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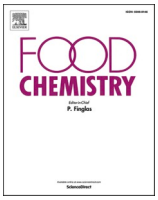

Microencapsulation of maillard reaction products from chicken bone protein hydrolysates: Retention and preservation of meat flavoring compounds

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ABSTRACT

The Maillard reaction is a natural process in foods and widely used in by-products to develop meat flavors. However, few research has focused on protecting the volatile compounds generated. This study investigated the Maillard reaction in chicken bone hydrolysate at pH 4 and 6, followed by spray drying encapsulation to assess volatile retention, preservation, and sensory properties. Forty-five volatile compounds were identified, with pH 6 showing a higher volatile profile. The pH 6 flavoring demonstrated the formation of aldehydes and furans, including hexanal, heptanal, benzeneacetaldehyde, nonanal, and 2-pentyl furan, which contributed to the characteristic aroma of cooked chicken. Microencapsulated flavorings were evaluated for sensory properties, with lower acceptance than the control, but no significant differences ($p < 0.05$) in volatile profiles across carrier concentrations. This study is the first to develop a powdered flavoring from chicken bone hydrolysate, demonstrating good aromatic retention and making it viable for food industry applications.

1. Introduction

Chicken bones are an essential by-product of broiler processing, accounting for approximately 8 % to 17 % of the animal's total weight. As they are a rich source of protein, the food sector is very interested in applying methodologies to transform and use this by-product (Bezerra et al., 2020; Guan et al., 2024).

The literature well describes the use of protein hydrolysate from bone by-products as an alternative for obtaining aroma precursors, demonstrating the use of free-range chicken bones (Cunha et al., 2023), goat bones (Silva, Gomes, et al., 2024), bovine bones (Chiang et al., 2019), cod bones (Tan et al., 2018), and sheep bones (Zhan et al., 2013).

The flavoring action conferred by hydrolysates is due to the presence of soluble compounds of low molecular weight, free amino acids, sugars, and fatty acids, which undergo complex reactions such as the Maillard reaction (MR), lipid oxidation, and vitamin degradation (Cordeiro et al., 2022). The intensity of the MR depends mainly on the sugars present or added, following this order: pentoses (xylose) > aldohexoses (glucose, mannose, galactose) > ketohexoses (fructose, sorbose) > reducing disaccharides (maltose), resulting in different degrees of browning (Farmer, 1994). Depending on the pH (de Sousa Fontes et al., 2024) or temperature (Guo et al., 2010), the volatile products of the Maillard reaction (MR) can contribute to an overall meaty aroma at lower pH or shift toward a sweet, fatty characteristic at higher pH.

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Commonly used in various food formulations, most flavorings consist of volatile components with relatively low molecular mass, making them susceptible to degradation during industrial processing or exposure to pH variations, light, oxygen, and high temperatures. Given its instability, microencapsulation by spray drying could provide a protective environment that helps maintain their integrity. Additionally, the use of carrier agents enhances stability and facilitates the conversion of liquid flavorings into powders. These agents may be polysaccharides, proteins, lipids, or their complexes, with maltodextrin being the most widely used encapsulating agent for protecting bioactive compounds (Breternitz et al., 2017; Carneiro et al., 2022).

Therefore, this study aimed to enhance the understanding of chicken aroma production under different pH conditions, its retention and preservation, and to propose an alternative for reusing chicken bones by producing a flavoring powder with potential applications in the food industry.

2. Material and methods

2.1. Materials

Broiler chickens were purchased from a certified supplier in João Pessoa (Paraíba, Brazil). Slaughtered was performing using electronarcosis in an immersion vat to induce humane stunning, following federal regulations, with carcasses bearing the Federal Inspection Service (SIF) seal. Protein hydrolysate was obtained using Flavourzyme® (*Aspergillus oryzae*), supplied by Novozymes Latino Americana Ltda (Paraná, Brazil). All reagents used were analytical grade, except for those employed in chromatographic analyses, which were of chromatographic-grade purity.

2.2. Production of chicken bones hydrolysate

2.2.1. Pre-treatment

To obtain the bones, deboning was performed after removing the head, skin, feet, and internal organs. The remaining waste was rinsed with distilled water, and the bones were packed, labeled, and stored at −20 °C until use, following the method of Zhan et al. (2013). The chicken bones were submitted to a high-pressure pre-treatment, autoclaved (Phoenix, Araraquara, Brazil) at 121 °C for 2 h. Subsequently, they were dried in an oven at 55 °C for 5 h, according to Cunha et al. (2023), with adaptation about heat treatment time. The bones were processed in a knife mill to obtain ground dry bone.

2.2.2. Enzymatic hydrolysis (HF)

In a jacketed reactor with a heating system, ultrapure water was added to the ground dry chicken bone (DCB) in a 1:2 (w:v) ratio, to which a burette filled with 0.5 M NaOH was attached to control the pH. Proteolysis was performed using Flavourzyme® according to a previous study by Cunha et al. (2023). The pH and temperature parameters were maintained according to the ideal values recommended, as supplied by the enzyme manufacturers. The reaction time was 180 min under agitation, and at the end of the hydrolysis, the enzyme was inactivated by heating at 90 °C for 15 min as stated by Cunha et al. (2023).

2.2.3. Degree of hydrolysis (DH)

The degree of hydrolysis (DH) was determined by Eq. (1), where the pH adjusted by NaOH 0.5 mol/L:

$$DH(\%) = B \times Nb \times (1 \div \propto) \times (1 \div m) \times (1 \div htotal) \times 100$$

Eq. (1). Calculation to determine the degree of hydrolysis.

where DH (%) is the degree of hydrolysis; B is the base consumption in mL; N_b is the base concentration; $1/\alpha$ is the average degree of dissociation of the α-NH2 group; $\alpha = 0.5267$; m is the protein mass in the sample fraction in the solution (g); and h_{total} is the total number of peptide bonds in the protein matrix given for meat by the constant 7.6 (Adler-Nissen, 1986).

2.3. Maillard reaction induction

Maillard reaction was carried out following the method of Guo et al. (2010), with modifications. Briefly, cysteine (0.5 g), xylose (1 g), and thiamine (1 g) were added to the chicken bone hydrolysate. The pH of the samples was adjusted to 4.0 and 6.0, and the final volume was brought to 100 mL. The solution was then placed in screw-cap bottles and heated in an autoclave at 121 °C for 1 h. To stop the reaction, the samples were cooled in an ice bath.

2.4. Microencapsulation of maillard reaction products

Spray drying was used to microencapsulate the flavoring in different concentrations of maltodextrin. A Büchi mini spray dryer, model B-290 (Büchi Labortechnik AG, Switzerland), was used. The control sample (C0) was dried without adding a carrier agent, while samples C1 and C2 were added with 50 % and 100 % 10DE maltodextrin, respectively, the percentages were calculated based on the solid content of the samples. The inlet drying air temperature was 180 °C, and the outlet air temperature was maintained at 95 °C by controlling the feed rate, ranging from 9.62 to 11.46 mL/min. The atomization was made with a dual-fluid glass cyclone to separate the powder from the drying nozzle of 0.7 mm in a glass drying chamber of 0.165 × 0.60 m. Fixed parameters were the flow rate of the drying air of 35 m^3/h and atomization air pressure of 6 bar with a flow rate of 1 m^3/h, following the method Breternitz et al. (2017) described.

2.4.1. Scanning electron microscopy (SEM)

The samples were air-dried at room temperature and mounted onto aluminum stubs (Ted Pella, Inc., Redding, CA) using conductive carbon adhesive tape (Ted Pella). No conductive coating was applied. Micrographs were obtained using a FEI Quanta 450 scanning electron microscope (Thermo Fisher Scientific, MA, USA) at an accelerating voltage of 10 kV.

2.4.2. Yield determination and physicochemical characterization of microparticles

After spray drying, the percentage yield of microparticles was determined by calculating the ratio of solids in the powder to solids in the feed solution. Moisture content was determined using the Association of Official Analytical Chemists - AOAC (2016). Water activity (Aw) was measured using an Aqualab digital thermo-hygrometer (3TE, Decagon, Pullman, USA) at 25 °C. The water solubility and hygroscopicity were determined and expressed as percentages, following the methods described by Rocha et al. (2019). Color was determined using a colorimeter (Model CR300, Minolta, Osaka, Japan) by directly measuring the parameters L* (0 = dark, 100 = light), a* (− = green, + = red), b* (− = blue, + = yellow), C* (chroma), and h° (hue angle; 0° = red, 90° = yellow, 180° = green, 270° = blue), according to the specifications of the Commission Internationale de l'Éclairage (CIE, 1986).

2.5. Physical-chemical analysis of bones (DCB) and hydrolysates

2.5.1. Characterization of DCB

The DCB was characterized for determining moisture, ash, and protein contents by the Association of Official Analytical Chemists - AOAC (2016), as described in the respective numbered procedures: 39.1.03, 39.1.09, and 39.1.15. Lipid content was measured using the Folch et al. (1957) methodology.

2.5.2. Soluble proteins

Soluble proteins were quantified according to the Lowry method of Peterson (1979). Bovine albumin (1 mg/mL) (A4503, Sigma Aldrich, St. Louis, USA) was standard at concentrations of 0, 2, 5, 10, 15, 20, 25, 30,

35, and 40 mg/mL. The amount of protein was expressed in mg/mL.

2.5.3. Total and free amino acid profile

Total amino acids were extracted based on the methodology described by Hagen et al. (1989). Briefly, the sample was subjected to hydrolysis with 6 M hydrochloric acid at 110 °C for 22 h. Free amino acids were extracted using a milder process, involving orbital shaking with 0.1 M hydrochloric acid for 60 min, as described by Bezerra et al. (2020). The resulting extracts were then derivatized with phenylisothiocyanate (PITC) and analyzed according to Hagen et al. (1989) and White et al. (1986). Separation of the free and total amino acids derivatized (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 comprised an acetate buffer pH 6.4 and a 40 % acetonitrile solution. Sample injection was performed automatically (50 μL), and detection occurred 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 sample. Quantification was performed by adding the internal α-aminobutyric acid standard and identified by comparison to a mixture of standards, according to White et al. (1986).

2.5.4. Sugars profile

The sugar profile was determined by Zeppa et al. (2001). Soluble extracts were obtained by weighing 2 g of DCB and 2 mL of hydrolysate with a final dilution of 10 mL in ultra-pure water. The solution was ground in an ultraturrax (IKA Works, Wilmington, USA) for 10 min, followed by centrifugation (Solab, Piracicaba, Brazil) at 2607*g* at room temperature for 10 min and subsequent filtration on qualitative filter paper. Next, the samples were filtered through a 0.45 μm porosity syringe filter and injected into an Agilent Liquid Chromatography system (VARIAN, Waters, CA, USA) coupled to a refractive index detector (RID) (model 356 LC-RID). The processing software used was the GALAXIE Chromatography Data System. The separation column was an Agilent Hi-Plex Ca (7.7 × 300 mm, 8 μm), heated to 85 °C. The injection volume of the sample was 20 μL with an isocratic solvent system in which ultrapure water was used. The run time was 30 min at a flow rate of 0.6 $mL.min^{-1}$ (Ball and Lloyd, 2011. Identification was performed by comparison with external standards (Sigma-Aldrich, St. Louis, MO, USA).

2.5.5. Volatile compound retention (GC–MS)

To determine the retention efficiency of the volatile components for each concentration of maltodextrin used, volatile components were extracted from samples C0, C1, and C2 at different storage times: T1 (30 days), T2 (60 days), and T3 (90 days). The powders were stored in a hermetically sealed container until they were used in the analysis at ambient temperature and protected from light.

For the extraction of volatiles, the Headspace solid-phase microextraction technique (HS-SPME) was used with an SPME device (Supelco, Bellefonte, USA), according to the methodology adapted from Cunha et al. (2023). An aliquot of 2 mL of the diluted powder (0.4 g for 20 mL of water) and 3 μL of the internal standard 1,2-dichlorobenzene in methanol (50 $\mu g.mL^{-1}$) were transferred to a 20 mL glass vial and immediately closed with a Teflon-coated septum cap.

Volatiles were extracted at 50 °C for 10 min (equilibrium). Then, the 50/30 μm Divinylbenzene/Carboxene/Polydimethylsiloxane (DVB/CAR/PDMS) fiber was exposed to headspace for 30 min of adsorption under agitation. The fiber was conditioned according to the manufacturer's specifications before extraction. Chromatographic analyses were performed using Gas Chromatograph 7890B (Agilent Technologies 5977B, Little Falls, DE, USA) equipped with VF- 5MS columns (30 m × 0.25 mm, 0.25 μm) and HP-INNOWAX (60 m × 0. 25 mm, 0.25 μm), coupled to a mass spectrometer under the conditions of the methodology by Cunha et al. (2023): initial oven temperature of 40 °C for 3 min, followed by 5 $°C.min^{-1}$ to 120 °C and 10 $°C.min^{-1}$ to 230 °C, where it remained for 5 min, for a while 35 min run total. 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 splitless 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 50 to 400 *m/z* at 4.44 scan/s. Compounds were identified using the NIST library database (2014) (National Institute of Standards and Technology, USA) combined with the mass spectrum and linear retention index. Each compound's linear retention index (LRI) was calculated from the retention times of a homologous series of C6-C_{20} *n*-alkanes.

Aroma characteristics were obtained according to databases https://www.flavornet.org/flavornet.html.

2.5.6. Sensory evaluation

The sensory methodology used was the Optimizade Descriptive Profile (ODP) by da Silva et al. (2012). All procedures performed in this study involving human participants were previously approved by the ethics committee of the Federal University of Paraiba (CAAE: 51549721.5.0000.5188) by the Declaration of Helsinki with the consent of the sensory panelists included in this research. At the final moment, 16 panelists, ranging from 24 to 41 years old, of both female and male genders, analyzed the samples alongside the reference materials in the sampling booth, allowing for consistent evaluation and presentation of quantitative information.

The analysis was carried out in the Sensory Analysis laboratory at the Federal University of Paraíba. The descriptors adopted for aroma characteristics were "Stewed chicken", "Sweet", "Roast chicken", "Artificial chicken flavoring" and the samples were evaluated using nine-centimeter interval scales between "weak" and "strong" intensities.

The references used for the aroma attributes associated with chicken were boiled chicken breast for "Stewed chicken", roasted chicken breast for "Roast chicken", sucrose diluted in water for "Sweet" and Nissin instant broth for "Artificial chicken flavoring".

2.6. Statistical analysis

The data obtained from volatile compounds retention were evaluated by analyses of variance (ANOVA) two way, using Statistical software Analysis System version 11.0 (SAS Institute Inc, 2014), based on significance levels of 5 %, followed by the Tukey test or Student's *t*-test (flavorings CF4 and CF6) to compare the means. Principal component analysis was carried out using the R programming language and its graphical interface, RStudio, using the Chemometrics Web application (Darzé, Lima, Luna, & Pinto, 2022; Darzé, Lima, Pinto, & Luna, 2022), while the heat map with hierarchical clustering was done using the TBtools-II v2.105 software (Chen et al., 2023).

3. Results and discussion

3.1. Physical-chemical characterization of chicken bones (DBC) and protein hydrolysate

The DBC had a high protein (26.96 %) and lipid (13.36 %) content, making it a potential raw material for hydrolysates and subsequent applications. Among the protein content, 17 amino acids were identified (Table 1), with higher concentrations of Gly, Pro, and Glu, which are characteristic of bone raw material, as analyzed by Silva, Gomes, et al. (2024) in goat bones, Cunha et al. (2023) in free-range chicken bones and Zhan et al. (2013) in sheep bones.

Other by-products are chicken bones, a potential source of protein content, and enzymatic hydrolysis, which has been used as one of the most effective methods for recovering proteins from animal processing. (Tang et al., 2023).

Protein hydrolysates have large amounts of peptides and free amino

Table 1
Physical-chemical characterization and profile of sugars and amino acids present in the chicken bones and protein hydrolysate.

Parameter	DCB	HF
Proximate composition (g/100 g)		
Moisture	23.35 ± 0.21	na
Ash	40.89 ± 0.94	na
Protein	26.96 ± 0.64	17.47 ± 0.01
Lipids	13.36 ± 1.16	3.79 ± 0.16
Sugars (mg/100 g)		
Maltose	na	
Glucose	na	
Fructose	na	
Ribose	na	
Total (g/100 g) and free (mg/100 g) amino acids		
Aspartic acid	1.00 ± 0.00	13.83 ± 0.08
Glutamic acid	2.57 ± 0.00	74.89 ± 0.13
Serine	1.08 ± 0.00	71.86 ± 0.55
Glycine	4.77 ± 0.01	94.83 ± 0.14
Histidine	0.80 ± 0.00	80.70 ± 0.05
Arginine	2.48 ± 0.00	211.17 ± 0.08
Threonine	1.13 ± 0.00	87.68 ± 0.11
Alanine	2.56 ± 0.00	120.01 ± 0.06
Proline	3.04 ± 0.01	31.50 ± 0.06
Tyrosine	0.67 ± 0.01	41.39 ± 0.41
Valine	1.12 ± 0.00	93.39 ± 0.03
Metionina	0.47 ± 0.00	37.63 ± 0.08
Cysteine	0.06 ± 0.00	41.25 ± 0.09
Isoleucine	0.74 ± 0.00	54.08 ± 0.10
Leucine	1.66 ± 0.00	226.57 ± 3.78
Phenylalanine	0.97 ± 0.00	155.89 ± 0.91
Lysine	1.65 ± 0.00	90.92 ± 0.39
Tryptophan	nd	15.32 ± 0.08

Results are expressed as mean ± standard deviation; DCB: Dry chicken bone; HF: Chicken bone protein hydrolysate obtained by Flavourzyme.
Note: "nd", not detected; "na", not applicable.

acids that act in flavor formation, including the formation of meat aroma, umami flavor, kokumi, and bitterness reduction (Sun et al., 2022). In its free form, glutamic acid showed an interesting average of 74.89 mg/100 g, essential to developing umami flavor and furan formation. Gly (94.83 mg/100 g), which has a considerably intense sweetness, stood out among the free amino acids, along with Arg (211.17 mg/100 g) and hydrophobic amino acids such as Leu (226.57 mg/100 g) and Phe (155.89 mg/100 g), which can impart bitterness, familiar to many hydrolysates.

The hydrolysate obtained from chicken bones reached a degree of hydrolysis of 4.83 % in our previous study (Cunha et al., 2023). The degree of hydrolysis is mainly regulated by the type of enzyme used, the reaction temperature, the hydrolysis time, and the pH. In particular, Flavourzyme® is an enzyme used to modify small molecules or macromolecular components to improve the unpleasant taste of peptides and, as has been well reported in the literature, is widely used by the food industry to obtain flavorings (Cui et al., 2022; Grossmann et al., 2021).

Although this percentage does not represent intense enzymatic action, the enzyme in question is a mixture of endoprotease and exoprotease that hydrolyzes bonds inside polypeptides and N- or C-terminal bonds, which ends up resulting in interesting aroma compounds for foods (Cui et al., 2022).

Meat flavor is obtained through chemical reactions, including the thermal degradation of amino acids and peptides, sugar degradation, thiamine degradation, lipid oxidation, MR, and interactions between MR and lipid oxidation (Sun et al., 2022).

3.2. Volatilomic content of DCB, HF, and flavorings

The aromatic profile of chicken bones (DBC), protein hydrolysate (HF), and flavorings made with the hydrolysate at pH 4 (CF4) and 6 (CF6) were evaluated to verify the influence of pH on the formation of volatile compounds.

Forty-nine volatile components were identified among the samples (Table 2), which fall into the following classes: aldehydes (18), alcohols (10), ketones (7), sulfur-containing (8), furans (3), pyrazine (1), pyrrole (1) and pyridine (1).

The hydrolytic process carried out on the chicken bones with Flavourzyme® (temperature 50 °C) resulted in the formation of four more compounds in the HF than in the DCB, as well as allowing the presence of interesting compounds such as decanal, (E)-2-decenal, (E,E)-2,4-decadienal, 1-pentanol, 1-hexanol and 1-octanol in the aromatic bouquet of the product.

Decanal (aldehydic, sweet, waxy), (E)-2-decenal, (E,E)-2,4-decadienal (fatty, oily, citric, chicken fat-like), 1-pentanol (pungent, fermented, yeasty), 1-hexanol, 2,3-octanedione and 1-octanol were present. In contrast, hexanal, benzaldehyde (almond, oily), nonanal, and 1-octen-3-ol were intensified, indicating possible greater oxidation of fatty acids as a result of the mild heating caused by enzymatic hydrolysis, as pointed out by Fontes et al. (2024).

Regarding flavorings CF4 and CF6, the change in pH led to the formation of volatiles differently, favoring the flavoring formulated at a less acidic pH. A total of 15 aroma compounds were identified for CF4, while 29 were identified for CF6. The CF6 flavoring showed the formation of aldehydic compounds and furans, especially hexanal, heptanal, benzeneacetaldehyde, and nonanal, as well as furans such as 2-pentyl furan, providing the aromatic characteristic of cooked chicken meat according to Qi et al. (2021).

At a more acidic pH, there is a greater availability of H+ ions, which makes the amino groups less reactive (due to hydrogen bonds) for interaction with carbonyls in the MR. This explains the marked formation of furfural in CF4 (126.35 μg/mL) compared to CF6 (0.04 μg/mL) since the lower pH induces 1.2 enolization of the Amadori compounds in which furfural production is intensified as the reaction progresses (Madruga & Mottram, 1995).

Therefore, in general, the volatiles formed and identified in the flavorings produced are characteristic of chicken meat, and sample CF6 had the highest number of compounds and intensity. On the other hand, aromatic compounds are considered unstable and sensitive to factors such as temperature, oxidation, and volatilization, making it necessary to apply techniques such as microencapsulation with carrier agents to protect them during storage and improve the conversion process of liquid flavorings into powder, adding possibilities for application in various products (Breternitz et al., 2017).

3.3. Characterization of chicken flavoring microparticles

3.3.1. Physical and physicochemical properties

The percentage yield of solids between powdered samples C0, C1, and C2 differed significantly ($p < 0.05$). As expected, C2 obtained the highest yield (Table 3) due to the more significant amount of maltodextrin added compared to the other samples, demonstrating a reasonable maltodextrin recovery rate. Adding wall material to encapsulated products increases weight and volume, resulting in a higher powder yield (Silva, Marques, et al., 2024).

The moisture content of the powders is a critical parameter for the stability of the microparticles obtained via spray drying during storage. The moisture content of the samples varied between 0.56 and 4.85 % (Table 3), with significant differences ($p < 0.05$) between the concentrations of maltodextrin, which, despite interacting with water in the same way, are in very different concentrations about the encapsulant. In this study, the lowest moisture content was found in the sample with the highest maltodextrin content, which is explained by the action of the encapsulant, which has low hygroscopicity (Nguyen et al., 2021).

The microparticles' water activity (Aw) showed low values, between 0.20 and 0.28 (Table 3). This parameter is essential from the point of

Table 2
Volatilomic content of DCB, HF and flavorings.

Nr	IRL	Compounds	Concentration (μg/mL)			
			DCB	HF	CF4	CF6
Aldehyde						
1	<800	3-Methylbutanal	nd	nd	nd	0.39
2	<800	Pentanal	51.26	5.31	nd	0.08
3	800	Hexanal	162.6	36.85	0.14b	11.78a
4	900	(Z)-4-Heptenal	1.1	0.42	nd	nd
5	901	Heptanal	10.5	4.02	0.07b	2.88a
6	958	(Z)-2-Heptenal	25.4	6.20	nd	nd
7	962	Benzaldehyde	19.63	16.79	1.36b	2.55a
8	1012	(E,E)-2,4-Heptadienal	5.0	1.47	nd	nd
9	1045	Benzeneacetaldehyde	nd	nd	0.25b	1.19a
10	1060	(E)-2-Octenal	28.3	8.15	nd	nd
11	1104	Nonanal	66.9	34.97	0.95b	17.41a
12	1115	(E,E)-2,4-Octadienal	1.3	0.71	nd	nd
13	1168	3-Ethylbenzaldehyde	17.0	0.65	nd	0.68
14	1206	Decanal	nd	1.42	nd	1.22
15	1216	(E,E)-2,4-Nonadienal	3.4	2.63	nd	nd
16	1263	(E)-2-Decenal	nd	2.72	nd	nd
17	1317	(E,E)-2,4-Decadienal	nd	6.31	nd	nd
18	1817	Hexadecanal	nd	nd	0.25	nd
Alcohol						
19	<800	1-Pentanol	nd	0.73	nd	nd
20	859	2-Furanmethanol	nd	nd	nd	0.08
21	868	1-Hexanol	nd	0.22	nd	1.12
22	970	1-Heptanol	7.2	4.60	nd	2.17
23	980	1-Octen-3-ol	61.0	13.4	nd	5.64
24	1003	4-Ethylcyclohexanol	3.0	nd	nd	nd
25	1030	2-Ethyl-1-hexanol	nd	nd	nd	0.40
26	1038	3,5-Octadien-2-ol	nd	6.65	nd	nd
27	1067	(E)-2-Octen-1-ol	8.8	3.86	nd	1.07
28	1071	1-Octanol	nd	16.59	nd	4.89
Ketones						
29	809	Dihydro-2-methyl-3-furanone	nd	nd	0.11	nd
30	891	2-Heptanone	nd	nd	nd	3.97
31	984	2,3-Octanedione	nd	21.26	nd	nd
32	985	2-Methyl-3-octanone	88.3	nd	nd	2.97
33	1040	3-Octen-2-one	4.1	nd	nd	nd
34	1091	3,5-Octadien-2-one	13.5	9.51	nd	nd
35	1193	2-Decanone	nd	nd	0.16b	1.62a
Sulfur-containing						
36	870	2-Methyl-3-furanthiol	nd	nd	3.42a	0.16b
37	911	2-Furfurylthiol	nd	nd	nd	3.10
38	1022	2-Acetylthiazole	2.4	nd	nd	0.81
39	1118	5-Methyl-2-thiophenecarboxaldehyde	nd	nd	0.40	nd
40	1121	3-Methyl-2-thiophenecarboxaldehyde	nd	nd	nd	5.25
41	1167	2-Pentylthiophene	nd	nd	0.12b	2.03a
42	1271	4-Methyl-5-thiazolethanol	nd	nd	nd	80.23
43	1540	bis(2-methyl-3-furyl)disulphide	nd	nd	0.62	nd
Furan						
44	<800	2-Ethylfuran	nd	nd	nd	1.69
45	833	Furfural	nd	nd	126.35a	0.04b
46	993	2-Pentylfuran	3.9	3.91	1.24b	110.30a
Pyrazine						
47	917	2,5-Dimethylpyrazine	0.8	nd	nd	nd
Pirrole						
48	1187	1-Furfurylpyrrole	nd	nd	0.10	nd
Pyridine						
49	1202	2-Pentylpyridine	nd	0.84	nd	1.96

Results are expressed as mean ± standard deviation. Means followed by equal letters do not differ from each other by the t-student test at the 5 % significance level. DCB: Dry chicken bone; HF: Chicken bone protein hydrolysate; CF4: Chicken flavoring pH 4,0; CF6: Chicken flavoring pH 6,0. Note: "nd", not detected.

Table 3
Physical and physicochemical properties of microparticles containing chicken flavorings from chicken bones.

Parameters	Samples		
	C0	C1	C2
Moisture (%)	4.85 ± 0.23a	2.11 ± 0.20b	0.56 ± 0.33c
Water activity	0.26 ± 0.00b	0.28 ± 0.00a	0.20 ± 0.00c
Solubility (%)	72.79 ± 0.58b	85.15 ± 0.21a	86.65 ± 0.71a
Hygroscopicity (%)	45.13 ± 0.01a	40.74 ± 0.02b	37.86 ± 0.01b
Yield (%)	17.64 ± 0.02c	37.94 ± 0.01b	44.5 ± 0.02a
L*	51.80 ± 1.00 c	75.19 ± 1.40b	81.54 ± 1.07a
a*	11.79 ± 0.48a	1.41 ± 0.36b	1.42 ± 0.10b
b*	28.77 ± 1.20a	23.73 ± 0.46b	18.10 ± 0.27c
h°	31.09 ± 1.29b	23.77 ± 0.47a	18.15 ± 0.27a
C*	67.72 ± 0.02a	86.59 ± 0.82b	85.50 ± 0.33c

C0: Control sample without addition of maltrodextrin; C1: encapsulated chicken flavor added 50 % maltodextrin; C2: encapsulated chicken flavor added 100 % maltodextrin.
Results are expressed as mean ± deviation.
a-e Different lowercase letters in the same row differ significantly by Tukey's test ($p \leq 0.05$).

view of the safety and stability of food powders since it influences their chemical properties and shelf life. Aw <0.3 allows reactions to take place too slowly, which allows the food product to be considered stable (Faria et al., 2020), indicating good stability of the powders obtained.

The applicability of microparticles prepared by the food industry presupposes good solubility in water (Bajac et al., 2022). For the values of this critical parameter, the control sample (72.79 %) differed significantly from C1 (85.15 %) and C2 (86.65 %). Still, all showed satisfactory solubility values, similar to those reported in the literature regarding microencapsulation with maltodextrin.

Hygroscopicity is the ability of a material to absorb moisture from the atmosphere. When high, it can contribute to lipid oxidation and powder aggregation (Bajac et al., 2022). Regarding hygroscopicity, the control sample had a significantly higher value than the C1 and C2 formulations, attesting to the importance of encapsulation. The C2 sample showed lower hygroscopicity due to the higher concentration of maltodextrin since the concentration of this encapsulant is the variable that most affect the hygroscopicity of powders. This is due to the low hygroscopicity of maltodextrin, which contributes to its efficiency as an encapsulating material (Tonon et al., 2008).

About the color analysis, a difference was observed between the samples for the L* parameter (Table 3), with values ranging from 51.80 to 81.54, increasing according to the maltodextrin concentration. This refers to the whiter (lighter) color of the C2 powder compared to C0, which lacked encapsulating material. For the a* values, the means differed significantly only for the control sample, indicating a redder color. The b* values represent a movement toward a yellow color, which was also more pronounced in the control sample, indicating that the increase in maltodextrin leads to a decrease in the a* and b* values. Maltodextrin is white, which justifies the changes caused by adding it to the flavoring (Silva, Marques, et al., 2024).

For h° values, sample C0 differed significantly from the others. However, they remained in the reddish region, with C0 at the threshold with a yellowish region, possibly due to the color of the flavoring.

3.3.2. *Scanning electron microscopy (SEM)*

The SEM analysis showed microparticles with a typical morphological structure (Fig. 1) of spray-dried particles. Fig. 1 illustrates the particles of the samples obtained by spray drying. All samples exhibited a spherical appearance, typical of products obtained through atomization. Breternitz et al. (2017), when microencapsulating mussel protein hydrolysate for application in instant noodles, obtained particles with similar characteristics.

By observing Image A, which contains a size reference of 10 μm, it is evident that the particles vary slightly above and below this value. This size is commonly observed for particles obtained via spray drying. The characteristics of low moisture content, low hygroscopicity, and small particle diameters are considered crucial in the industrial handling of powders to prevent the separation of ingredients in a fine powder mixture.

3.3.3. *Retention of volatile compounds by chicken flavoring microparticles over time*

The retention of volatile components by the three samples of powdered chicken flavorings, C0 (control flavoring) dried without the addition of maltodextrin, C1 (flavoring microencapsulated with 50 % maltodextrin) and C2 (flavoring microencapsulated with 100 % maltodextrin), stored for 90 days was evaluated: T1 (30 days), T2 (60 days) and T3 (90 days) (Supplementary table).

Forty-five volatile compounds were identified among the C0, C1, and C2 samples in the three storage periods mentioned (Table S1). They are distributed in seven classes: aldehydes (24), alcohols (8), ketones (6), furan (1), sulfur-containing (3), ester (1), and phenol (2).

The volatiles identified in the powders showed different profiles (Fig. 2) between the control sample and the microencapsulated samples with maltodextrin. The maltodextrin-added samples (C1 and C2) showed the highest concentrations of most of the volatile compounds identified, with similar profiles. Storage time significantly influenced the increase in the concentration of most of the aldehydes and alcohols. The highlight was the sample containing 100 % maltodextrin after 60 days (C2T2), which showed the highest abundance of volatile compounds.

In contrast, although in smaller quantities, the C0 sample still had

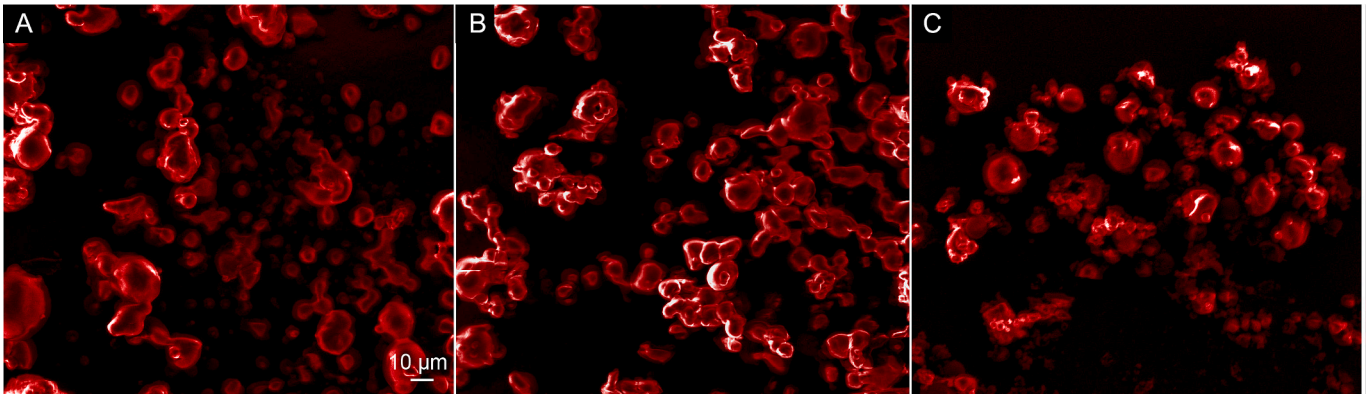

Fig. 1. Micrographs obtained by spray drying for C0, C1, and C2 samples.
Scanning electron microscopy (SEM) micrographs obtained by spray drying. A) C0: control sample; B) C1: sample added with 50 % maltodextrin; C) sample added with 100 % with maltodextrin. Magnification of 1500× for all images. The scale bar in fig. A represents 10 μm.

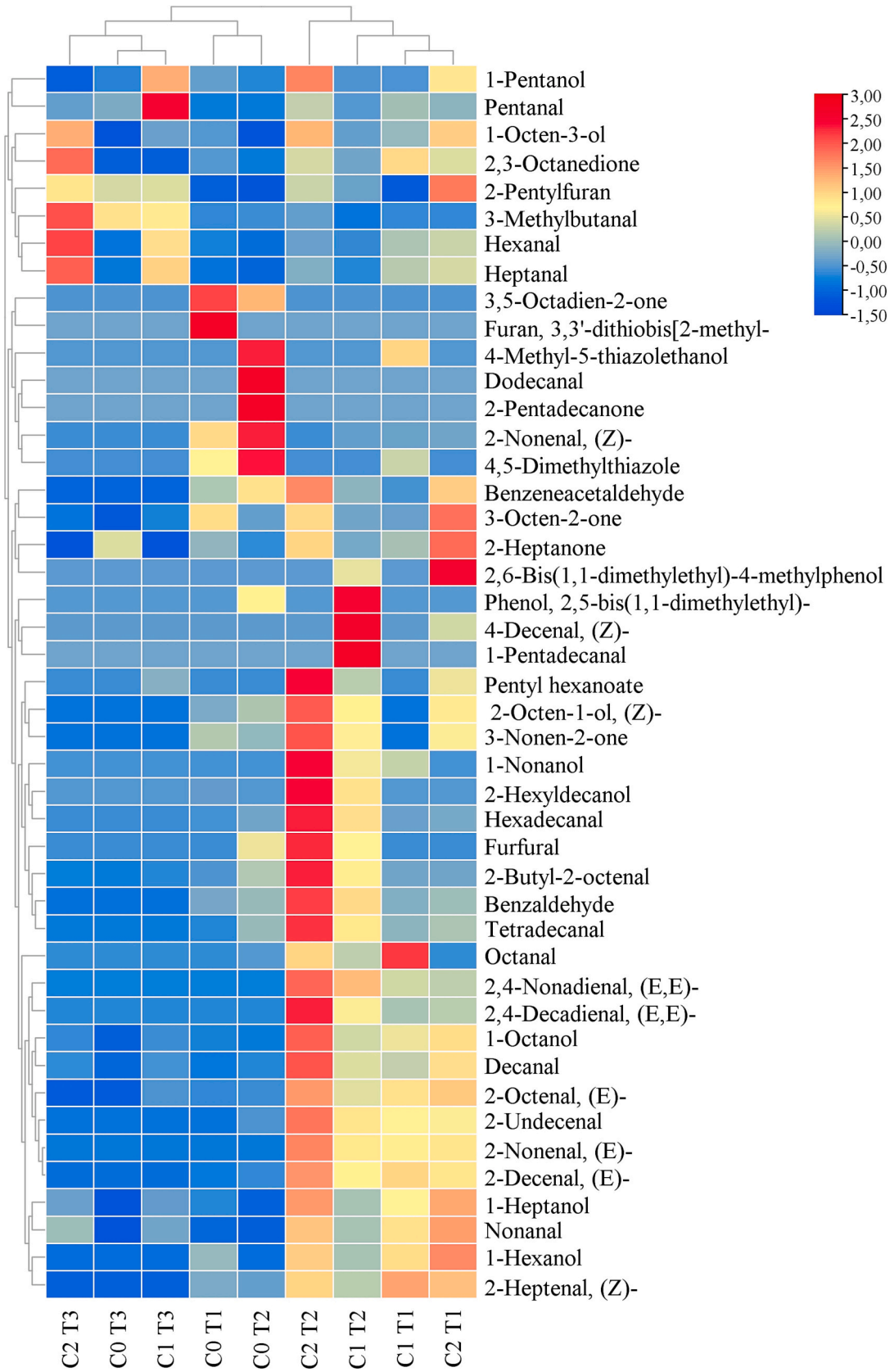

Fig. 2. Heat map generated based on volatile retention by microparticles at different times.
C0T3, control powder at final time; C1T3, microencapsulated powder with 50 % maltodextrin at final time; C2T3, microencapsulated powder with 100 % maltodextrin at final time; C1T1, microencapsulated powder with 50 % maltodextrin at initial time; C2T1: powder microencapsulated with 100 % maltodextrin at the initial time; C1T2: powder microencapsulated with 50 % maltodextrin at the intermediate time; C2T2: powder microencapsulated with 100 % maltodextrin at the intermediate time; C0T1: control powder at the initial time; C0T2: control powder at the intermediate time.

significant volatile compounds, including furans, such as furan, 3,3′-dithiobis[2-methyl-]. This class of compounds, originating from the Maillard reaction (MR), represents one of the main categories of aromatic substances formed during the heating of chicken (Yao et al., 2022). Unlike the samples microencapsulated with maltodextrin, the C0 sample contained a more significant amount of available xylose, favoring the progression of the MR during spray drying and promoting the formation of volatile compounds distinct from those generated in the samples supplemented with the glucose polymer.

Aldehydes were the predominant class of volatiles identified in the powders. Compounds such as octanal, nonanal and 2-undecenal, present in some samples, are products of the lipid oxidation of oleic acid. At the same time, pentanal and hexanal are derived from the oxidation of linoleic acid (Shahidi, 1994).

The most abundant aldehyde identified in the aroma of roast chicken meat is hexanal, and this result was observed in samples C1 and C2 during the longest storage time. This can be explained by the fact that when linoleic acid is submitted to thermal processes, it undergoes autooxidation, producing 13-hydroperoxide as one of the intermediates, which, when homolytically cleaved, synthesizes hexanal (Josephson & Lindsay, 1987). Similarly to hexanal, (E,E)-2,4-decadienal is also associated with a greasy odor. However, this compound is considered one of the aromatic marker compounds for chicken volatiles (Feng et al., 2018).

When it comes to this compound, specifically during storage (Fig. 3b), microencapsulation helped to retain and preserve it. The flavoring without microencapsulation did not include this compound in its aromatic profile (C0T1, C0T2, and C0T3), indicating instability during storage.

The same behavior was observed for 2,4-nonadienal-(E,E) (Fig. 3a), in samples C1T3 and C2T3, the volatile related to the sensory descriptor “chicken fat”, also considered a potential volatile marker for chicken aroma. This confirms the importance and efficiency of the microencapsulation method in retaining and preserving volatile compounds in products.

With almond flavor, the formation/retention of benzaldehyde, derived from the Strecker degradation of phenylalanine and isoleucine in proteins (Ouyang et al., 2024), occurred in samples C1 and C2 up to 60 days and was not retained during the 90-day storage period (T3), regardless of microencapsulation. With specific regard to the retention of decanal, an essential compound for roast chicken (Cunha et al., 2023), it was possible to verify that the control sample did not retain the compound for up to 60 days, as observed for the samples protected by maltodextrin, C1T2, and C2T2.

In addition to being metabolites of lipid degradation, alcohols can also be intermediates in the MR (Fontes et al., 2024). 1-octanol was the most significant alcohol for the maltodextrin-added samples. The powders’ retention of this alcohol could be observed at 30 and 60 days of storage, with degradation after 90 days. 1-octen-3-ol, an alcohol with important aromatic characteristics (chicken, earthy, and green), showed the best retention during T3 for the sample with the highest maltodextrin content (C2), signaling protection by the wall material under C2T3 conditions.

3.3.4. *Volatile compounds retention by principal component analysis*

To evaluate the formation and retention of volatile components throughout storage in powdered and microencapsulated flavorings, a principal component analysis was applied to reduce the dimensionality of the data and observe behaviors involving the aroma compounds.

As shown in Fig. 3c, the volatile constituents were distributed in all regions of the PCA plot. It was possible to see the separation of the groups as a function of storage time (Fig. 3d). The samples from T3 are grouped in the positive quadrant of PC1, correlating longer storage times with volatile compounds derived from lipid oxidation, such as the aliphatic aldehydes hexanal, pentanal, and heptanal. 3-Methylbutanal, formed through the Strecker degradation of leucine via MR (Varlet

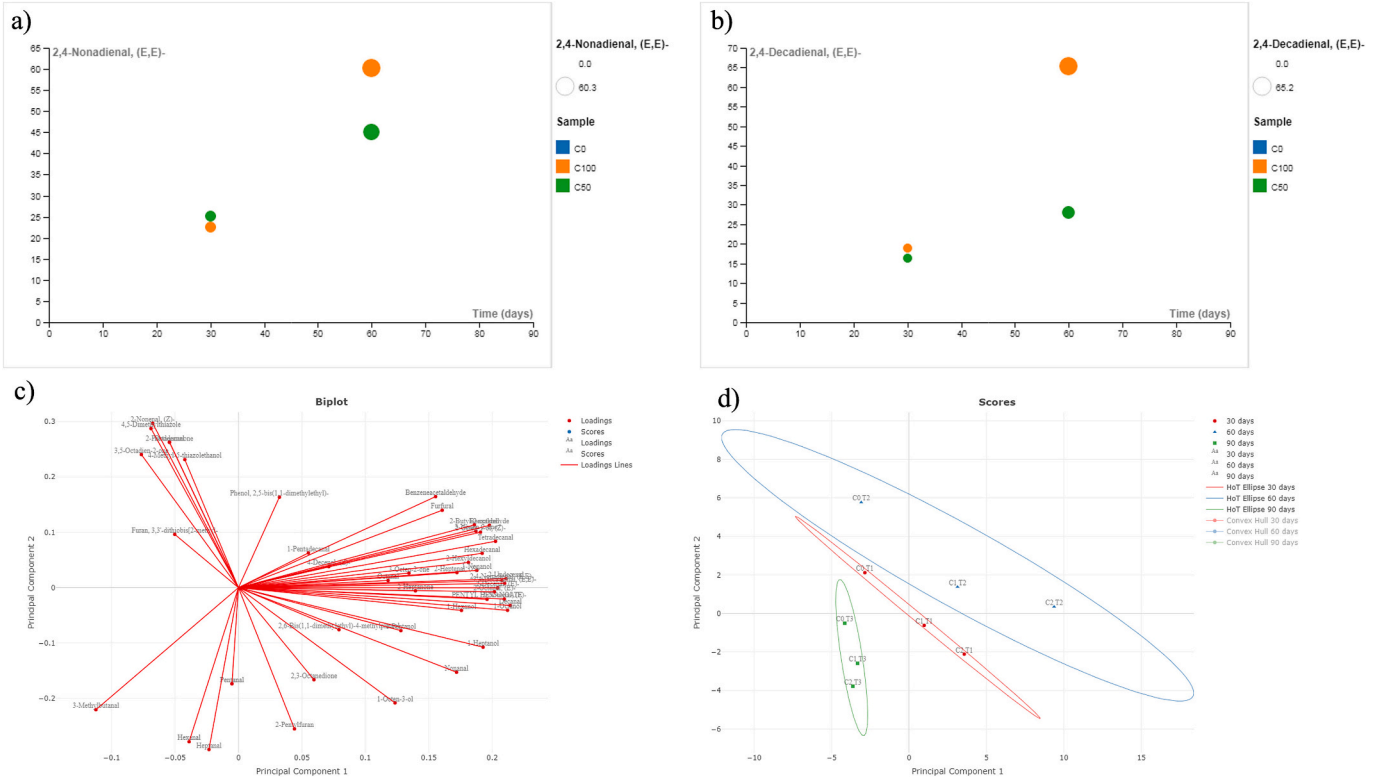

Fig. 3. Analysis of main components of volatile compounds present in powdered flavorings over storage time.
A) Concentration of 2,4-Nonadienal, (E,E)- in samples C0, C1, and C2 over storage time. B) Concentration of 2,4-Decadienal, (E,E)- in samples C0, C1, and C2 over storage time. C—D) Analysis of main components of volatile compounds present in powdered flavorings over storage time.

et al., 2007), also showed higher concentrations in T3. Its low detection threshold contributes to the easy perception of a fatty aroma (Fontes et al., 2024).

On the other hand, the negative quadrant of PC1 and PC2 had a more heterogeneous profile of volatiles, grouping samples related to T2 and T1. The grouping referring to the intermediate storage time correlates compounds such as (E)-2-decenal and (E,E)-2,4-decadienal, which Qi et al. (2021) showed a positive relationship with the fleshy trait when correlating variables of flavor and aroma characteristics during evaluation of short-term frozen storage for chicken meat.

In addition, it is possible to observe that at T2 there is the opposite grouping between C1 and C2 about C0, and compounds such as nonanal, decanal, (E,E)-2,4-decadienal and (E,E)-2,4-decadienal, are closer to C1 and C2, thus favoring the meaty aroma in the microencapsulates stored at T2.

3.3.5. *Efficiency of microencapsulation*

Table 4 shows the quantity of components in the dry sample without excipient (C0) and those containing 50 % or 100 % maltodextrin after drying. In all the samples containing maltodextrin, except Benzeneacetaldehyde in C1, the component values were equivalent or higher, demonstrating the retention of compounds in the matrix formed by maltodextrin during the spray drying process of the liquid flavoring.

3.3.6. *Sensory evaluation*

The powdered and microencapsulated chicken flavorings at an early stage (T1) were evaluated using Optimized Descriptive Sensory Analysis. The sensory panel selected four (4) aromatic attributes representative of the samples: stewed chicken, roast chicken, artificial chicken flavoring, and sweet.

Fig. 4 shows that the three flavoring samples presented the four attributes highlighted by the panel but with different intensities, making it possible to characterize each sample sensorially.

The control flavoring (C0) had a higher intensity of roast and artificial chicken aroma. This sample went through the drying process without adding wall material (maltodextrin). It showed more concentrated aromatic characteristics than concentrations C1 and C2, which showed a less intense aroma diluted by the encapsulating material. This result corroborates the findings of the volatile profile discussed above.

Sample C1, a flavoring microencapsulated with 50 % maltodextrin, had a higher intensity of cooked chicken aroma. This characteristic may be due to the insertion of maltodextrin during microencapsulation, making the chicken aroma softer and more similar to cooked chicken.

While the flavoring microencapsulated with 100 % maltodextrin (C2) was characterized by lower intensities of aromatic descriptors. However, the sweet descriptor was associated with a higher value for this sample, possibly due to the formation of compounds characteristic of reactions such as caramelization since maltodextrin is a carbohydrate made up of glucose oligomers.

Table 4
Efficiency of microencapsulation on the concentration of volatile compounds.

Compounds (μg/g)	C0 T1	C1 T1	C2 T1
Aldehydes			
3-Methylbutanal	5.8 ± 0.0a	6.3 ± 3.4a	6.5 ± 0.2[a]
Pentanal	7.2 ± 0.0b	37.6 ± 14.2a	30.4 ± 2.7a
Hexanal	166.7 ± 0.0b	680.7 ± 51.2a	809.2 ± 106.1a
Heptanal	39.2 ± 0.0b	123.5 ± 12.1a	141.4 ± 14.6[a]
Benzaldehyde	17.3 ± 0.0b	20.1 ± 3.6ab	25.7 ± 4.5a
Benzeneacetaldehyde	15.3 ± 0.0b	6.4 ± 0.2c	27.7 ± 2.5[a]
Nonanal	144.7 ± 0.0b	914.5 ± 177.4a	1147.2 ± 41.8a
Decanal	6.9 ± 0.0c	54.3 ± 11.7b	83.4 ± 0.8[a]
Alcohols			
1-Hexanol	3.3 ± 0.0b	7.2 ± 1.2a	10.0 ± 2.1a
1-Heptanol	26.9 ± 0.0c	72.0 ± 7.8b	94.8 ± 8.5[a]
1-Octen-3-ol	61.2 ± 0.0b	79.4 ± 12.6b	127.4 ± 6.8a
1-Octanol	76.8 ± 0.0b	223.5 ± 31.9a	269.9 ± 12.1[a]
Ketone			
2-Heptanone	5.7 ± 0.0b	6.4 ± 0.7b	15.8 ± 1.8a
Furan			
2-Pentylfuran	54.1 ± 0.0b	51.5 ± 2.9b	190.6 ± 2.0[a]

C0 – Control flavoring; C1 – Flavoring with 50 % maltodextrin; C2 – Flavoring with 100 % maltodextrin; T1 – Stored for one month; Means with different letters within the same row showed significant difference ($p < 0.05$), by Tukey test.

4. Conclusion

This study describes the production and retention of aromas obtained by microencapsulating chicken flavoring. The hydrolytic process carried out on the chicken bones with Flavourzyme® allowed the presence of interesting compounds such as decanal, (E)-2-decenal, (E,E)-2,4-decadienal, 1-pentanol, 1-hexanol, and 1-octanol in the aromatic bouquet. Additionally, the Maillard reaction, induced at pH 4 and 6, demonstrated that the less acidic pH was more favorable for the formation of meat-like aroma, particularly enhancing the characteristics associated with cooked chicken flavor. The results related to the encapsulated product suggest its quality in terms of parameters such as yield and stability, which allows for the applicability of the microparticles obtained. A total of 45 volatile components were identified, including volatiles of importance for chicken flavoring, which differed quantitatively and qualitatively between samples C0, C1, and C2. Characteristic compounds such as 2,4-Decadienal, (E,E)- and 2,4-Nonadienal, (E,E)- were identified in C1T1, C1T2, C2T1, and C2T2, suggesting retention capacity by the samples with added maltodextrin when compared to control samples, in which the aforementioned compounds were not identified. Sensorial data suggest that the different profiles of the volatile components between the powders make it viable to add chicken flavoring to satisfy different objectives for the food industry, whether with a more intense flavor close to artificial or a smoother flavor close to natural flavoring, such as roast or boiled chicken.

CRediT authorship contribution statement

Rebeka Correia de Souza Cunha: Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Viviane Maria de Sousa Fontes:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Eike Guilherme Torres de Souza:** Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Gezaildo Santos Silva:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Layane Rosa da Silva:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mercia de Sousa Galvão:** Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Izabela Dutra Alvim:** Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Angela Matilde da Silva Alves:** Methodology, Investigation, Formal analysis. **Maria Teresa Bertoldo Pacheco:** Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Felipe Lopes Brum:** Formal analysis, Investigation, Methodology. **Sandro Marden Torres:** Methodology, Supervision. **Marta Suely Madruga:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization. **Valéria Paula Rodrigues Minim:** Supervision, Project administration, Conceptualization. **Taliana Kênia Alencar Bezerra:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

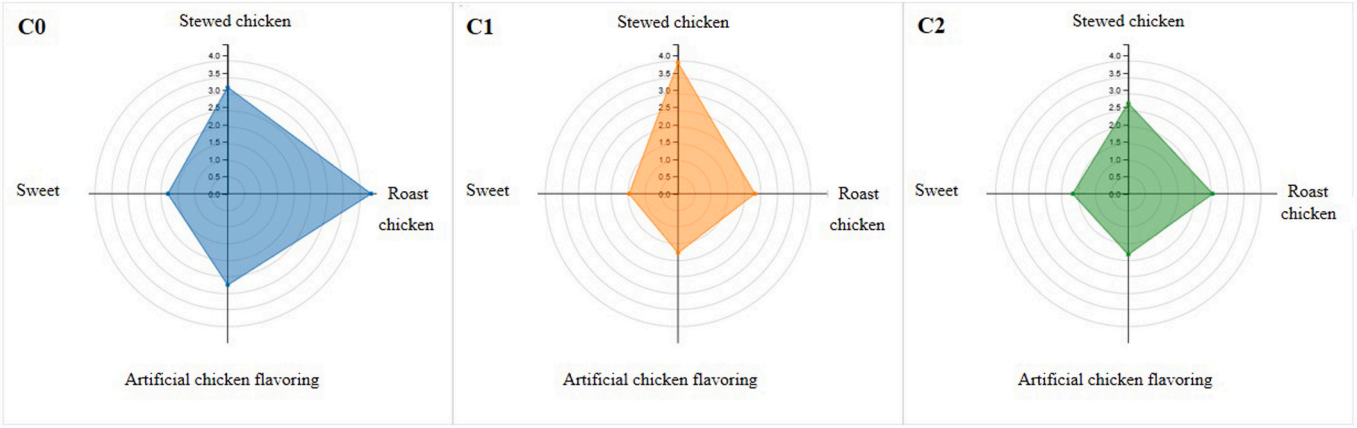

Fig. 4. Sensory evaluation of powdered flavorings.
C0, control sample without addition of maltodextrin; C1, sample microencapsulated with 50 % maltodextrin; C2, sample microencapsulated with 100 % maltodextrin.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.144313.

Data availability

No data was used for the research described in the article.

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