar result for crude protein content in chicken bone extract (25.59%), and as for free-range chicken bone lipid content, Wang et al. (2016) reported 13.41%, corroborating the present study.

These compositions make free-range chicken bone by-products an excellent source for protein hydrolysis. The method uses the Maillard Reaction with proteins to obtain peptides and amino acids (important flavor precursors), that present bioactive and technological potential. The degradation of fats such as aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters also contributes greatly to the volatiles found in cooked meat (Mottram, 1998).

The moisture content of chicken bones is well described in the literature by Fonkwe and Singh (1996), as being approximately 51%. However, after thermal processing in an oven, the free-range chicken bones dry residue presented a moisture content of 29.59 likely due to the concentration (resulting from the treatment) of components in the sample.

At 27.26%, the ash content in the free-range chicken bone dry residues differed from values seen in the literature, such as 15% by Dong et al. (2019), in industrial chicken bones. However, it is likely that this difference reflects, variety, and type of raising (whether free-range or not). Free-range chickens enjoy an increase in bone density stimulated by physical activity (walking, landing, etc.). Such behavior is recurrent for free-range birds, and provides a mineral reservoir in the skeletal system, which plays an important role in chicken health (Evaris, Sarmiento-Franco, & Sandoval-Castro, 2021).

As expected, the mineral profile of free-range chicken bones (Table 1) shows a greater predominance of calcium and phosphorus, counting for approximately 95.83% of the mineral profile. As in other industry by-products, the mineral content of free-range chicken bones brings potential to their use as an ingredient in the food industry, contributing to the daily dietary need for minerals, and enriching the final product.

3.2. Degree of hydrolysis

During the proteolytic process, the glucose hydrolysis (GH) of the three hydrolysates (HF, HA, and HFA) was evaluated and the curves are shown in Fig. 1. Analyzing the values, it can be seen that the Alcalase® enzyme presented the highest GH (20.59%). Known for its intense ability to break peptide bonds, this enzyme is nonspecific and has excellent hydrolytic power (Toldrá, Reig, Aristoy, & Mora, 2018). Thus, Cordeiro et al. (2020) and Queiroz et al. (2017) found similar results when reporting that the highest level of protein hydrolysis in goat viscera was obtained when using Alcalase®.

Differing peptide patterns result when using different peptidases. Toldrá et al. (2018) reports exopeptidase activity in enzymes, among which Alcalase® 2.4 L (Novozymes), and Flavourzyme® 1000 L (Novozymes) were cited. Exopeptidase activity refers to the action of aminopeptidase and carboxypeptidase at the N and C terminals and a progressive decrease in the size of the peptides with the release of free amino acids. In the case of HFA, glucose hydrolysis was lower than with HA. This may be related to the non-specificity of Alcalase® and consequent competition for the same substrate.

During MR, modulating the glucose hydrolysis of the protein hydrolysate provides different aromas and flavors (Xu, Zheng, Song, Gong, & Pan, 2019). To obtain a flavoring, GH control is important, though in the food industry, a higher GH does not always release better aromatic substances or precursors.

3.3. Aminoacids profiling: total (TAAs) and free (FAAs)

In the total amino acid profile, 17 amino acids were identified: aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine, and lysine (Table 2).

The total amino acid content was similar to that reported by Dong et al. (2014), in chicken bone extract, with the presence of essential amino acids such as lysine, threonine, leucine, valine, tyrosine, and isoleucine. The highest quantified values were arginine (751.98 mg/100g), threonine (299.62 mg/100g), valine (375.96 mg/100g), leucine (492.84 mg/100g) and lysine (425.62 mg/100g).

The efficiency of the proteolytic process in releasing amino acids is essential, and free amino acids were not found in the dry residue sample - free-range chicken bones (raw material).

The free amino acid profile of the hydrolysates revealed the presence of 17 amino acids, with different concentrations according to the specificity of the proteolytic enzyme used (Alkalase®, Flavourzyme, or the 1:1 mixture). From Table 2, the HFA hydrolysate presented higher proportions for most of amino acids identified. However the leucine and phenylalanine values were similar for HF and HFA. These amino acids are important precursors in thermal generation of aroma compounds characteristic of roasted meat (Zhan et al., 2013).

Free amino acids play a fundamental role in the Maillard reaction due to their association with volatile compounds formation (Sun et al., 2014). Kang, Alim, and Song (2019) mention the importance of the peptide N-terminal amino acid residue (in non-enzymatic reactions), since Maillard reactive peptides have structural characteristics containing Leu (Ile)-X, Val-X, Phe -X, and cysteine and that among them, Leu - Ala, Phe - Ser, Ala - Tyr and Val-Met are flavor related precursors in chicken hydrolysates. Further, when the N-terminal amino acid is cysteine, leucine, isoleucine, or phenylalanine, the peptide will exhibit higher reactivity.

Together with the MR and the production of aromatic compounds, amino acids are detected via taste receptors in tongue and palate epithelium taste buds. One of the flavors known as umami is specifically attributed to the amino acids glutamic acid, alanine, and aspartic acid (Zhao, Schieber, & Gänzle, 2016). Sun et al. (2014) mention that amino acids with hydrophobic side chains usually present an unpleasant, bitter taste. This was corroborated by Ramalingam, Song, and Hwang (2019), who also point out that glycine and alanine present considerably strong sweetness.

Some amino acids present more than one flavor characteristic. Arginine has a bitter though slightly sweet taste, while serine has a sweet and sour taste, an umami touch. Glutamic acid presents as a combination of sour and umami, and alanine presents as a sweet with a slight umami taste (Dashdorj, Amna, & Hwang, 2015).

Although HA presented the highest degree of hydrolysis, HFA and HF were much higher in the number of free amino acids such as serine, threonine, valine, isoleucine, and leucine, important precursors for the formation of meat flavor; providing Strecker aldehydes and aromatic compounds such as pyrazines (Madruga, Elmore, Oruna-cancha, Balagiannis, & Mottram, 2010).

3.4. Sugar profile

The sugar content and hydrolysates revealed the presence of maltose, glucose, fructose, and ribose. Maltose was present at the highest concentration, (342.97 mg/100g), followed by glucose (221.10 mg/100g), fructose (75.00 mg/100g), and ribose (4.18 mg/100g). The presence of these sugars has been reported in meat by-products, and it is known that the content of these sugars, during cooking, (application of heat), is essential for the development of meat flavor (Cordeiro et al., 2020; Madruga et al., 2010).

Evaluating sugar content in relation to the enzymatic process (Table 3), it was observed that all presented significant reductions from the raw material (bones). This demonstrates consumption of these sugars during processing, and the potential for production of aromatic and color compounds.

The hydrolysate obtained with Alcalase® (HA) stood out from the others due a greater reduction in glucose and fructose. It was observed that the HF and HFA hydrolysates presented greater maltose reduction, and as expected, ribose presented the lowest content, whether in bone or hydrolysate, this was also reported by Madruga et al. (2010), mentioning glucose, followed by fructose, and finally ribose in terms of lessening proportions in cattle, swine, sheep and chickens.

3.5. Volatile profile of free-range chicken bone protein hydrolysate

Eighty-four volatile compounds were identified in the analyzed samples of dry chicken bone residues, and the protein hydrolysates for HF, HA, and HFA (Table 4), were distributed in thirteen (13) chemical classes: aldehydes (34), alcohols (13), hydrocarbons (9), ketones (9), esters (5), terpenes (3), aromatics (2), acids (1), furans (1), pyrazines (1), pyridine (1) sulfur components (1) and others (4).

Of the total, 55 compounds were not identified in the raw material; this demonstrates the importance of enzymatic activity when using by-product proteins from animal processing.

Aldehydes were the main class identified, in which 11 compounds were found in the dry chicken bone residues, and 30, 35, and 33 compounds were found respectively in the HA, HF, and HFA hydrolysates. Nonanal was the principal compound detected in the HA hydrolysate, as was also described by Kerth and Miller (2015) who reported nonanal and hexanal as common volatiles in fatty acid thermal hydrolysis. In item 3.1, the dry residue presented a lipid concentration of approximately 12%. Therefore, during the hydrolysis process, changes both in proteins and the lipid fraction occurred.

Most of the components of this chemical class, including alcohols, ketones, esters, carboxylic acids and aliphatic hydrocarbons, find their origin in lipid oxidation of fatty acids. This type of reaction causes rancid flavors, known as *off-flavors*. However, when dealing in cooking meats interactions are quicker, and the resulting volatile profile contributes to more desirable flavors (Mottram, 1998).

It is reported in the literature that lipid degradation is responsible for the particular flavor of each animal species, and that the resulting compounds present high detection thresholds for aroma. Most of these compounds include saturated and unsaturated aldehydes (six to ten carbons), are quite volatile, and play an important role in meat flavor (Kerth & Miller, 2015).

Aldehydes, considered important in the aroma of roasted chicken, reached higher concentrations after hydrolytic treatment. Hexanal obtained a maximum value in the HF hydrolysate. Benzaldehyde concentrations were high in both the HF and HFA hydrolysates. In the HF hydrolysate, octanal and (E)-2-octenal achieved their highest averages, while one of the key compounds in roast chicken, decanal, achieved its highest average in the HA hydrolysate.

Because of their unsaturated components, and low detection thresholds, aliphatic alcohols, also associated with fatty acid degradation, are known to contribute to the flavor of roasted meat (Cordeiro et al., 2020). 1-octen-3-ol is considered an important compound for the overall aroma of meats (Liu et al., 2020; Ma, Zhan, Tian, Zhisheng, & Wang, 2020). In the present study, the HF hydrolysate presented higher proportions of these compounds.

Though known as key descriptors for nuts and toasts, alkylpyrazines were not well identified in our hydrolysates. 2,6-dimethylpyrazine was not detected in either the dry bone residue or in the HA hydrolysate, but

did present values of 0.22[5] and 53.81[5], respectively in the HF and HFA hydrolysates. Despite being considered a key compound for the aroma of roasted chicken meat, the low values of 2,6-dimethylpyrazine can be explained by lower formation temperatures, being mostly between 120 and 150 °C.

In general, the volatile profile found in the present study corroborates that obtained by Wang et al. (2016) who analyzed the effect of time and temperature on aromatic compounds in chicken bone extract. During cooking, there are many thermally induced reactions between non-volatile tissue components, however the volatiles formed determine the aroma attributes and the important characteristic flavors of the meat (Mottram, 1998).

3.5.1. Hierarchical cluster analysis and heat map applied to the volatile profile of dry bone residue and protein hydrolysates

A hierarchical cluster analysis and heat map (Fig. 2) was performed considering all identified compounds (Table 4) in dry bone residue from free-range chickens and the corresponding protein hydrolysates (HF, HA and HFA). The volatiles were grouped vertically and the intensity of the compound (peak area) was represented by different colors: the brighter green, the higher the peak area; the brighter red, the lower the peak area. The hierarchical cluster analysis grouped the samples into three clusters. The first cluster, consisting of the dry bone residue's, is grouped separately from the others due to the lower concentration of most of the identified compounds except for the last five compounds: Limonene, 2-acetylthiazole, dodecane, toluene and 1-hexanol.

The second cluster, to which HF belongs, showed a predominance of peaks with higher intensity compared to the third cluster formed by HA and HFA. This predominance is due to the stronger expression of the following volatiles: Tetradecanal, 2-pentylpyridine, (E-Z)-2,4-decadienal, (Z)-2-undecenal, (Z)-4-decen-1-ol, decane, (Z)-2-octenal, 2-heptyn-1-ol, octylbenzene, (E,E)-2,4-decadienal, Z-4-heptenal, (E,E)-2,4-heptadienal, 2,4-di-*tert*-butylphenol, 1-nonen-3-ol, methyl decanoate, hexadecanal, (E,E)-2,4-nonadienal, phenylacetaldehyde, methyl dodecanoate, 3-ethylbenzaldehyde, 1-pentanol, hexadecane, 2,6,10-trimethyltetradecane, (E)-2-dodecenal, dibutylformamide, (Z)-non-2-enal, (E,Z)-2,4-nonadienal, (Z)-2-decenal, 1-octen-3-ol, tridecane, geranylacetone, dodecanal, (E)-2-octen-1-ol, (E)-2-octenal, 2-hexenal, 3,5-octadien-2-one, (Z)-hept-2-enal, 3-undecanone, hexanal, and 2,5-octanedione.

This difference shows the expression of volatiles important to HF such as (E,E)-2,4-nonadienal (chicken fat), (E,Z)-2,4-nonadienal (fatty, chicken soup), (E,Z)-2,4-decadienal (sweet, fatty, chicken soup). In addition to (E,E)-2,4-decadienal, which was reported by Feng et al. (2018) was found to be the predominant aroma compounds in chicken broth.

3.6. Characterization of the flavoring products

3.6.1. Instrumental color: brightness (L*)

The instrumental color parameter luminosity (L*) of the flavoring samples was evaluated before and after thermal processing (autoclaving) (Table 5). We noted that there was a decrease in L* values (variation from black to white) in both MR products (GF and XF, respectively glucose and xylose). However, XF presented greater browning intensity. This can be explained by the greater reactivity of pentose (xylose) in relation to hexose (glucose), and thus more browning (Zou, Kang, Yang, Song, & Liu, 2019).

3.6.2. Volatile profile of free-range chicken bone flavors

Seventy-five volatiles were identified for the XF and GF flavors (Table 6), distributed in fourteen (14) chemical classes: aldehydes (36), alcohols (12), ketones (9), hydrocarbons (3), esters (3), furans (3), terpenes (2), aromatics (1), acids (1), pyrazines (1), pyridines (1), sulfur components (1), phenol (1) and starches (1).

Of the total, eight of the compounds were absent in GF, demonstrating the greater variety of compounds generated by XF, and the influence of sugar choice. These compounds are generated by reaction during heating of amine groups with carbonyl groups in the reducing sugar. Subsequently, glycosylamines are produced, which are then rearranged and dehydrated to form furfural, furanone derivatives, hydroxyketones, and dicarbonyl compounds. These are essential in determining meat flavor (Ramalingam et al., 2019).

During the initial stage of the MR, an Amadori rearrangement can occur between aldose sugars and amino acids, to constitute important intermediates for the final MR products. The rearrangement products can be decomposed into α-dicarbonyls of various chain lengths, such as: 3-deoxyhexos-2-ulose, 1-deoxy-2,3-hexodiulose, 2-oxopropanal, butane-2,3-dione, and glyoxal. A-dicarbonyls are very reactive and trigger cascades of additional reactions, these can result in a complex mixture of many MR products, especially volatiles and brown pigments (Hou et al., 2017). We noted that phenylacetaldehyde, an important compound formed by Strecker degradation of phenylalanine, was present in higher values in XF averaging 14.32[5] as compared to GF, at only 2.51[5].

Sulfur compounds constitute an important meat flavoring group. The main mechanism by which sulfur-containing volatiles are generated is through the formation of hydrogen sulfide from glutathione mercapto in the Maillard reaction (Zou et al., 2019). In this study, only 2-acetylthiazole was identified as a sulfur compound, presenting a higher average concentration in GF.

In addition to MR products, degradation of vitamins during cooking such as thiamine, lipid oxidation, and synergism between oxidized lipid products all produce the volatile flavor components characteristic of cooked meat. These compounds are organic in nature, and have low molecular weights. Lipids reduce the vapor pressure for most flavor compounds, and thus exert great influence on the production of these aromas (Khan, Jo, & Tariq, 2015).

The hydrolysis of lipids gives rise to free fatty acids, which when heated (saturated and unsaturated) undergo thermal decomposition to form hydro-peroxides which then react to form aldehydes. Hexanal, heptanal, octanal, nonanal, and (E,E)-2,4-decadienal are generated by heating unsaturated fatty acids (Liu et al., 2020). XF exhibited higher averages for these compounds than GF, with the exception of hexanal. The higher averages of these compounds are likely due to the high fat content found in chicken bones. Aldehydes were identified as the main class. Yet of a total of 36, only 28 appeared in the GF. Nonanal, already mentioned as a key compound, presented respective averages of 23.93 [5] and 49.78 [5] for GF and XF. Hexanal, another compound common in fatty acid thermal hydrolysis was (as well as benzaldehyde) its highest in GF.

3.6.3. Sensory analysis of the free-range chicken bone flavorings

The results of the sensory evaluation of the free-range chicken bone flavorings are shown in Fig. 3. Six descriptors, distributed as attributes of appearance (brown intensity), and aroma (roasted chicken, cooked chicken bone, artificial chicken broth, seafood and rancid) were evidenced.

Yet, the sensory panel did not identify a significant difference between the flavorings analyzed; (flavoring with Xylose: XF, or flavoring with glucose: GF), demonstrating no perceptible sensorial difference in the sugars (xylose or glucose) when used in the processing. However, it can be seen in the figure that both flavorings exhibited brown coloring and aromatic attributes more intensely - whether as roasted chicken, cooked chicken bone, seafood, or rancidity. The characteristic aroma of these descriptors corroborated the volatile profiles of the samples, presenting key compounds (in higher proportions) of each aldehyde class, whether alcohols, ketones, esters and pyrazine.

4. Conclusion

This study describes the aromas obtained by hydrolysis with Alcalase®, Flavourzyme®, or a mixture of both, as well as the Maillard

reaction for the Flavourzyme® flavorings when supplemented with glucose or xylose. The results suggest that Flavourzyme® is the best alternative for the formation of flavor compounds, this with xylose as the most interesting sugar for flavor formation. A total of 75 compounds were identified for XF, while only 67 compounds were identified for GF, including the most important meat flavor compounds. This allows their use in the food industry as flavoring agents and as a basis for new flavors.

CRediT authorship contribution statement

Rebeka Correia de Souza Cunha: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Re- sources, Writing – review & editing. **Leila Moreira de Carvalho:** Software, Methodology, Validation, Writing – review & editing. **Viviane Maria de Sousa Fontes:** Software, Methodology, Validation, Writing – review & editing. **Mércia de Sousa Galvão:** Formal analysis, Software, Methodology, Resources, Writing – review & editing. **Lary Souza Olegário:** Software, Methodology, Writing – review & editing. **Lorena Lucena de Medeiros:** Software, Methodology, Validation, Writing – review & editing. **Marcelo Antonio Morgano:** Software, Methodology, Validation, Writing – review & editing. **Maria Teresa Bertoldo Pacheco:** Visualization, Supervision, Methodology, Writing – review & editing. **Marta Suely Madruga:** Conceptualization, Methodology, Writing – review & editing. **Taliana Kênia Alencar Bezerra:** Conceptualization, Methodology, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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