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Hydrolyzed collagen, KCl, and arginine: A successful strategy to reduce fat and sodium while maintaining the physicochemical, sensory, and shelf life quality of mortadella

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ABSTRACT

Mortadellas were reformulated by replacing animal fat with hydrolyzed collagen (HC) at 50 % and 70 % levels, substituting 50 % NaCl with KCl, and adding 1 % arginine. The effects of these changes on physicochemical properties, sensory attributes, and shelf life over 60 days at 4 °C were evaluated. The results showed that substituting 50 % and 70 % of fat with HC reduced fat content by 42.6 % and 55.3 %, respectively, while increasing protein levels by 37 % and 55 %. Replacing 50 % of NaCl with KCl reduced sodium content by 33.2 % and increased potassium content by 234.7 %. The 50 % HC substitution was the most suitable, as it preserved sensory attributes and structural integrity, unlike the 70 % replacement, which negatively affected texture and taste. Although substituting NaCl with KCl introduced sensory defects like bitter and metallic tastes, adding arginine effectively masked these issues, resulting in sensory quality comparable to the control. Oxidative stability, evaluated through TBARS values and sensory assessments, was similarly maintained across all treatments throughout storage. Counts of aerobic mesophilic microorganisms and lactic acid bacteria evolved similarly in reformulated products and the control, ensuring microbiological stability. The pH levels remained consistent across treatments, and color stability, assessed by ΔE values, was preserved throughout storage. These results highlight the feasibility of reformulating mortadellas with HC, KCl, and arginine to produce healthier products without compromising sensory quality, physicochemical stability, or shelf life.

1. Introduction

Mortadella is a traditional emulsified cooked sausage widely recognized for its rich flavor, smooth texture, and culinary versatility. This meat product is typically prepared from finely ground pork or a mixture of pork and beef, seasoned with select spices to achieve its distinctive taste. However, it is traditionally characterized by high levels of saturated fats and sodium chloride (NaCl) (Campagnol, Lorenzo, Da Rosa, Dos Santos, & Cichoski, 2021), components directly linked to increased risks of cardiovascular diseases, hypertension, and other health issues when consumed excessively (MacDonald, Madkia, Mounier-Vehier, Severi, & Boutron-Ruault, 2023; Rust & Ekmekcioglu, 2017). In light of growing public health concerns, consumer demand has shifted toward healthier versions of traditional products that maintain their sensory appeal and technological quality while offering improved nutritional profiles (Saldaña, Eduardo, Mayta-Hancco, Huamán-Castilla, & Escobedo-Pacheco, 2024). Reformulating mortadella to meet these expectations presents significant challenges, particularly in preserving its physicochemical and sensory properties and shelf life.

Hydrolyzed collagen (HC) has emerged as a promising alternative for reducing animal fat in meat products like mortadella. Composed of hydrophilic amino acids, HC enhances water retention, stabilizes emulsions, and improves texture, which, in turn, can enhance the technological and sensory quality of emulsified meat products (Gomez-Guillen, Gimenez, Lopez-Caballero, & Montero, 2011). Beyond its technological benefits, HC is associated with health advantages such as

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Table 1
Formulation of low-sodium and low-fat mortadella produced with hydrolyzed collagen, potassium chloride, and arginine.

(%)	Control	HC_{50}	HC_{50} + KCl	HC_{50} + KCl + Arg	HC_{70}	HC_{70} + KCl	HC_{70} + KCl + Arg
Beef	70	70	70	70	70	70	70
Pork backfat	15	7.5	7.5	7.5	4.5	4.5	4.5
Sodium chloride	2.5	2.5	1.25	1.25	2.5	1.25	1.25
Potassium chloride	0	0	1.25	1.25	0	1.25	1.25
Arginine	0	0	0	1	0	0	1
Hydrolyzed collagen	0	7.5	7.5	7.5	10.5	10.5	10.5
Sodium nitrite	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Sodium tripolyphosphate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium erythorbate	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Monosodium glutamate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Garlic	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Coriander	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Black pepper	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Water	11.06	11.06	11.06	10.06	11.06	11.06	10.06

supporting joint, skin, and bone health (Porfírio & Fanaro, 2016; Proksch et al., 2014). Studies have shown that HC can replace up to 50 % of animal fat in sausages without negatively impacting sensory or technological properties (Sousa et al., 2017). However, higher substitution levels may compromise sensory attributes, emphasizing the need for optimized applications in meat products. In this context, a 70 % substitution level was included in the present study to investigate the upper limit of HC replacement and to identify the potential challenges associated with its application at higher levels.

Sodium chloride is critical in meat product processing by contributing to flavor, microbial stability, and texture (Campagnol, Lorenzo, Dos Santos, & Cichoski, 2022). Nevertheless, excessive sodium consumption has driven efforts to reduce NaCl content using salt replacers (Khan et al., 2024). Among the available sodium substitutes, KCl stands out as the most widely used and promising due to its molecular similarity to NaCl and its Generally Recognized as Safe (GRAS) status. However, its use often produces sensory defects such as bitter and metallic tastes at substitution levels above 40–50 % (dos Santos Alves et al., 2017). To address these sensory challenges, recent studies suggest that specific amino acids, such as lysine, arginine, and histidine, can mask these defects by interacting with taste receptors to counteract bitterness and enhance overall flavor in products with reduced NaCl (Wai, Zhong, Feng, & Xu, 2024). Building on this, a previous study conducted by our research group demonstrated that adding 1 % arginine to mortadellas reformulated with high levels of KCl significantly improved sensory acceptability. This improvement is attributed to arginine's ability to interact with taste receptors, reducing their response to bitter and metallic flavors associated with KCl (da Silva et al., 2020).

Although HC, KCl, and arginine have been individually explored in meat product reformulations, their combined effects have not been thoroughly studied. This study uniquely investigates their synergy as a comprehensive strategy to reduce sodium and fat in mortadella. A critical challenge addressed is the interaction between fat reduction and salt perception, as lower fat levels can attenuate salty taste intensity (Desmond, 2006), potentially compromising sensory quality. By exploring this synergy, the study establishes a novel approach for developing healthier mortadella that maintains technological, sensory, and shelf-life properties.

In this study, mortadellas were reformulated by substituting 50 % or 70 % of animal fat with HC, replacing 50 % NaCl with KCl, and adding 1 % arginine. The effects of these modifications on physicochemical properties, sensory attributes, and shelf life were comprehensively evaluated to develop a nutritionally improved product that meets consumer demand for healthier options.

2. Materials and methods

2.1. Treatments and preparation of Mortadellas

Eight treatments were prepared according to the formulations presented in Table 1. The control treatment was formulated with 2.5 % sodium chloride (NaCl) and 15 % pork backfat. In the treatments coded as “HC_{50}” and “HC_{70}”, 50 % and 70 % of the pork backfat were replaced, respectively, with HC (Peptan B 2000 HD, Rousselot do Brasil Ltda, São Paulo, Brazil). The HC used in this study had a chemical composition of 92.3 ± 1.1 % protein, 7.4 ± 0.2 % moisture, and 0.2 ± 0.01 % ash. Its amino acid profile (g/100 g of protein) was as follows: alanine (8.1), arginine (8.4), aspartic acid (6.6), glutamic acid (12.4), glycine (20.6), histidine (0.8), hydroxylysine (1.2), hydroxyproline (11.4), isoleucine (1.5), leucine (2.9), methionine (0.6), phenylalanine (2.1), proline (11.5), serine (3.4), threonine (1.9), tyrosine (0.5), and valine (2.4). In the treatments coded as “KCl”, 50 % of the NaCl was replaced with potassium chloride (KCl), and in the treatments coded as “Arg”, 1 % arginine (Merck, Darmstadt, Germany) was added. The amount of water was adjusted in all treatments to ensure that the total sum reached 100 %.

The manufacturing process was conducted three times, and during each run, 15 mortadellas were produced per treatment, each weighing approximately 200 g. The beef and pork backfat were separately ground using 5 mm discs (PJ-22 Plus Professional, Jamar, Brazil). The ground meat was then transferred to a cutter (R.i60, Cutter Industrial, Brazil), where it was mixed with NaCl, sodium tripolyphosphate, and half of the water for 60 s to extract the myofibrillar proteins. Next, the remaining ingredients, including the HC, were incorporated directly into the batter without prior hydration. Pork backfat was then added, and the comminution process continued until complete homogenization was achieved. Throughout the comminution process, the temperature of the mass was maintained below 12 °C (Pinton et al., 2019). The emulsion was stuffed into impermeable artificial casings (46 μm thickness, 32 mm diameter) and cooked in water at 80 °C until reaching an internal temperature of 72 °C. After cooking, the mortadellas were immediately cooled in an ice bath until they reached an internal temperature below 10 °C and then stored under refrigeration at 4 (±0.1) °C for 60 days.

Samples for the different analyses were taken directly from the refrigerated storage at the specified time points (e.g., days 1, 15, 30, 45, and 60). For sensory evaluations, the mortadellas were sliced and prepared fresh for tasting on the designated days of analysis. Physicochemical and microbiological analyses were performed on freshly prepared samples immediately after removal from refrigeration to ensure the integrity of the products.

2.2. Physicochemical analyses

2.2.1. Emulsion stability and cooking losses

The emulsion stability was evaluated in quintuplicate immediately after processing by measuring water and fat exudation, as Colmenero, Ayo, and Carballo (2005) described. Five grams of emulsion were placed in 25 mL Falcon tubes, centrifuged at 2 °C for 5 min at 388*g*, followed by heating at 95 °C for 40 min. The exuded liquids were collected and quantified after cooling in an ice bath to 10 °C.

Cooking losses were determined in quintuplicate. Approximately 20 g of emulsion samples were packaged in polyethylene bags, sealed, and cooked in water at 70 °C for 60 min. The cooking losses were calculated based on the weights before and after cooking, as described by Parks and Carpenter (1987).

2.2.2. Chemical composition and minerals

The mortadellas' chemical composition and mineral concentration were determined in triplicate immediately after manufacturing. The moisture, protein, and ash contents were evaluated according to the methodology of the Association of Official Analytical Chemists (AOAC, 2010). Protein content was determined using the Kjeldahl method; moisture was measured by oven drying, and ash content was determined by incineration in a muffle furnace. Fat content was determined using the solvent extraction method described by Bligh and Dyer (1959).

The minerals (Na, K, P, Ca, Mg, Zn, Fe, Cu, Mn) were quantified after dry digestion, as described by AOAC (2010). Samples (2.5 g) were incinerated in a muffle furnace at 450 °C, and the resulting ashes were dissolved in a 5 % (*v*/v) hydrochloric acid solution. Determining minerals was conducted using an inductively coupled plasma optical emission spectrometer (ICP OES), model Agilent 5100 VDV ICP OES (Agilent Technologies, Tokyo, Japan). The equipment operated with axial and radial views, using a 27 MHz solid-state radio frequency source, peristaltic pump, double-pass cyclonic spray chamber, and seaspray nebulizer. The radio frequency power was set to 1.20 kW, the argon flow rate was 12 L/min, and the auxiliary flow rate was 1.0 L/min. The nebulizer flow rate was 0.70 L/min, with a read time of 7 s and a stabilization time of 15 s. The argon used as plasma gas had a minimum purity of 99.996 %.

2.2.3. Water activity (Aw)

The mortadellas' water activity (Aw) values were determined in triplicate immediately after manufacturing. The water activity was measured using the Aqualab 4TE device (Decagon, Pullman, WA), calibrated with standard salt solutions (NaCl and LiCl), and operated at 25 °C according to the manufacturer's instructions.

2.2.4. Texture profile

The mortadellas' texture profile analysis (TPA) was performed in sextuplicate immediately after manufacturing. Three mortadellas per treatment were used, with a 5 cm thick slice taken from the central portion of each. Two cylinders measuring 2 cm in height and 2 cm in diameter were extracted from each slice, totaling six cylinders per treatment. The analysis used a texture analyzer (TA.XT.plus, Stable Micro Systems Ltd., Surrey, England) with a 25 kg load cell. The cylinders were compressed in two successive cycles with 50 % compression of their original height, using a P/40 probe (40 mm diameter) with a test speed of 1 mm/s. The following texture parameters were determined: hardness (N), elasticity (mm), cohesiveness, gumminess (N), and chewiness (N).

2.2.5. Instrumental color

For the instrumental color analysis, three 5 cm thick slices were taken from the central portion of three mortadellas per treatment (one slice per mortadella). The color was evaluated at three different points on the internal portion of each slice, using a colorimeter model CR-700d (Konica Minolta, Japan), equipped with spectral reflectance and previously calibrated with a white calibration plate. The L^* (lightness), a^* (redness intensity), and b^* (yellowness intensity) values were determined using a D65 illuminant with a 10° observation angle and an aperture of 1.5 cm.

2.3. Sensory analyses

2.3.1. CATA (check-all-that-apply) and acceptance tests

The CATA and acceptance tests were performed immediately after manufacturing in a Sensory Analysis Laboratory equipped with individual booths with light and temperature control. The tests were conducted with 100 regular mortadella consumers aged between 18 and 65. All tasters signed an informed consent form agreeing to participate in the study. For the CATA test, participants received a list of sensory attributes, including three related to appearance (pink color, pale, and unpleasant), two to aroma (pleasant aroma and rancid), eleven to taste (rancid taste, metallic, pleasant, bitter, acidic, unpleasant, astringent, very salty, little salty, right amount of salt, and pleasant seasoning), and four to texture (juicy, pleasant texture, hard, and dry). The terms used in the questionnaire were developed by a group of fifteen trained tasters from the sensory panel described in Section 2.4.1, in addition to including sensory descriptors from the literature (Stone & Sidel, 2004).

The tasters were instructed to select all the sensory attributes they perceived in each sample. They were also instructed to mark the attributes they deemed important to be present in a sample they considered ideal. Subsequently, the tasters performed the acceptance test evaluating the attributes of color, aroma, taste, texture, and liking of the samples using a 9-point unstructured hedonic scale, ranging from "dislike extremely" to "like extremely". The mortadellas were cut into slices of 0.3 cm thickness and 3 cm diameter and served in white plastic cups coded with three random numbers. The sensory analysis was conducted in two sessions, and all tasters evaluated all samples. The samples were served in monadic order in a balanced way using balanced complete blocks (Macfie, Bratchell, Greenhoff, & Vallis, 1989).

2.4. Shelf life analyses

2.4.1. Oxidative stability

Oxidative stability during storage of the mortadellas was evaluated through TBARS analysis and descriptive sensory analysis. The TBARS analysis was performed in triplicate on days 1, 15, 30, 45, and 60 of storage using the method described by Bruna, Ordóñez, Fernández, Herranz, and de la Hoz (2001), and the results were expressed in milligrams of malonaldehyde per kg of sample. The attributes of oxidized color, rancid aroma, and rancid taste, which are associated with the degradation of lipid quality in mortadellas (da Silva et al., 2019), were evaluated through a descriptive sensory analysis on days 1, 30, and 60 of storage. The sensory panel consisted of 15 trained tasters (9 women and 6 men) aged between 20 and 42. The tasters were trained to identify these attributes in four sessions, each lasting four hours. In the first session, the descriptive terms related to lipid degradation were defined, along with their references (Table S1). After training, the tests were conducted using a 9 cm unstructured scale, whose extremes ranged from little/none to very much. The samples were served as described in item 2.3.1.

2.4.2. Counts of aerobic mesophilic microorganisms and lactic acid Bacteria

The counts of aerobic mesophilic microorganisms and lactic acid bacteria were performed in triplicate on days 1, 30, and 60 of storage, according to the methodologies described in ISO 7218:2007 (International Standard Organization, 2007).

2.4.3. pH

The pH values of the mortadellas were determined in triplicate on days 1, 15, 30, 45, and 60 of storage. For the pH analysis, 10 g of sample

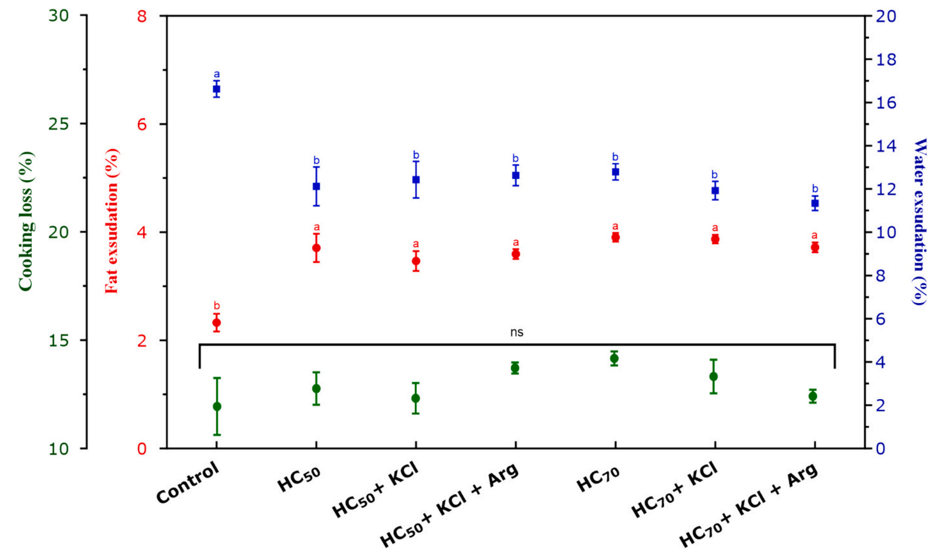

Fig. 1. Cooking loss and emulsion stability (fat and water exudation) of mortadella samples.
Values are presented as mean ± SEM. Different superscript letters indicate significant differences ($P < 0.05$). Treatments: see Table 1.

Table 2
Physicochemical properties of low-fat and low-sodium mortadellas produced with hydrolyzed collagen, potassium chloride, and arginine.

	Control	HC_{50}	HC_{50} + KCl	HC_{50} + KCl + Arg	HC_{70}	HC_{70} + KCl	HC_{70} + KCl + Arg	SEM	SIG
				Chemical composition (g/100 g)					
Moisture	65.3^{a}	65.4^{a}	64.3^{a}	63.1^{b}	65.9^{a}	65.5^{a}	63.7^{b}	1.2	*
Fat	14.1^{a}	8.1^{b}	8.3^{b}	7.9^{b}	6.5^{c}	6.1^{c}	6.3^{c}	0.4	***
Protein	17.1^{c}	23.7^{b}	22.7^{b}	23.9^{b}	26.5^{a}	26.8^{a}	26.2^{a}	0.8	***
Ash	4.2^{c}	4.2^{c}	4.2^{c}	4.4^{b}	4.2^{c}	4.2^{c}	4.5^{a}	0.01	***
				Minerals (mg/100 g)					
Na	1177.1^{a}	1119.6^{a}	767.3^{b}	752.1^{b}	1115.7^{a}	804.6^{b}	822.3^{b}	29.2	***
K	240.2^{b}	223.1^{b}	745.4^{a}	811.9^{a}	234.3^{b}	853.3^{a}	805.4^{a}	14.3	***
P	191.7^{a}	172.4^{c}	160.0^{d}	179.6^{bc}	173.2^{c}	187.2^{ab}	182.6^{b}	2.5	***
Ca	6.8^{d}	26.5^{c}	26.2^{c}	34.1^{b}	40.6^{a}	40.7^{a}	41.2^{a}	1.8	***
Mg	15.9^{a}	13.8^{b}	12.4^{c}	15.7^{a}	15.2^{a}	16.2^{a}	15.6^{a}	0.3	***
Zn	3.3^{b}	2.0^{e}	1.2f	3.8^{a}	2.3^{d}	3.0^{c}	3.3^{b}	0.1	***
Fe	1.1^{d}	0.9^{d}	0.7^{e}	1.9^{a}	1.0^{d}	1.4^{c}	1.6^{b}	0.06	***
Cu	0.04^{b}	0.01^{e}	0.01^{e}	0.05^{a}	0.02^{d}	0.03^{c}	0.04^{b}	0.00	***
Mn	0.02^{bc}	0.02^{bc}	0.018^{c}	0.04^{a}	0.03^{b}	0.02^{bc}	0.04^{a}	0.00	***
Aw	0.981^{a}	0.973^{ab}	0.974^{ab}	0.968^{b}	0.973^{ab}	0.974^{ab}	0.972^{b}	0.002	*
				Texture profile					
Hardness (N)	112.9^{a}	105.5^{ab}	102.5^{b}	98.0^{b}	86.8^{c}	82.1^{c}	65.4^{d}	2.7	***
Springiness (mm)	0.91^{a}	0.88^{a}	0.89^{a}	0.91^{a}	0.90^{a}	0.90^{a}	0.88^{a}	0.01	n.s.
Cohesiveness	0.76^{a}	0.75^{a}	0.77^{a}	0.74^{a}	0.74^{a}	0.76^{a}	0.75^{a}	0.01	n.s.
Gumminess (N)	85.3^{a}	78.6^{ab}	79.1^{ab}	72.9^{b}	64.6^{c}	62.1^{c}	48.8^{d}	2.1	***
Chewiness (N)	77.6^{a}	69.4^{b}	70.6^{b}	66.4^{b}	58.3^{c}	56.3^{c}	43.2^{d}	2.3	***
				Color parameters					
L^*	56.8^{a}	50.6^{c}	51.6^{bc}	48.2^{d}	52.2^{b}	51.0^{c}	50.8^{c}	0.3	***
a^*	11.9^{d}	12.7^{c}	14.0^{ab}	14.0^{ab}	13.5^{b}	14.2^{ab}	14.7^{a}	0.2	***
b^*	12.6^{b}	13.7^{b}	13.7^{b}	12.9^{b}	13.4^{b}	13.6^{b}	15.1^{a}	0.3	**

Values are presented as mean. Different superscript letters in the same row indicate significant differences ($P < 0.05$).
Treatments: See Table 1.
SEM: standard error of the mean.
SIG (level of significance): n.s. (not significant); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

was homogenized in distilled water at 1:10 (sample/water). The measurement was performed using an electrode coupled to a digital pH meter (Digimed - DM-23 DC, São Paulo, SP, Brazil) calibrated with standard pH 4 and 7 solutions per the manufacturer's guidelines before use.

2.4.4. Overall color difference (ΔE)

The mortadellas' L^*, a^*, and b^* values were determined on days 1, 15, 30, 45, and 60 of storage as described in item 2.2.5. The overall color difference values (ΔE) were calculated for each treatment by comparing the storage days (15, 30, 45, and 60) with the color values obtained immediately after manufacturing. The following equation was used to calculate ΔE:

Table 3
Consumer test results of low-sodium and low-fat mortadella produced with hydrolyzed collagen, potassium chloride, and arginine.

	Control	HC_{50}	HC_{50} + KCl	HC_{50} + KCl + Arg	HC_{70}	HC_{70} + KCl	HC_{70} + KCl + Arg	SEM	SIG
Color	6.4^{ab}	7.0^{a}	6.9^{ab}	6.8^{ab}	6.4^{ab}	6.2^{bc}	5.7^{c}	0.08	***
Aroma	6.4^{a}	5.9^{abc}	6.0^{abc}	6.2^{ab}	5.5^{c}	5.6^{bc}	5.4^{c}	0.07	***
Taste	6.5^{ab}	6.8^{a}	6.3^{abc}	6.4^{abc}	6.2^{abc}	5.8^{c}	6.0^{bc}	0.07	***
Texture	6.0^{ab}	6.6^{a}	6.3^{a}	6.4^{a}	6.2^{ab}	6.0^{ab}	5.6^{b}	0.07	***
Liking	6.3^{abc}	6.8^{a}	6.2^{abcd}	6.4^{ab}	6.1^{bcd}	5.7^{d}	5.9^{cd}	0.07	***

Averages within the same line followed by the same letters did not show any significant difference ($P > 0.05$) by Tukey's test. Treatments: Described in Table 1. SEM: standard error of the mean; Sig.: significance: *** ($P < 0.001$).

$$\Delta E = \left[(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2\right]^{0.5} \quad (1)$$

Where ΔE is equal to the square root of the sum of squares of the differences between the coordinates L^*, a^*, and b^* of the same treatment on days 15, 30, 45, and 60 with day 1 (de Oliveira Faria et al., 2015).

2.5. Statistical analysis

The study utilized a completely randomized experimental design, with three independent batches for each treatment, processed and analyzed individually to ensure true replication. A generalized linear model was used to analyze the results, considering treatments and storage days as fixed effects and replicates as a random effect. Sessions and tasters were also included in the model as random factors to analyze the sensory results; however, their effects were not significant. The interaction between the fixed effects was examined in the shelf life analyses. Tukey's test at a 5 % level was applied for mean comparisons. The results of the CATA test were evaluated through correspondence analysis, and a principal component analysis was applied to assess the correlation between the descriptors of the CATA test and the liking scores. A Generalized Procrustes Analysis (GPA), including the tasters in the model, was applied to jointly evaluate the data from the three days (1, 30, and 60 days) on which the descriptive sensory analysis was performed. All statistical analyses were conducted using XLSTAT Version 2016.02.28451 (Addinsoft, Paris, France).

3. Results and discussion

3.1. Physicochemical analyses

3.1.1. Cooking loss and emulsion stability

The results for cooking loss and emulsion stability are presented in Fig. 1. The cooking loss of the samples ranged between 11.9 % and 13.7 % ($P > 0.05$). The emulsion stability analysis revealed that the HC treatments exhibited higher fat exudation and lower water exudation than the control ($P < 0.05$), which can be attributed to the composition of the hydrolyzed collagen. This outcome can be linked to the composition of the HC, which is predominantly composed of hydrophilic amino acids such as glycine, proline, and hydroxyproline. These major amino acids are known for their strong water-binding properties, which likely enhanced moisture retention in the emulsion while simultaneously reducing fat retention (Gomez-Guillen et al., 2011; León-López et al., 2019). Studies have observed similar results using collagen to replace fat in emulsified meat products (Araújo, Lima, Pereira, & Madruga, 2019; Sousa et al., 2017). Additionally, the partial replacement of NaCl with KCl and the addition of arginine did not significantly influence emulsion stability, highlighting that this sodium reformulation can be applied alongside HC without compromising emulsion integrity.

3.1.2. Chemical composition and minerals

The samples' moisture, fat, protein, and ash contents ranged from 63.1 % to 65.9 %, 6.1 % to 14.1 %, 17.1 % to 26.8 %, and 4.2 % to 4.5 %, respectively (Table 2). The fat replacement with HC and NaCl with KCl did not affect the samples' moisture; however, treatments containing arginine showed lower moisture content, consistent with the amount of water added to the formulations (Table 1). Treatments with arginine also had higher ash content, which can be attributed to the mineral residues of this amino acid.

Replacing 50 % and 70 % of the fat with HC resulted in average reductions in fat content of 42.6 % and 55.3 %, respectively, and average increases in protein content of 37.0 % and 55.0 %, respectively. These modifications confer healthier characteristics to the products, as lower fat intake is associated with reduced risk factors related to cardiovascular diseases (Hooper et al., 2020). Moreover, the proteins present in HC are easily absorbed and contribute to the health of skin, joints, and bones, promoting structural and functional benefits to the body (Inacio et al., 2024).

The substitution of NaCl with KCl resulted in an average reduction in sodium content of 33.2 % and an average increase in potassium content of 234.7 % (Table 2). These modifications impart healthier characteristics to the mortadellas, as reducing sodium intake and increasing potassium consumption benefit cardiovascular health (Kim, Yu, & Shin, 2024). The fat replacement with HC significantly increased the calcium content of the mortadellas (Table 2). This effect is likely due to residual calcium in HC, which is derived from animal tissues that are naturally rich in calcium. Although this is a positive aspect, the increase does not confer significant nutritional benefits, as calcium in the mortadellas (26.2 to 41.2 mg/100 g) is far below the recommended daily intake of 1000 mg (Institute of Medicine, 2011). Despite some statistical differences observed, the reformulation conducted in this study did not cause significant changes in the other minerals analyzed (magnesium, zinc, iron, copper, and manganese), which was an expected result given the profile of the ingredients used.

3.1.3. Water activity (aw)

The samples' water activity (Aw) values ranged from 0.968 to 0.981 (Table 2). The fat replacement with HC and NaCl with KCl did not significantly affect the Aw values of the mortadellas. However, adding arginine reduced the Aw values compared to the control ($P < 0.05$). This effect can be explained by the hygroscopic capacity of arginine, which attracts and retains water molecules, decreasing the availability of free water in the product (Hackl, Darkwah, Smith, & Ermolina, 2018a, 2018b). This reduction in Aw can benefit the microbiological quality of the mortadellas, as it may reduce microbial growth, contributing to greater stability and safety of the product during storage (Rifna, Dwivedi, & Chauhan, 2022). Although this effect was not observed in the present study, it may prove advantageous in future research, as this type of treatment can potentially reduce microbiological spoilage. However, further studies are needed to evaluate the interaction of these treatments with different meat products to validate this hypothesis.

3.1.4. Texture profile

Replacing fat with HC reduced the hardness of the samples, with this effect being significant in the HC_{70} formulation compared to the control (Table 2). This behavior can be attributed to the water-holding capacity of HC, as evidenced in the emulsion stability analysis (Fig. 1), which contributes to a softer matrix. The substitution of NaCl with KCl and the addition of arginine also influenced hardness. However, only the HC_{70}

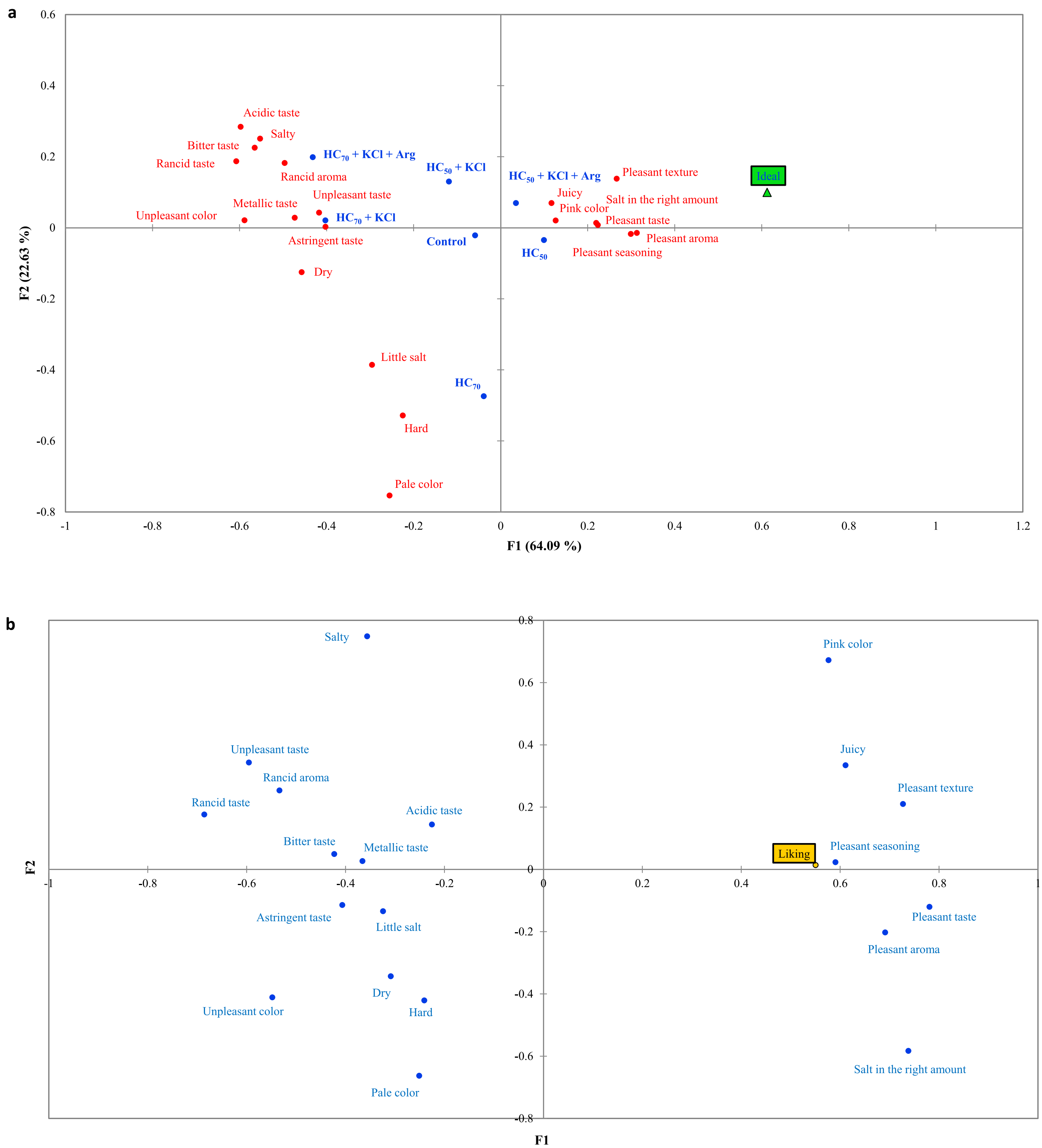

Fig. 2. (a) Correspondence analysis map for the sensory attributes of mortadella samples from the CATA test. (b) Principal component analysis of sensory attributes most associated with liking scores from the CATA test.
Treatments: see Table 1.

+ KCl + Arg sample showed significantly lower values than the samples with the same level of HC (HC_{70} and HC_{70} + KCl). This effect can be explained by the hydrophilic property of arginine, which increases moisture retention in the meat matrix (Zhu et al., 2018).

Gumminess and chewiness followed a similar pattern for hardness, with more pronounced reductions in the HC_{70} + KCl + Arg treatment. In contrast, the reformulation did not affect the cohesiveness and elasticity of the samples ($P > 0.05$). The preservation of cohesiveness, even with the reduction in hardness, gumminess, and chewiness, is a positive result, as this parameter is related to the structural integrity of the matrix, ensuring that the product maintains its shape and texture when handled or chewed (Bourne, 2002).

3.1.5. Instrumental color (L, a*, and b*)*

Replacing fat with HC significantly reduced the L^* values (lightness) and increased the a^* values (redness intensity) in the HC_{50} and HC_{70}

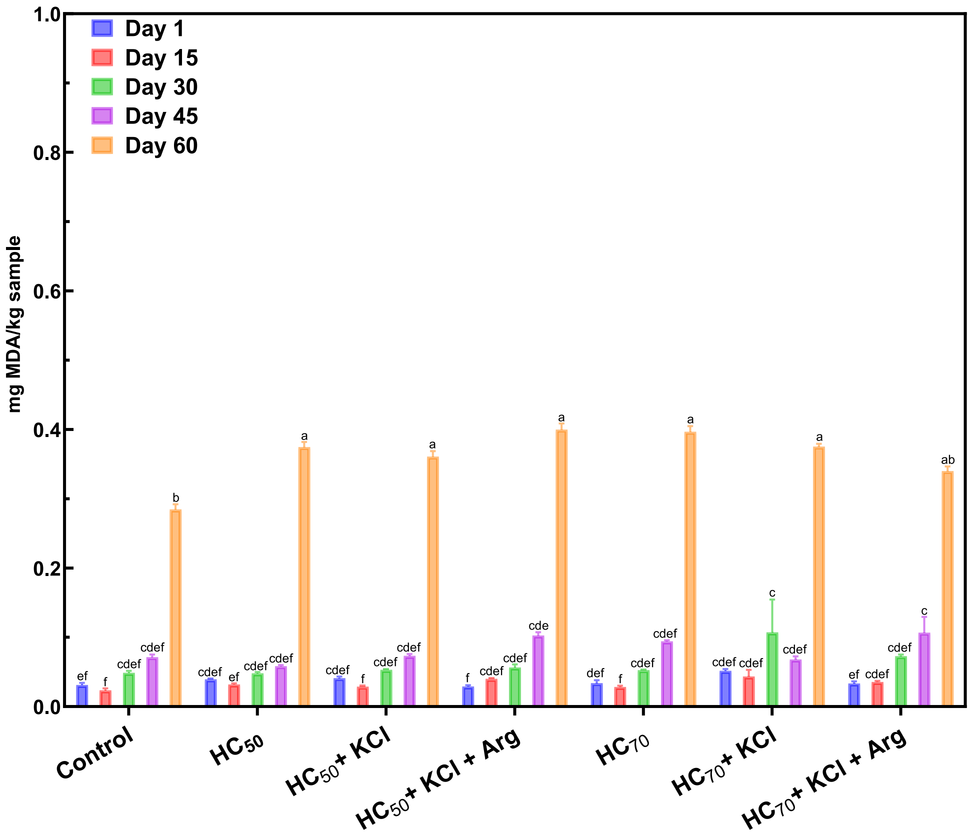

Fig. 3. TBARS values of mortadella samples during 60 days of storage at 4 °C.
Values are presented as mean ± SEM. Different superscript letters indicate significant differences ($P < 0.05$). Treatments: see Table 1.

samples compared to the control, without affecting the b^* values (yellowness intensity) (Table 2). The concentration and chemical state of heme pigments primarily determines the characteristic color of mortadella. Nitrosylmyoglobin (NO-MbFe^{2+}), a ferrous complex of myoglobin (MbFe^{2+}) and nitric oxide (NO), is the predominant pigment responsible for the pink coloration in cured raw meat. During thermal treatment, NO-MbFe^{2+} undergoes denaturation to form nitrosylhemochrome, the main pigment in cooked meat products (Takeda et al., 2024). The observed reduction in L^* values and the intensification of red-pink coloration in HC-treated samples may be explained by improved water retention, which likely reduced pigment dilution during processing.

Additionally, substituting NaCl with KCl and adding arginine further reduced the L^* values and increased the a* values. It also raised the b^* values in the HC_{70} + KCl + Arg sample. This effect can be attributed to the aforementioned oxidizing effects, which could be affected by NaCl and KCl concentrations in meat pigments. Studies have indicated that NaCl has a more substantial oxidizing effect than KCl on myoglobin, favoring metmyoglobin formation (dos Santos, Campagnol, Fagundes, Wagner, & Pollonio, 2017). The combination of arginine, a basic amino acid, and HC, which contains basic amino acids as major components, may have worked synergistically to stabilize the matrix pH (Platts & Falconer, 2015), promoting the preservation of nitrosylhemochrome. However, this combination may have influenced how pigments reflect light, which could explain the observed increase in b^* values, accentuating yellowish tones.

3.2. Sensory analyses

3.2.1. Acceptance test

The results of the sensory acceptance test are presented in Table 3. The replacement of 50 % fat with HC did not affect the attributes of color, aroma, flavor, texture, and liking compared to the control. However, at 70 % fat replacement, the HC_{70} sample showed significantly lower aroma scores, although it did not differ from the control in color, taste, texture, and liking attributes. These findings suggest that at 50 % replacement, HC's ability to enhance water retention and form a structural matrix similar to fat may have preserved the sensory profile. In contrast, at 70 % replacement, the higher levels of HC may have diluted volatile compounds responsible for aroma or altered their release from the product matrix, reducing perceived aroma intensity.

In the samples with 50 % fat replacement by HC, substituting 50 % NaCl with KCl did not depreciate the sensory attributes analyzed, maintaining acceptance levels similar to the control. This is an interesting result, as it is widely known that replacing 50 % of NaCl with KCl usually generates significant sensory defects in meat products (da Rosa et al., 2023; dos Santos Alves et al., 2017). On the other hand, besides obtaining lower aroma scores, the HC_{70} + KCl sample also showed depreciation in the attributes of taste and liking compared to the control. This difference between the HC_{50} + KCl and HC_{70} + KCl samples may be related to the fat content, as it is commonly reported that the perception of saltiness, which significantly influences taste acceptance, is positively correlated with fat content (Desmond, 2006). Fat contributes to the modulation of flavor perception and the gradual release of salt during mastication. The reduction in fat content at 70 % replacement may have diminished the saltiness perception, contributing to the lower scores. Additionally, the HC content itself may have influenced taste perception, as this ingredient contains amino acids such as arginine, glutamic acid, glycine, and histidine, which could interact with flavor compounds or modify the perception of saltiness and bitterness. The fact that the HC_{50} + KCl sample achieved scores similar to the control in taste and liking attributes suggests that these components may have partially masked the sensory defects commonly associated with KCl.

Interestingly, the HC_{70} + KCl + Arg treatment had taste scores similar to the control, indicating that adding arginine reduced the

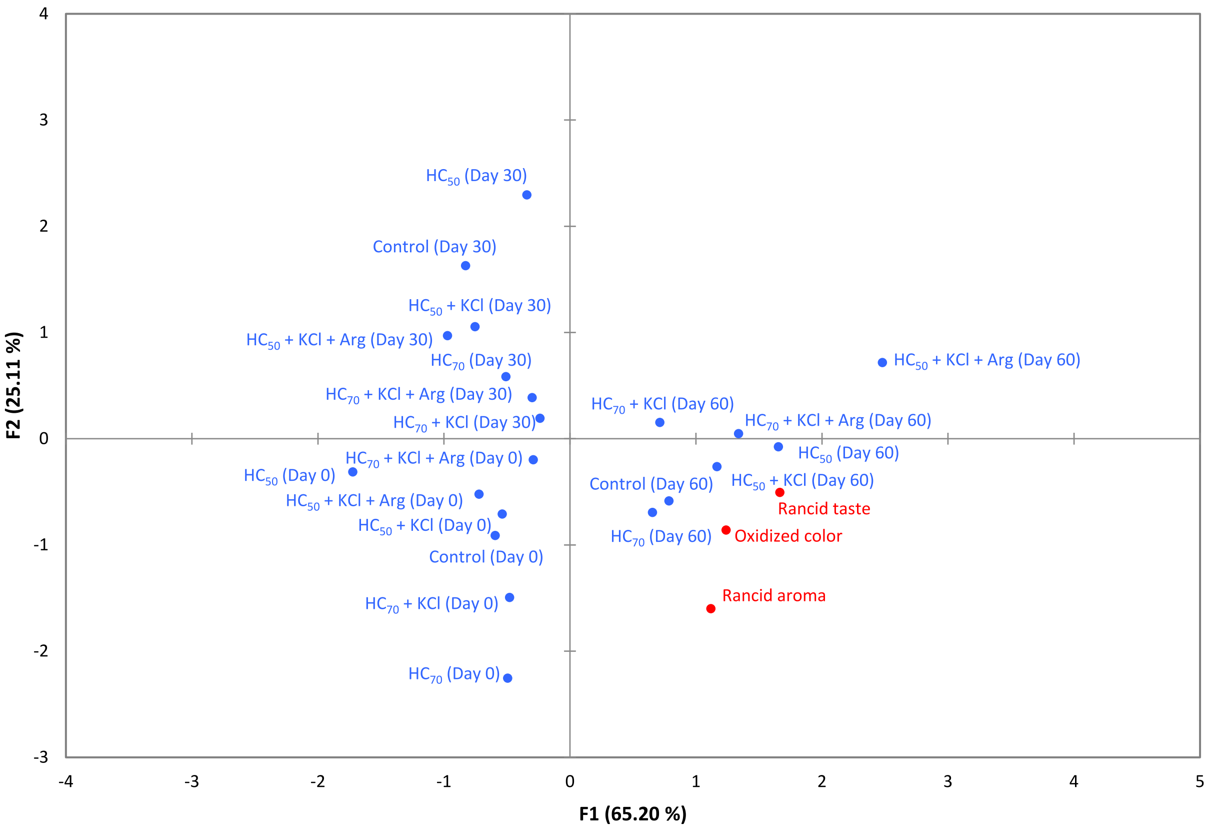

Fig. 4. GPA map evaluating the lipid oxidation sensory descriptors of mortadella samples at days 1, 30, and 60 of storage.
Treatments: see Table 1.

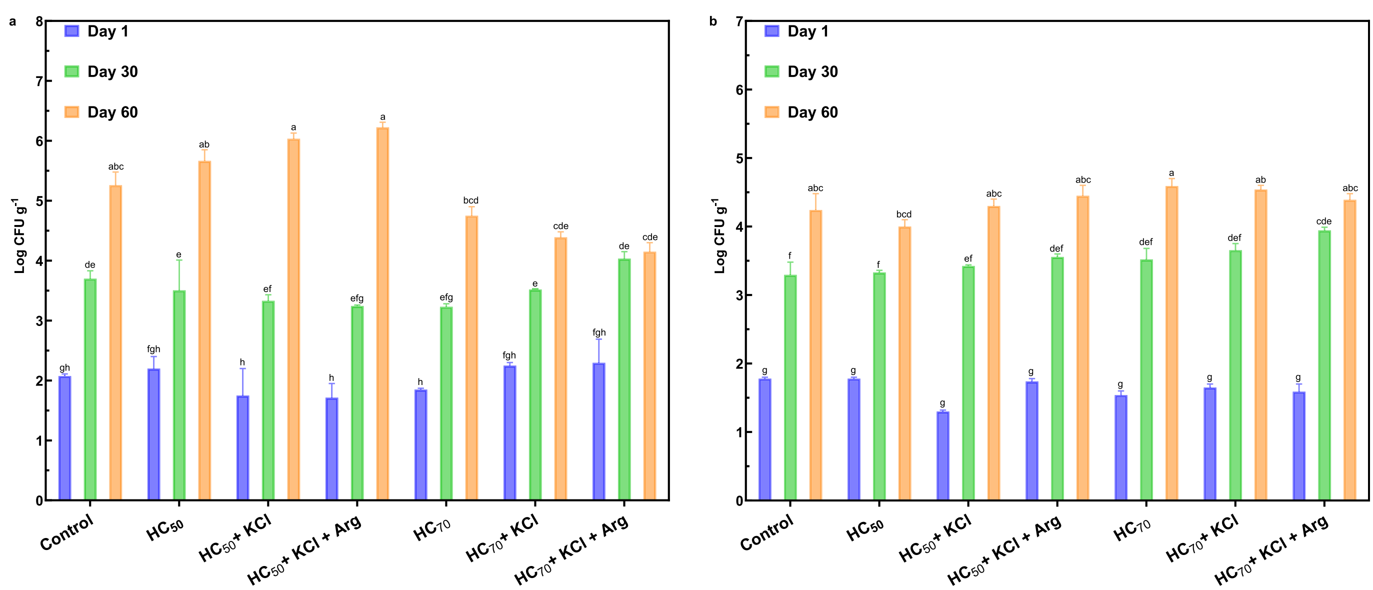

Fig. 5. Aerobic mesophilic microorganism (a) and lactic acid bacteria (b) counts in mortadella samples during storage.
Values are presented as mean ± SEM. Different superscript letters indicate significant differences ($P < 0.05$). Treatments: see Table 1.

sensory defects caused by adding KCl. Moreover, the liking scores for this treatment were similar to those of the control, even with lower scores in the attributes of color and aroma, reinforcing the importance of taste for overall product acceptance.

3.2.2. CATA test

The correspondence analysis map used to interpret the CATA test data is presented in Fig. 2a. The first dimension (F1) explained 64.09 % of the data variation, while the second (F2) explained 22.63 %. It is observed that the HC_{70} samples with NaCl replaced by KCl were positioned close to negative sensory attributes such as metallic, astringent, bitter, and unpleasant. These attributes are commonly reported in meat products containing KCl above 1 % (dos Santos Alves et al., 2017).

The HC_{70} sample was characterized as hard and low in salt,

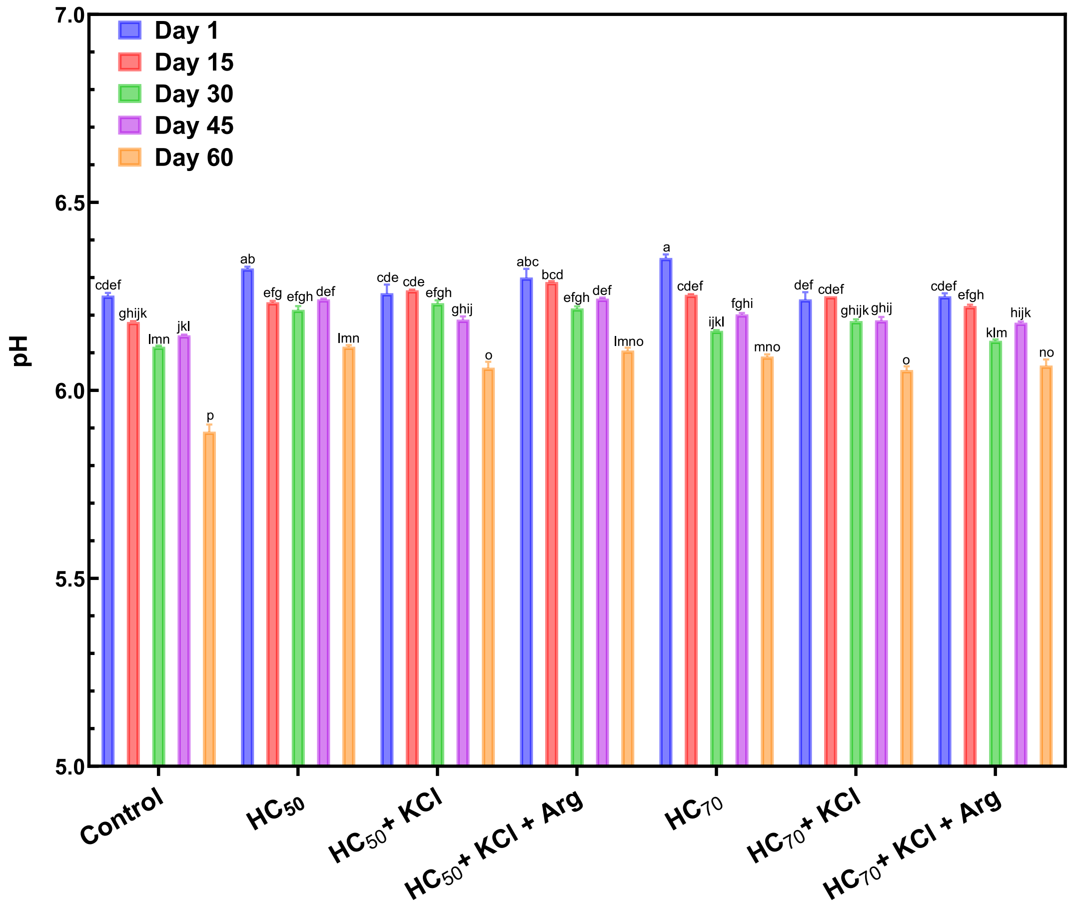

Fig. 6. Evolution of pH values of mortadella samples during 60 days of storage at 4 °C.
Values are presented as mean ± SEM. Different superscript letters indicate significant differences ($P < 0.05$). Treatments: see Table 1.

highlighting that fat replacement with HC should be carried out cautiously, as HC could not fully mimic the action of fat at a 70 % replacement level. On the other hand, replacing 50 % of fat with HC was effective, as the HC_{50} sample was closer than the control to the sensory attributes considered ideal by consumers for this meat product, such as pleasant texture, juiciness, pink color, right amount of salt, pleasant taste, pleasant aroma, and pleasant seasoning.

Furthermore, the addition of arginine to the sample with 50 % fat replacement by HC and 50 % NaCl replacement by KCl (HC_{50} + KCl + Arg) was also associated with these desirable sensory attributes, demonstrating the positive effect of this amino acid in mitigating the sensory defects caused by KCl. This result is consistent with the data obtained in the sensory acceptance test (Table 3), reinforcing the beneficial action of arginine. These results are also aligned with previous studies that showed the positive effect of arginine on the sensory quality of emulsified products with high levels of KCl (da Silva et al., 2020); however, this is the first time this effect is demonstrated in products with reduced fat content.

Fig. 2b presents the correlation of sensory attributes with the most significant influence on the product's liking scores. The essential attributes for increasing liking were those that characterized the HC_{50} and HC_{50} + KCl + Arg samples, demonstrating that, from a sensory standpoint, it is possible to reduce fat by 50 % using HC and reduce NaCl by 50 % by replacing it with a combination of KCl and arginine.

3.3. *Shelf life analyses*

3.3.1. *Lipid oxidation*

The TBARS values were significantly influenced by the interaction between treatments and storage time (Fig. 3). An increase in TBARS values was observed over the storage period, especially on the 60th day, which can be attributed to the formation of malonaldehyde due to the oxidation of unsaturated fatty acids (Botsoglou et al., 1994). While the HC treatments did not reduce lipid oxidation, as evidenced by TBARS values similar to or slightly higher than the control on the 60th day, the low overall values indicate good oxidative stability across all formulations, suggesting that the reformulation did not significantly impact lipid oxidation during storage.

The GPA map (Fig. 4), used to evaluate the sensory descriptors related to lipid oxidation, presented results consistent with the evolution of TBARS values. It was observed that, on all evaluation days, the modified treatments and the control were grouped very closely on the GPA map, reinforcing that the reformulation did not affect the oxidative stability of the products. Only the samples stored for 60 days were characterized by the attributes of rancid flavor, oxidized color, and rancid aroma, confirming the relationship between the increase in TBARS values and the sensory perception of lipid oxidation in the samples.

3.3.2. *Microbiological analyses*

The counts of aerobic mesophilic microorganisms (Fig. 5a) and lactic acid bacteria (LAB) (Fig. 5b) were significantly influenced by the interaction between treatments and storage time. The initial count of mesophilic aerobes was close to 2 log CFU/g in all treatments ($P > 0.05$), demonstrating the raw material's good hygienic and sanitary conditions and processing conditions. During storage, an increase in mesophilic aerobic counts was observed, reaching values between 4 and 6 log CFU/g at the end of 60 days. The modified treatments showed an evolution in mesophilic aerobic growth similar to the control. However, it was noted that the HC_{70} treatments presented lower counts of mesophilic aerobes

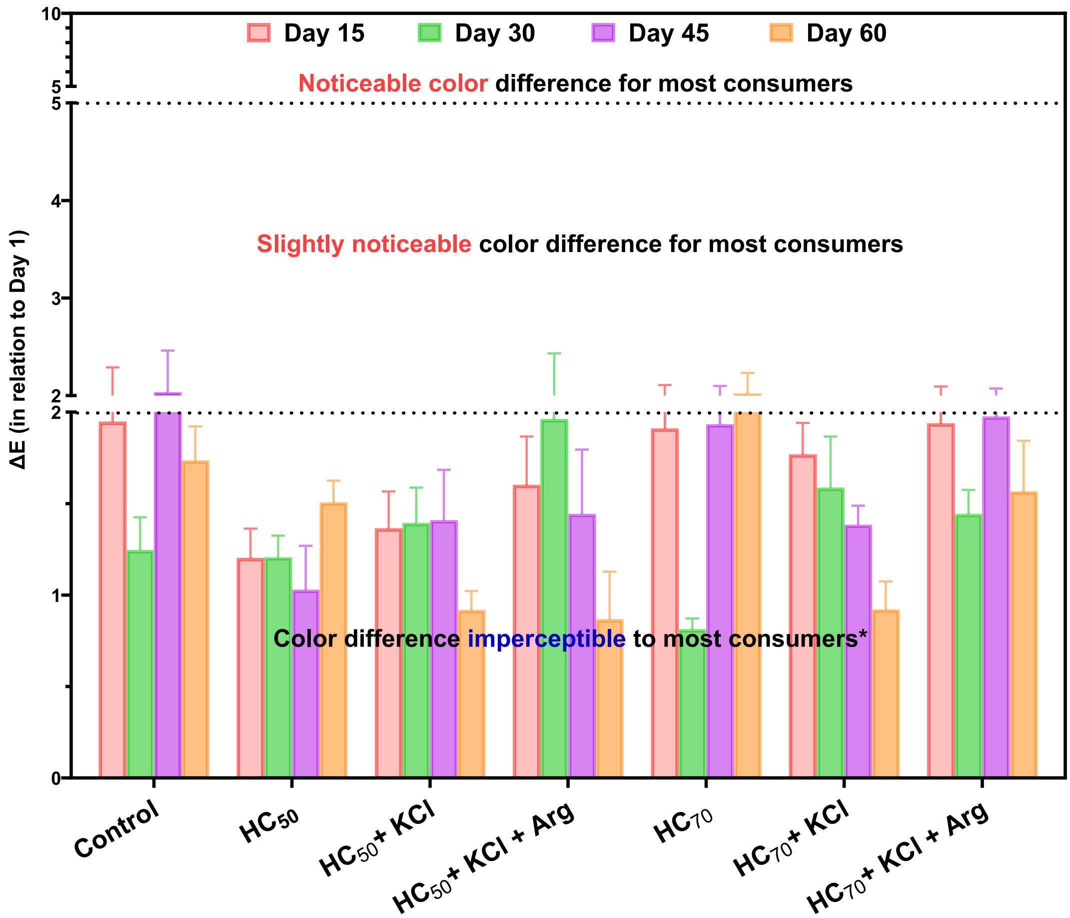

Fig. 7. Overall color difference (ΔE) of mortadella samples during storage compared to day 1.
Values are presented as mean ± SEM. Treatments: see Table 1. * (Francis & Clydesdale, 1975).

at the end of storage compared to the HC_{50} treatments. One possible explanation is that the higher proportion of HC in the HC_{70} treatments may have resulted in a denser matrix, which could limit the diffusion of nutrients and oxygen, creating a less favorable environment for microbial growth. However, this hypothesis requires further investigation.

The initial LAB count was less than 2 log CFU/g in all treatments. During storage, LAB counts increased, reaching values between 4 and 5 log CFU/g. The evolution of LAB counts in the modified treatments was very similar to that observed in the control, indicating that the formulation changes did not significantly influence the growth of these bacteria. Thus, considering that LAB are the main microorganisms responsible for the end of shelf life in cooked meat products (Menezes, Martins, Longhi, & de Aragão, 2018), the stability observed in their counts reinforces the feasibility of reformulation without compromising shelf life.

3.3.3. pH and ΔE

The evolution of pH values of the mortadellas was significantly influenced by the interaction between treatments and storage time (Fig. 6). The pH values of the samples at the beginning of storage ranged between 6.24 and 6.35, which is within the range commonly reported for this meat product (dos Santos et al., 2023). The replacement of fat with HC resulted in an increase in the pH of the samples, which can be attributed to the differences in pH between collagen and pork backfat (6.5 vs. 6.0, respectively). Adding KCl in the samples with HC promoted a reduction in pH, making it similar to that of the control. This effect can be explained by the ability of KCl to alter the ion balance in the meat matrix, resulting in a slight acidifying impact (Vidal, Bernardinelli, Paglarini, Sabadini, & Pollonio, 2019). On the other hand, the addition of arginine, an alkaline amino acid, did not significantly affect the samples' pH. This result may be attributed primarily to the relatively low concentration of arginine added, which was insufficient to overcome the buffering capacity of the meat matrix. Additionally, interactions between arginine and other components, such as proteins and salts, may have neutralized its alkalizing effect. During storage, the pH of the samples decreased due to the production of lactic acid by lactic acid bacteria (Fig. 5a), a result consistent with what has been reported in other studies (da Rosa et al., 2023; Khorsandi, Eskandari, Aminlari, Shekarforoush, & Golmakani, 2019).

The ΔE values were calculated for each treatment by comparing days 15, 30, 45, and 60 of storage with day 1 to assess the effect of storage on the overall color difference of the samples (Fig. 7). The results show that all samples exhibited a similar trend in the evolution of ΔE values throughout the 60 days of storage. This indicates that the reformulation did not significantly impact the color stability of the mortadellas. The ΔE values remained between 1 and 2 during storage, which can be classified as imperceptible to slight differences for most consumers (Altmann et al., 2022; Larraín, Schaefer, & Reed, 2008).

4. Conclusion

This study evaluated the effect of combining HC, KCl, and arginine as a strategy to reduce fat and sodium in mortadellas. The results showed that reformulation with 50 % fat replacement by HC and 50 % NaCl replacement by KCl, with the addition of arginine, was effective in maintaining the overall quality of the product throughout storage. This combination allowed for a significant reduction in fat and sodium contents, besides increasing potassium levels, giving the mortadellas a healthier nutritional profile. Although the reformulation increased the product's protein content, it is important to note that collagen protein itself is considered low-quality due to its lack of several essential amino acids. However, HC may offer additional nutritional benefits through the potential bioactivity of its peptides, a possibility that warrants further investigation in future studies. Adding arginine played an essential role in masking the sensory defects often associated with KCl, ensuring sensory quality similar to the control. Fat replacement with HC up to the 50 % level proved viable for preserving texture and structural integrity, although higher replacements may compromise sensory quality. Additionally, the reformulation did not alter the oxidative and microbiological stability of the product, ensuring a shelf life compatible with traditional mortadellas.

Consent form

The authors declare that informed consent was obtained for experimentation with human subjects.

CRediT authorship contribution statement

Leticia Pereira Correa: Conceptualization, Investigation, Writing – original draft. **Mariana Basso Pinton:** Investigation. **Bibiana Alves dos Santos:** Investigation, Writing – review & editing. **Marcelo Antonio Morgano:** Investigation. **Márcio Vargas-Ramella:** Validation, Visualization, Writing – review & editing. **Alexandre José Cichoski:** Validation, Visualization, Writing – review & editing. **Roger Wagner:** Investigation, Writing – review & editing. **Paulo Cezar Bastianello Campagnol:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – original draft.

Declaration of competing interest

The author has no conflicts of interest in disclosing to the paper entitled **" Hydrolyzed Collagen, KCl, and Arginine: A Successful Strategy to Reduce Fat and Sodium While Maintaining the Physicochemical, Sensory, and Shelf Life Quality of Mortadella".**

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Data availability

Data will be made available on request.

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