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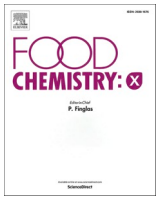

# Enhancing mortadella formulations: Exploring the impact of curcumin microcrystals, cochineal carmine, and annatto dyes on sensory preferences, stability, and antioxidant potential

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ABSTRACT

The effects of adding cochineal carmine and annatto dyes in five mortadella formulations made with curcumin microcrystals were compared, and the preference was evaluated and described sensorially. Based on the optimized formulation obtained with color parameters, two formulations were elaborated: curcumin microcrystals and cochineal carmine were added. During 60 days, pH, objective color, water retention capacity, lipid oxidation, and texture profile analyses were performed. The results demonstrate the possibility of excluding sodium erythorbate from formulations containing curcumin microcrystals. There was no significant difference in lipid oxidation between the samples, presenting at the end of 60 days a value of 0.11 mg and 0.10 mg of MDA $kg^{-1}$ for the two samples, respectively. There were also no significant differences between the two samples or the evaluated storage times, and the average values obtained for pH, WRC, objective color, and TPA were expected for this type of cooked meat sausage. In the presence of curcumin microcrystals, the synthetic antioxidant, sodium erythorbate, can be eliminated from the formulations, as it does not affect the physical-chemical parameters studied, such as pH, water retention capacity, color objective, and texture profile.

## 1. Introduction

The increased demand for meat makes industries invest more and more in processed products such as meat sausages. Industrialization is the main alternative for the disposal of raw materials, which, in addition to increasing the shelf life of the food, adds value to it. The consumer has various meat derivatives, including hams, sausages, and many others (Troy & Kerry, 2010).

Among the meat sausages sold in Brazil, mortadella stands out for being a fast-food item at an affordable price (Grizotto et al., 2012). Mortadella is an industrialized meat product obtained from an emulsion of meat from butchery animals, with or without bacon, added ingredients, embedded in a natural or artificial casing, in different forms, and subjected to the appropriate heat treatment (Brasil, 2000).

Mortadella has 30% fat in its composition (Brasil, 2000) and is subject to several factors that influence its stability (Silva et al., 2003). Oxidation causes the transformation of its characteristics, promoting the development of an unpleasant taste and odor, as well as a decrease in the product's nutritional value, negatively affecting consumer acceptability (Campagnol et al., 2011). Chemical compounds known as antioxidants are used to inhibit or delay these transformations (Ramalho & Jorge, 2006).

According to Bauer et al. (2001), the number of questions regarding synthetic antioxidants used in the food industry is increasing, demonstrating the possibility of these antioxidants presenting some toxicity.

When inadequate amounts of antioxidants are applied to food, they

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can cause harm to the health of the consumer and may also contain harmful compounds (Kunrath et al., 2017). Antioxidants include synthetic and natural ones, which act similarly under different conditions (Lemos Leão et al., 2017). Antioxidants must be used respecting the maximum limits allowed by legislation, and this amount varies according to each food (ANVISA, 1998). Due to the growing health concern, there the demand for foods that use natural additives, which can be extracted from plants and vegetables, is significantly increasing due to their antioxidant and antimicrobial properties; the application of natural preservatives in food has been increasingly studied (Mariutti et al., 2008).

Plants with significant biological activities, such as *Curcuma longa* L., also known as curcumin, have sparked considerable interest in the food industry due to their potent biological activities. The rhizomes of this plant contain curcumin as the main component, known for its vibrant orange color and remarkable antioxidant and antimicrobial properties (Sueth-Santiago et al., 2015; Wang et al., 2009). Incorporating natural additives derived from plants like *Curcuma longa* L. into food formulations is gaining popularity, driven by growing concerns for consumer health and stringent regulatory requirements (ANVISA, 1998). Curcumin, as mentioned earlier, not only gives curcuma its characteristic color but also possesses a range of bioactive properties of particular interest to the food industry. In addition to curcumin, other bioactive compounds present in the rhizomes of *Curcuma longa* L., such as essential oils and other polyphenols, contribute to its antioxidant and antimicrobial properties. These aspects are critical for contextualizing the relevance of the plant not only as a natural dye but also as a valuable functional ingredient in food products formulated with a focus on consumer health and well-being (Sueth-Santiago et al., 2015; Wang et al., 2009).

In addition to the antioxidant and antimicrobial properties already mentioned, using curcumin derived from *Curcuma longa* L. microcrystals can offer significant advantages in the food industry. These microcrystals are a form of encapsulation that enhances the stability and bioavailability of curcumin, thereby increasing its effectiveness as a functional ingredient. Curcumin microcrystals are designed to protect curcumin from degradation during food processing and storage, which can be challenging due to its sensitivity to light, heat, and pH. Furthermore, encapsulation in microcrystals can improve the solubility of curcumin in aqueous food systems, allowing for a more uniform distribution of the bioactive compound in food products Júnior et al. (2019).

According to the experiments by Júnior et al. (2019), in which the artificial antioxidant was replaced in mortadella by microcrystals of curcumin, the results were satisfactory, with little interference in the physical-chemical parameters studied. However, this addition of microcrystals to the mortadella color, when analysing the objective color parameter, obtained a lower intensity of the red color and a greater incidence of the green color, thus presenting a greenish coloration that is not typical for this product, which should have a pink color, displaying divergences from the trading pattern. Therefore, the appearance of curcumin-containing meat sausage formulations can benefit from adding natural pigments such as annatto and cochineal carmine, which tend to collaborate with developing a pink color, typical of this type of product.

De Souza et al. (2022) developed mortadella made from alligator meat from the Pantanal (*Caiman yacare*); because alligator meat has a white color similar to that of fish, the acceptance of the sausage is impaired. Thus, food coloring is an essential attribute that the consumer must evaluate at the time of purchase. Therefore, additives must be added to the original formulation for products made with white meat to obtain the characteristic pink color. Thus, the food industry incorporates pigments during food processing, providing a more attractive color, among which pigments of natural origin are preferably used, such as annatto and cochineal carmine (Brasil, 2006).

Therefore, food color becomes a parameter for sensory evaluation, indicating consumer preference, which can be measured by sensory descriptive methods that detect, describe, and quantify the sensory attributes present in a food. The food industry uses these methods to develop new products, control quality, change ingredients and/or formulations, and study food storage (De Alcantara & De Grandi Castro Freitas-Sá, 2018).

According to De Alcantara and De Grandi Castro Freitas-Sá (2018), sensory techniques help companies in product reformulations that may occur due to various reasons such as legal requirements, supplier changes, changes in the manufacturing process and/or equipment, due to competitive aspects, etc. There are several fundamental tests in sensory analysis, and your choice will depend on which attributes of the food you want to evaluate or know.

Since appearance is the first attribute that impacts the acceptance of a product, the present work aimed to evaluate the preference for the color of mortadella formulations made with different natural pigments, to describe them sensorially through the Flash Profile analysis, and evaluate the physical characteristics-chemicals over 60 days of shelf life.

## 2. Material and methods

### 2.1. Material

Curcumin was purchased from Sigma-Aldrich (C1386-10G) with a purity content of 99.5%. The pigments and additives used were supplied by the Brazilian Additives and Condiments Industry (IBRAC). The other raw materials were purchased from local businesses, using glassware and utensils located in the laboratories of the Federal Technological University of Paraná in the interior of Brazil.

### 2.2. Methods

#### 2.2.1. Curcumin microcrystals

The methodology used to obtain the microcrystals was the non-solvent precipitation technique (Yen et al., 2010), with some changes. In this work, the stabilizer polyvinylpyrrolidone (PVP) used by Yen was not applied because, according to the study by Santos (2015), the same procedure for obtaining with PVP was performed, there was no formation of microcrystals, but curcumin crystals. In addition to producing smaller-sized microcrystals, the preparation carried out in the absence of stabilizers has the advantage of being easier to create. Its dispersion characteristics were as good as those of microcrystals made with stabilizers.

The non-solvent precipitation technique was performed by weighing a sample of ±0.600 g of powdered curcumin in a beaker, then solubilized in 180 mL of 99.8% ethyl alcohol. As curcumin is sensitive to the incidence of light, the beaker was wrapped with aluminum foil until the next step.

An ice bath with a claw was attached to the Ultra Turrax homogenizer (digital IKA T25). A second gripper was attached so that a beaker with approximately 900 mL of water could be connected to the center of it. With the homogenizer at 15000 rpm, the alcoholic solution was poured into the aqueous medium to form the crystals simultaneously and in size. The final mixture was kept under stirring for 10 min, then poured into Erlenmeyer flasks, and taken to the metabolic bath (D1 - 950 M) at 50 °C, with minimum stirring of 20 rpm for 48 h so that the organic solvent was evaporated entirely. The content was frozen in an ultra freezer at 90 °C and lyophilized (Lyophilizer L101 - Liotop) for approximately 72 h to obtain the product. After lyophilization, the microcrystals were stored in Eppendorf and frozen, as shown in Fig. A (supplementary material).

#### 2.2.2. Microscopic analysis: Curcumin and curcumin microcrystals

Two solutions were prepared, one containing in natura curcumin and water and the other containing curcumin microcrystals and water. A Pasteur pipette transferred a drop of each solution obtained to two glass slides; the coverslip was placed once over the solution on each slide to

avoid air bubbles. An optical microscope (Alltion) was used to visualize the slides at magnifications of 100×, 400×, and 1000×. The ScopePhoto program was used to take pictures of the samples.

### 2.2.3. Preparation of mortadella

Five formulations were prepared to replace the sodium erythorbate antioxidant in the original formulation with curcumin microcrystals. In this way, the curcumin microcrystals were maintained in all formulations. To provide the consumer with a more visually pleasing color, two pigments were added: annatto and cochineal carmine, as shown in Table 1, considering the amount of each additive allowed by ANVISA (1998).

The formulation without adding pigments was prepared in the cutter (Mado Garant model). The ingredients were weighed, added in a pre-established order, and homogenized until a meat emulsion was obtained. Adding ice is extremely important, as the dough temperature cannot exceed 16 °C at this stage.

After mixing the ingredients described in Fig. B (supplementary material), the dough was separated into five equal parts, and their respective concentrations of pigments were added. Subsequently, the homogenized meat emulsion was embedded in an artificial envelope, weighed, and cooked in an oven until it reached 72 °C (internal part). Soon after cooking, the thermal shock was carried out for 15 min in running water, and then the mortadella was weighed and conditioned under refrigeration temperature (7 °C) in a refrigerator.

### 2.2.4. Physical-chemical analyses

Mortadella samples were submitted for physicochemical analyses, including water retention capacity, pH, objective color, and weight loss from cooking.

*Water Retention Capacity (WRC).* The method described by Grau and Hamm (1953) was modified by Hoffmann et al. (1982) with some changes. Aliquots of 5 g of each sample were weighed in a semi-analytical balance (Shimadzu-UW620H) and pressed on two filter papers (Whatman n° 1) between plates weighing 10 kg for 5 min. Subsequently, the pressed sample was weighed again, and its respective mass was recorded.

*Hydrogenionic Potential (pH).* The pH measurements were performed using a contact potentiometer (Testo), according to the methodology suggested by Olivo et al. (2001), with the point of incision of the electrode being the central part of the mortadella.

*Objective Color.* The samples were split in Half, and the reading was done on the inside. Results were obtained in triplicate using a MiniScan EZ colorimeter (HunterLab, MSEZ-0231). The results were expressed in L* (which represents the percentage of brightness, 0 = dark and 100 = light); a* (where -a* represents the green direction and + a* the red direction); and b* (where −b * represents the blue direction and + b* yellow direction).

*Weight Loss Cooking (WLC).* The PPC analysis was determined according to the methodology proposed by Silva et al. (2007) by weighing the samples before and after cooking on a semi-analytical scale (Shimadzu-UW620H).

*Lipid oxidation.* For the lipid oxidation analysis in the mortadellas, following the methodology described by Crackel et al. (1988), 5 g of each treatment was weighed in triplicate into a beaker, and 25 mL of a solution containing TCA (7.5%), propyl gallate (0.1%), and EDTA (0.1%) was added. The mixture was homogenized using an ultraturrax (Fisatom, 713D) and filtered through qualitative filter paper (Qualy) with a 12.5 cm diameter placed in a glass funnel. In a test tube, 5 mL of the filtrate was combined with 5 mL of TBA solution (0.02 M). A blank sample was prepared by adding 5 mL of TCA solution (7.5%) and 5 mL of TBA solution (0.02 M) to another test tube. Subsequently, all samples and the blank were placed in a water bath (WEA, 837–2) at 100 °C for 40 min. Finally, readings were taken on a spectrophotometer at a wavelength of 538 nm. The results were expressed in mg of malondialdehyde per kg of sample, determined according to the slope equation of a standard curve of tetramethoxypropane (TMP: $3 \times 10^{-4}$ mol $L^{-1}$).

*Texture Profile (TPA).* Texture analysis was conducted using the texture profile analysis (TPA) method, using a texturometer (Stable Microsystem - TA-XT Express Enhanced) equipped with a P/36R probe. With the aid of a mold, ten samples of each formulation were cut, with 2.2 cm in diameter, and the samples were compressed twice to 50% of their size. There was no rest time between the two compression cycles. The deformation curve was obtained with six texture parameters: adhesiveness, elasticity, chewiness, gumminess, cohesiveness, and resilience.

### 2.2.5. Microbiological analyses

To ensure the hygienic and sanitary conditions of the bologna, the samples were submitted to microbiological analyses for the search for *Coliforms* at 45 °C $g^{-1}$, *Staphylococcus* coagulase positive $g^{-1}$, *Clostridium* sulfite reducer at 46 °C $g^{-1}$ and the presence of *Salmonella* sp. 25 $g^{-1}$. The studies were carried out according to the methodologies described by Silva et al. (2021) and started 24 h after cooking the mortadella. The results obtained were analysed according to the standard established by RDC n° 12 (Brasil, 2001).

### 2.2.6. Sensory analyses

The Ethics Committee of UTFPR approved this project under opinion n° 89,638,518.6.0000.5547.

*Preference ordering test.* The preference for mortadella samples was evaluated using the preference ordering test (Haufe et al., 2018). The assessors ordered the samples, which were served simultaneously, in ascending order according to their preference.

The samples were submitted for evaluation by 64 assessors who received five mortadella samples prepared with the pigment concentrations described in Table 1. Each sample (cubes of approximately 25 g) was served to the assessor in a 50 mL glass identified with random numbers of three digits and a form for evaluation. Assessors were instructed to order the samples only on preference for the color attribute; that is, it was unnecessary to ingest the samples. Samples were ordered in ascending order of preference.

The results of each assessor were allocated in a matrix (assessors in the rows and samples in the columns), arranging the preference that each assessor attributed to each sample (least preferred receiving grade 1 and most preferred grade 5). The sum of the orders for each sample

**Table 1**
Ingredients and variations in pigment concentrations used in mortadella formulations.

| Basic formulation | |
|---|---|
| Ingredients | Quantity (%) |
| pork shank | 68.868 |
| ice/water | 12.000 |
| bacon | 12.000 |
| cassava starch | 3.000 |
| salt | 2.000 |
| isolated soy protein | 1.000 |
| condiments for mortadella | 0.400 |
| cure IBRAC | 0.250 |
| Acordini 701 - stabilizer | 0.250 |
| garlic powder | 0.100 |
| monosodium glutamate | 0.100 |
| pigments* | 0.030 |
| curcumin microcrystals | 0.002 |

| Formulations | absolute values | | real values | |
|---|---|---|---|---|
| | carmine | annato | carmine (g) | annato (g) |
| 1 | 0.75 | 0.25 | 0.0225 | 0.0075 |
| 2 | 0.25 | 0.75 | 0.0075 | 0.0225 |
| 3 | 0.50 | 0.50 | 0.0150 | 0.0150 |
| 4 | 1.00 | 0 | 0.0300 | 0 |
| 5 | 0 | 1.00 | 0 | 0.0300 |

* Used according to experimental design.

was obtained. The results were evaluated according to the Friedman test ($p < 0.05$), and, in case of significant differences between the samples, the Christensen Table (Christensen et al., 2006) was used to compare the sum of the orders obtained, identifying the differences.

*Flash profile.* Due to the similarity between the developed products and the commercial bologna, the latter was used for the attribute survey. Samples of industrialized pork and chicken mortadella were offered to assessors so that, through their comparison, they could find the most suitable attributes for their description. Samples were presented randomly inside disposable white cups coded with random digits. For the survey of the attributes, the assessors were instructed to evaluate the appearance (observing color and aspect), odor (smelling the sample twice), flavor (tasting the mortadella), and texture (biting the mortadella with the front teeth and evaluating the sensation given in the mouth). As the number of attributes was unlimited, assessors could describe as many attributes as necessary.

In the second part of the sensory analysis, five cups identified with random three-digit numbers were given to the assessor. Each cup had two portions of approximately 25 g of each of the five mortadella formulations (F1, F2, F3, F4, and F5), prepared with the pigment concentrations described in Table 2. The samples were submitted to the Profile Evaluation Flash by 34 assessors (A1 - A34). The assessors received, together with the samples, a form with the attributes and their respective definitions obtained in the first phase. They were instructed to order the samples for each attribute listed above in ascending order, from the weakest sensation to the most vital sense.

#### 2.2.7. Statistical analyses

The results of each assessor were allocated in a matrix (attributes in the columns and samples in the lines). The data processing was performed in the MATLAB R2016a software through the ComDim technique (Common dimensions), according to the algorithm proposed by Qannari et al. (2001) and described in detail by Jouan-Rimbaud Bouveresse et al. (2011). ANOVA was performed with Tukey's test at 5%, and Analyses Principal Components with Perason's correlation, and analyses and statistical graphs were generated with the OriginPro 2023b and Statistica 12.0 programs.

## 3. Results and discussion

### 3.1. Microcrystals

The yield of microcrystals was 0.6 g per 100%. Curcumin and microcrystals obtained through the non-solvent precipitation technique were analysed under an optical microscope (Fig. 1).

The images show the distribution and size of curcumin powder particles in natural solubilized water (Fig. 1(a)) and curcumin microcrystals (Fig. 1(b)). It can be seen from the images that there was a change in the size and distribution of the particles that went through the crystallization process of the samples that were not treated. An agglomerate of particles with no defined shape is observed for the untreated particles, which have a surface area more significant than the microcrystals in spicules. The developed micro crystals presented a format consistent with those produced by Thorat and Dalvi (2014) and Santos (2015), in which the same precipitation technique in non-solvent was applied.

**Table 2**
Values of physicochemical variables for mortadella formulations.

| Formulations | pH | WLC | WHC |
|---|---|---|---|
| F1 | $6.190^{a} \pm 0.012$ | $-0.505^{b} \pm -0.088$ | $95.230^{a} \pm 0.053$ |
| F2 | $6.147^{b} \pm 0.015$ | $-0.797^{a} \pm -0.046$ | $95.652^{a} \pm 0.291$ |
| F3 | $6.193^{a} \pm 0.007$ | $-0.432^{b} \pm -0.022$ | $95.331^{a} \pm 0.151$ |
| F4 | $6.190^{a} \pm 0.000$ | $-0.451^{b} \pm -0.057$ | $94.840^{a} \pm 0.139$ |
| F5 | $6.173^{ab} \pm 0.033$ | $-0.312^{b} \pm -0.071$ | $94.973^{a} \pm 0.636$ |
| Formulations | | Color | |
| | L* | a* | b* |
| F1 | $70.470^{a} \pm 0.491$ | $10.483^{a} \pm 0.588$ | $15.843^{ab} \pm 0.713$ |
| F2 | $70.183^{a} \pm 0.286$ | $10.783^{a} \pm 0.521$ | $18.053^{ab} \pm 1.602$ |
| F3 | $70.027^{a} \pm 0.826$ | $10.673^{a} \pm 0.101$ | $17.930^{ab} \pm 0.229$ |
| F4 | $65.050^{b} \pm 0.734$ | $10.417^{a} \pm 0.215$ | $14.570^{b} \pm 0.662$ |
| F5 | $71.360^{a} \pm 0.575$ | $10.290^{a} \pm 0.394$ | $19.753^{a} \pm 0.358$ |

This means that the same column, followed by different letters, differ from each other by Tukey's test, at a significance level of 5%.

### 3.2. Physicochemical analysis

The five mortadella formulations (Fig. C - supplementary material) were prepared by adding curcumin microcrystals to replace the synthetic antioxidant sodium erythorbate. In formulations F1, F2, and F3, cochineal carmine and annatto pigments were mixed, varying the pigment concentrations according to the experimental design (Table 1). In the F1 formulation, the proportion of pigments used was 75% cochineal carmine and 25% annatto; in F2, the proportion used was 25% cochineal carmine and 75% annatto; in F3, the concentrations for the two pigments were the same. Therefore, it received 50% of each pigment. Formulations F4 and F5 were prepared with only one of the pigments. Thus, F4 received only cochineal carmine, and F5 only annatto.

The results of the means and standard deviations of the parameters pH, WLC, WHC, and color are shown in Table 2.

It can be seen that the pH values varied between 6.147 (F2) and 6.193 (F3). Despite the minimal numerical variation, a significant difference was found between the values ($p < 0.05$). The mortadella pH must be in the neutral range, close to 7.0 (Brasil, 2000). In studies conducted by Dinalli, Auriema, & Soares (2016), in which *Moringa oleifera* Lam flour was added to mortadella, the pH found for the control sample, in which flour was not added in partial fat replacement, was 6.21. Orsolin et al. (2015) found that at time zero of the mortadella shelf life study, the pH ranged from 6.43 to 6.63. Saldaña et al. (2015) evaluated pH values between 6.66 and 6.76, where they partially replaced the amount of animal fat added to bologna with vegetable fat. Thus, the analysed pH value agrees with other studies involving meat sausages.

Regarding weight loss, only sample F2 differed significantly from the others ($p < 0.05$), showing less weight loss, while sample F5 lost the most weight. The values were negative, demonstrating an increase in final weight in all samples. Usually, these cooked meat products are embedded in synthetic casings with a low permeability rate to water vapor, thus reducing weight loss during cooking. As for the water retention capacity, the samples did not differ significantly from each other ($p < 0.05$).

The luminosity (L*) had a lower value for F4, presenting a lighter color and statistically differing from the other samples. However, the samples were similar in the chromatic coordinate a*, which measures the saturation of the red and green colors. There was statistical variation for the chromatic coordinate b*, which measures the saturation of the yellow and blue colors. Still, all the samples showed positive values for the yellow hue, noting that the F5 sample was the one with the highest shade (19.73), as it was the sample that had 100% annatto in its composition, with the yellow/orange color being the predominant color of this natural pigment. It is also observed that samples F4 (100% carmine) and F1 (75% carmine and 25% annatto) showed a lower intensity of the yellow color, probably due to the color of the natural carmine pigment, which predominates in the pink/red color.

In the experiments by Júnior et al. (2019), where curcumin microcrystals replaced the artificial antioxidant sodium erythorbate, the results of L*, a*, and b* found for day zero of the F2 formulation were 69.31, 4.95, and 15.86 respectively. The L* values are close to those found in this study for formulations F1, F2, F3, and F5, which all contained the natural pigment annatto. For the values of a*, the five evaluated formulations presented values between 10.290 and 10.783; the

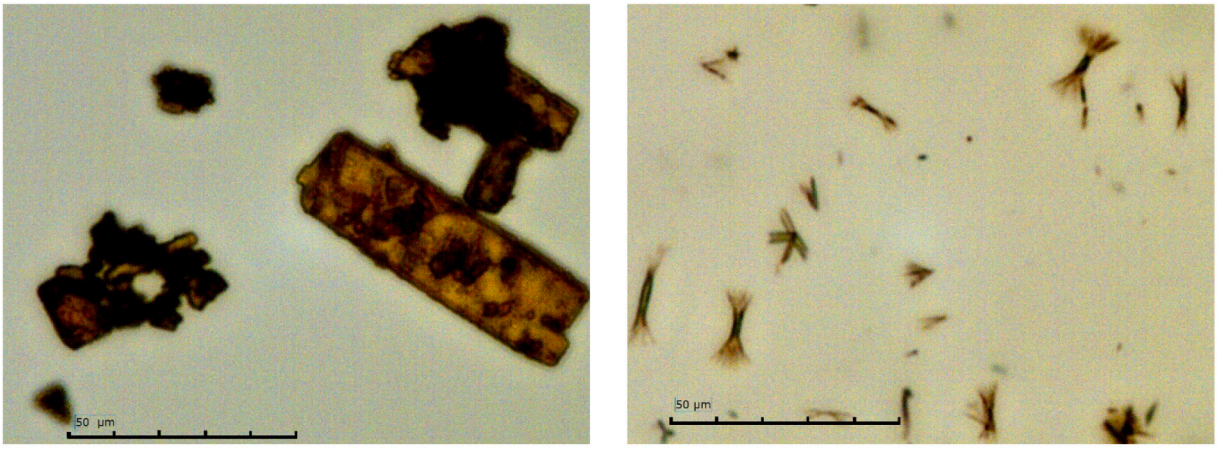



**Fig. 1.** Images of curcumin and curcumin microcrystals.

difference in the values of a* is due to adding pigments to the formulations since the values of +a* represent the direction towards red. Regarding the b* parameter, the formulations containing a higher concentration of annatto pigment obtained higher values than those using only cochineal carmine since +b* represents the direction towards yellow.

The desirability function was used to determine the optimized formulation, and the parameters b* and L* were considered for the color, given that these two factors showed a significant difference between the averages of the formulations.

The desirability function determined, according to physicochemical results, that the optimized formulation (Fig. 2) is given by the formulation composed of 100% carmine and, therefore, there is a definition that 93.660% of this formulation simultaneously reaches the smallest values of the parameters L* and b*. This optimized formulation is precisely the F4 formulation, which was the most accepted by the assessors

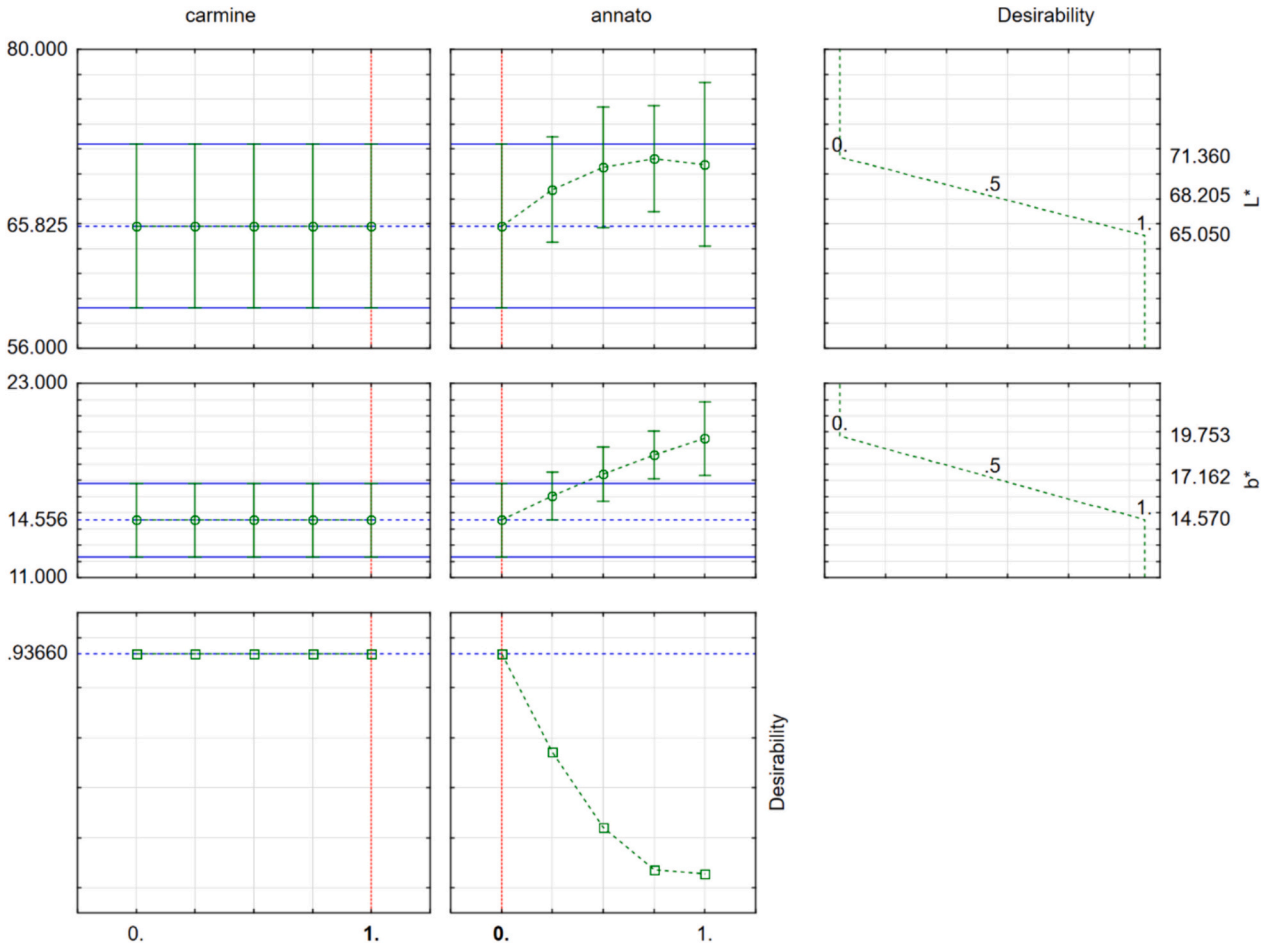



**Fig. 2.** Graph of influence of dyes in obtaining color parameters L* and b*.

of the sensory analysis, whose average did not differ significantly from the F1 formulation.

### 3.3. Microbiological analysis

According to Brazilian regulations, the microbiological standards to be investigated for meat products are *Coliforms* at 45 °C $g^{-1}$, *Staphylococcus* coagulase positive $g^{-1}$, *Clostridium* sulfite reducer, and *Salmonella* sp. 25 $g^{-1}$ (Brasil, 2001).

The results of the microbiological analysis are shown in Table A (supplementary material). All formulations were observed to be within the standards required for all microorganisms researched, thus ensuring their microbiological quality before conducting the sensory analysis.

### 3.4. Sensory analysis: Preference ordering

The color of the samples developed was submitted to the ordering test of your preference with 64 assessors, students, and employees at the University who were over 18 years old and regular consumers of mortadella.

The results of the sums of ordering totals obtained through a sensory analysis were submitted to the Friedman test ($p < 0.05$), and the ordering totals were compared with the table of minimum differences between samples (Christensen et al., 2006). By this method, all tested samples can be compared. Statistical analysis of the ordering test data revealed a significant difference between the color preferences of the developed samples ($p < 0.05$). F4 (100% cochineal carmine) and F1 (75% cochineal carmine and 25% annatto) are equal formulations and significantly more preferred colors than the other samples. F2 (75% annatto and 25% cochineal carmine), F3 (50% annatto and 50% cochineal carmine), and F5 (100% annatto) also did not differ among themselves in the preference of their colors. However, they are less preferred than F4 and F1, thus proving consumers' predilection for light red mortadella, tending towards pink.

### 3.5. Sensory analysis: Flash profile

The chemometric method for ComDim multi-table analysis was used to discriminate the five mortadella samples according to the Flash Profile sensory analysis results. The analysis of standard dimensions allows the evaluation of the more relevant variables in separating different samples (Stafussa et al., 2018). In the exploratory study, four common dimensions (CD1, CD2, CD3, and CD4) were necessary (Fig. 3) to represent 99.99% of the variance in the evaluated tables.

The first common dimension (CD1) extracts 72.59% of the total variance of the sample data; for the other dimensions, CD2, CD3, and CD4, the variances were 12.80%, 8.56%, and 6.04%, respectively. Each assessor has a different weight in each dimension, so it is possible to define which assessors were most important for constructing each dimension. From the consequences of each assessor, it is possible to plot the salience graph (Fig. 4) for the dimensions.

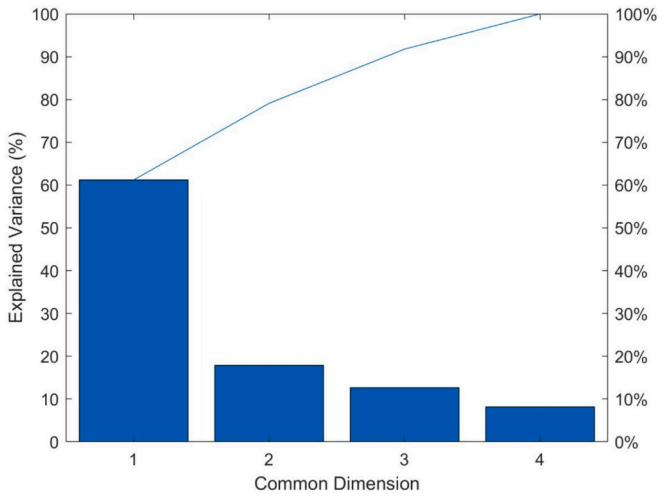



**Fig. 3.** Pareto Chart with the variance represented in each common dimension.

Fig. 4 (a) evaluates dimensions CD1 and CD2. The most relevant assessor for the construction of CD1 was A12, while A20 was the most important for the construction of CD2. For the construction of CD3, the assessor who contributed the most was A19, while for CD4, it was A2. It can be seen that while A2 was necessary for the construction of CD4, it needed to have significant relevance for the other dimensions.

Fig. 4 (b) represents the chart of scores for CD1 and CD2, representing a total variance of 79.12%, sufficient for describing the samples.

For the sensory characterization of the samples, the relationship between the scores of the common dimensions and the scores of the attributes used by the assessors is used (Cariou et al., 2019). Thus, with a 95% confidence level and analysing the positive and negative relationships of the evaluated attributes, obtaining a sensory description of the samples and, consequently, an association between the proportions and types of pigments on the relevant sensory characteristics is possible. Statistically significant relationships between attributes and common dimensions are listed in Table 3.

Analysing the color attribute, the samples that remained in the negative quadrant of the common dimension 1 have an intensification of the pink color (10 direct relations). The pink and orange colors for the positive quadrant showed only a direct relationship. The assessor who identified the pink color in the positive quadrant of CD1 was A13, but he needs a higher saliency in the other dimensions; therefore, statistically, he is not considered a good assessor. For the orange color, also identified in the positive quadrant of CD1, the assessor who determined this attribute was A18. Despite having low salience in CD3 and CD4, it was an essential assessor for constructing CD1 along with A12. Relating Fig. 4 to Table 3, it is observed that for CD1, samples F1 and F4 differed from samples F2, F3, and F5. Thus, samples F1 and F4 showed a more intense pink color as their main feature. While samples F2, F3, and F5 showed a greater intensity of orange coloration.

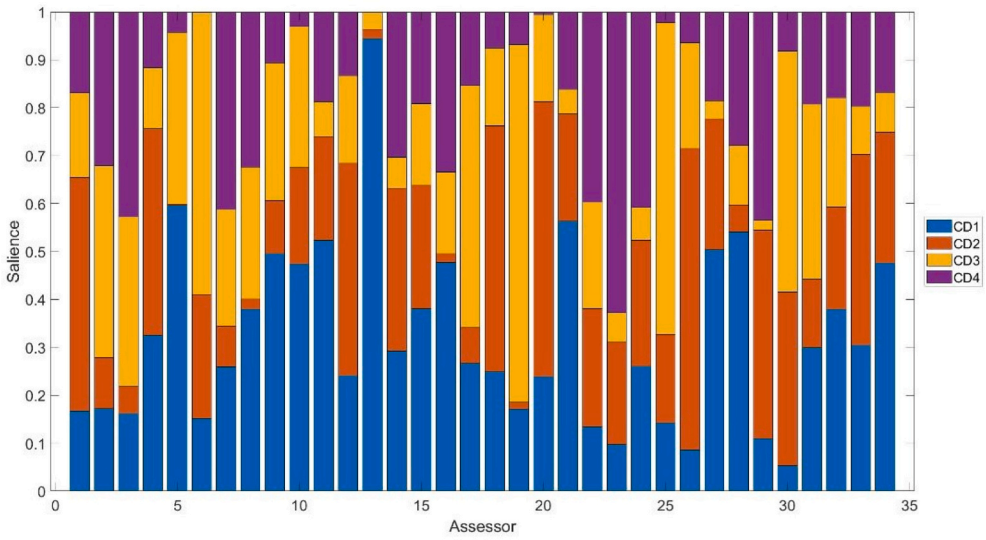
Although CD2 expresses 17.92% of the data variance, the attributes related to it were not significant for the description of the samples since, for this dimension, there were two direct relationships about firmness in the negative quadrant. In the positive quadrant, there were three direct relationships about the presence of fat. For CD2, in the negative quadrant, F1 and F2 showed greater firmness compared to the other formulations. As for the positive quadrant, F3, F4, and F5, I had more fat than F1 and F2. This is because the basic formulation needed to be completely homogeneous, thus presenting pieces of fat. The amount of fat in the dough is inversely proportional to the firmness of the sample; therefore, formulations with a smaller amount of fat present an intensification of the firmness attribute.

In the second stage, the physical-chemical analyses were carried out for samples F1 (now the optimized sample) and F2 (sample optimized with the insertion of sodium erythorbate), as shown in Table 4, over 60 days of the shelf. It is essential to highlight that the new formulations were produced using the same methodology as those presented by the experimental design in Table 1.

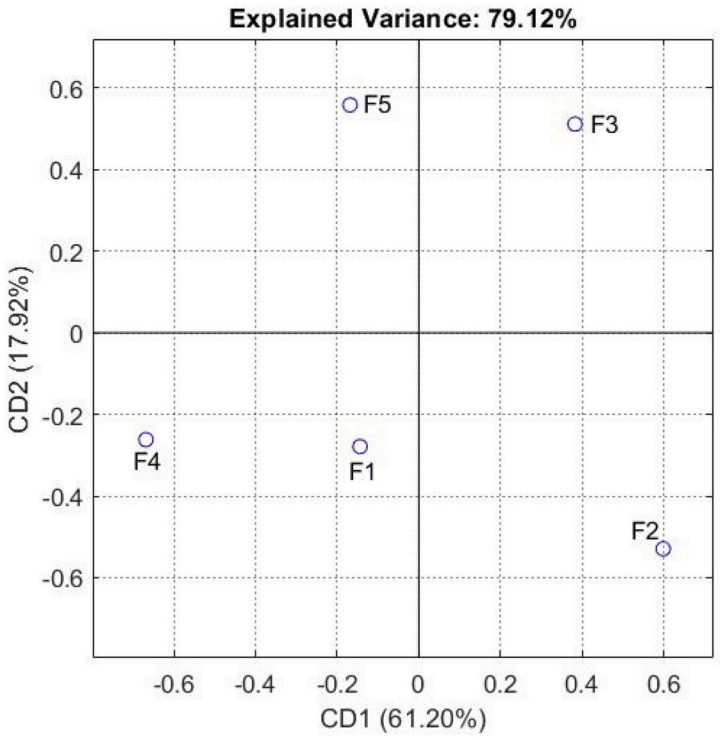
Fig. D (supplementary material) shows the mortadella formulations developed on day 0 of the analysis. Fig. E (supplementary material) shows the F1 and F2 formulations on day 60, the last day of the physical-chemical analyses.

Table 5 presents the results of the mean values and standard deviations of the physicochemical analyses of pH, objective color (L*, a*, and b*), and water holding capacity (WHC). All results were subjected to analysis of variance ($p < 0.05$) between treatments (F1 and F2) and storage time (0, 15, 30, 45, and 60 days) and, in case of significant difference, analysed according to the Tukey test.

Based on the data presented, it is possible to verify that both samples




(a)




(b)

**Fig. 4.** Flash Profile evaluation using ComDim.

had an increase in pH over the 60 days, and for F1 on day 0, it had a value of 6.19, and on day 60, 6.27, and for F2 of 6.12 and 6.25, on day 0 and 60, respectively.

The mortadella pH must be in the neutrality range, close to the value of 7.0 (Brasil, 2000b). Orsolin et al. (2015) found that at time zero of the mortadella shelf life study, the pH ranged from 6.4 to 6.63. Saldaña et al. (2015) evaluated pH values between 6.66 and 6.76, where they partially replaced the amount of animal fat added to bologna with vegetable oils. Thus, the analysed pH value agrees with other studies involving meat sausages.

In the L* color parameter, the samples showed a slight increase over the 60 days, indicating that the mortadella lightens over the days. For parameters a* and b*, higher values were observed on the first day for test formulation F1 (without synthetic additive) and F2 (with sodium erythorbate). The higher the values of these two parameters, the closer the coloration is to a reddish (+a*) and yellowish (+b*) tone, and the smaller they are, the coloration is closer to a greenish tone (−a*) and bluish tone (−b*). The a* chromatid is the most important for meat products because the more positive and higher its values are, the reddish; the samples are pink. Júnior et al. (2019) noticed that about the a* parameter, there was a lower tendency to red color in the samples with curcumin, where the values ranged from 4.91 to 8.42 throughout the

**Table 3**
Sensorial correlations in mortadella evaluations.

| Relationship with CD1 | | Relationship with CD2 | |
|---|---|---|---|
| Negative | Positive | Negative | Positive |
| pink color (10)<br>firmness (1) | pink color (1)<br>humidity (1) | firmness (2)<br>mortadella odor (1) | presence of fat (3)<br>firmness (1) |
| brightness (1) | homogeneity (1) | | flavor enhancer flavor (1) |
| pepper flavor (1) | I like fat (1) | | mortadella flavor (1) |
| fat flavor (1) | orange color (1)<br>mortadella flavor (1) | | sausage odor (1)<br>fat flavor (1) |
| | | | salty flavor (1) |

Significant relationships ($p < 0.05$) between sensory attributes and common dimensions.

**Table 4**
Ingredients and additives used in mortadella formulations (F1 and F2).

| Ingredients/Additives | Formulation 1 (%) | Formulation 2 (%) |
|---|---|---|
| pork shank | 68.868 | 68.868 |
| ice/water | 12.020 | 11.770 |
| bacon | 12.000 | 12.000 |
| cassava starch | 3.000 | 3.000 |
| salt | 2.000 | 2.000 |
| isolated soy protein | 1.000 | 1.000 |
| condiment for mortadella | 0.400 | 0.400 |
| cure IBRAC | 0.250 | 0.250 |
| acordini 701 - stabilizer | 0.250 | 0.250 |
| garlic powder | 0.100 | 0.100 |
| monosodium glutamate | 0.100 | 0.100 |
| pigment (cochineal carmine) | 0.010 | 0.010 |
| curcumin | 0.002 | 0.002 |
| antioxidant | 0.000 | 0.250 |

evaluated period due to the natural color of curcumin. Thus, this work noted that adding the natural dye cochineal carmine helped develop the red color. For Ongaratto et al. (2021), cochineal carmine is used to prepare mortadella and ham to attribute the long-lasting pink/reddish color characteristic during storage of these products designed without the addition of curing salts.

To evaluate the results presented by Anova, the Principal Component Analysis (PCA) was performed, considering the pH analyses and the parameters L*, a*, and b* of the objective color for samples F1 and F2 over 60 days. In Fig. 5, where observations were made for the samples, the first (PC1) and second (PC2) components explained 94.50% of the total data variation. Principal component 1 (PC1) captured 87.73% of the variance, while principal component 2 (PC2) was 6.77%. In Principal Component Analysis (PCA), the variables are represented as vectors, which characterize the samples related to them. The longer the vector, the greater the explanation of the sample variability. The values obtained by Pearson's correlation confirm the relationship between the parameters observed in the principal component analysis (Table 6), demonstrating relationships between some studied variables. The pH (0.52504) and the color parameter L* (0.50735) were positively related to the first component (PC1). The a* parameter (−0.64477) showed a negative correlation (found in PC2), and the b* parameter (0.75985) had an inverse positive relationship (PC2). With this information, it is possible to state that the values of the color parameter L* and the pH are magnitudes inversely proportional to the color parameters a* and b*. This can be explained due to peroxides, which in meat products cause discoloration and oxidation of pigments. Formation of metmyoglobin, consequently decreasing the positive values for chromatid a* (Alcantara

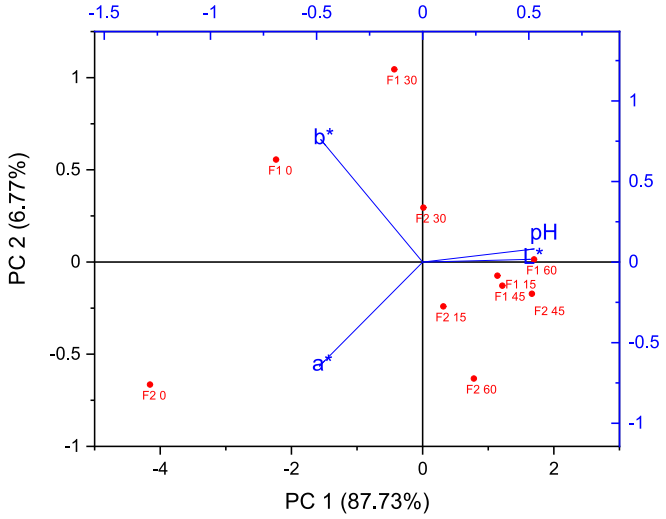



**Fig. 5.** Principal component analysis (PCA) for the evaluation.

**Table 6**
Correlations between the physicochemical parameters and the first two principal components.

| Parameters | Principal components | |
|---|---|---|
| | PC1 | PC2 |
| | r | r |
| pH | 0.52504 | 0.08118 |
| L* | 0.50735 | 0.01744 |
| a* | −0.48612 | −0.64477 |
| b* | −0.48023 | 0.75985 |

r = Pearson's correlation coefficient.

**Table 5**
Results of physicochemical analyses performed on samples containing curcumin microcrystals and curcumin microcrystals and sodium erythorbate.

| Variables | | Samples | Time (days) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 15 | 30 | 45 | 60 |
| pH | | F1 | $6.19^{aC} \pm 0.02$ | $6.27^{aA} \pm 0.00$ | $6.22^{aBC} \pm 0.01$ | $6.25^{bAB} \pm 0.00$ | $6.27^{aA} \pm 0.01$ |
| | | F2 | $6.12^{aB} \pm 0.03$ | $6.24^{aA} \pm 0.03$ | $6.22^{aA} \pm 0.01$ | $6.28^{aA} \pm 0.01$ | $6.25^{aA} \pm 0.01$ |
| Color | L* | F1 | $67.78^{aB} \pm 0.77$ | $69.56^{aAB} \pm 0.36$ | $67.83^{aB} \pm 0.39$ | $69.68^{aAB} \pm 0.69$ | $70.79^{aA} \pm 0.19$ |
| | | F2 | $64.78^{bC} \pm 0.30$ | $67.99^{bB} \pm 0.37$ | $68.12^{aB} \pm 0.10$ | $70.33^{aA} \pm 0.36$ | $69.57^{aAB} \pm 0.65$ |
| | a* | F1 | $9.17^{aA} \pm 0.36$ | $7.81^{aAB} \pm 0.25$ | $7.67^{aB} \pm 0.46$ | $7.62^{aB} \pm 0.16$ | $7.61^{bB} \pm 0.15$ |
| | | F2 | $10.15^{aA} \pm 0.67$ | $7.99^{aB} \pm 0.14$ | $7.69^{aB} \pm 0.29$ | $7.70^{aB} \pm 0.08$ | $8.34^{aB} \pm 0.13$ |
| | b* | F1 | $26.30^{aA} \pm 0.40$ | $19.45^{aB} \pm 0.60$ | $23.56^{aA} \pm 0.81$ | $18.79^{aB} \pm 0.26$ | $19.13^{aB} \pm 0.76$ |
| | | F2 | $25.07^{aA} \pm 1.98$ | $19.53^{aB} \pm 0.28$ | $20.86^{aAB} \pm 1.27$ | $18.66^{aB} \pm 0.54$ | $18.94^{aB} \pm 0.80$ |
| WRC (g per 100 g) | | F1 | $95.09^{aA} \pm 0.08$ | $95.03^{aA} \pm 0.38$ | $95.76^{aA} \pm 0.21$ | $96.04^{aA} \pm 0.24$ | $95.28^{aA} \pm 0.30$ |
| | | F2 | $93.82^{aA} \pm 0.61$ | $95.04^{aA} \pm 1.05$ | $94.38^{bA} \pm 0.11$ | $95.27^{aA} \pm 0.16$ | $95.12^{aA} \pm 0.40$ |

Means on the same line, followed by distinct capital letters, differ by Tukey's test at a significance level of 5% for each sample over time 0, 15, 30, 45, and 60 days. Means in the same column, followed by different lowercase letters, differ by Tukey's test at a significance level of 5% between samples F1 and F2.

et al., 2012; Demodaran & Parkin, 2018). In Fig. 5, it is also possible to verify that higher values of parameters a* and b* are observed at time 0, both for formulation F1 and F2, and that higher values of parameter L* are verified over the 60 days, corroborating the information presented by Table 5. In the color analysis of fresh sausage in the study by Baldin et al. (2018), the control sausage (with curing salt) and the fresh sausage with 4% cochineal carmine obtained a significant reduction in a* values during storage for only 15 days, demonstrating that the red color promoted by the action of these color agents is not very stable, corroborating the results found in this study. Ongaratto et al. (2021) also verified this reduction in the a* parameter in mortadella with curing salts and cochineal carmine.

As for the water retention capacity, both samples, F1 and F2, did not show significant differences either between them (Table 5) or individually over time.

For lipid oxidation (Table 7), formulation F1 (0.11 mg of MDA $kg^{-1}$ at 60 days) showed a slightly higher value than formulation F2 (0.10 mg of MDA $kg^{-1}$ at 60 days). days), but both differed significantly, neither between them nor overtime individually, which may be an excellent option to add curcumin microcrystals without the addition of the synthetic antioxidant (sodium erythorbate) for the conservation of shelf life of cooked meat sausages. Júnior et al. (2019) obtained values between 0.67 and 2.73 mg of MDA $kg^{-1}$ of sample for the mortadella formulation containing microcrystals of curcumin and 0.26 to 2.44 mg of MDA $kg^{-1}$ of sample for the formulation containing the synthetic antioxidant, sodium erythorbate, over the studied period. The country's current legislation does not provide a maximum limit of malondialdehyde for mortadella. Still, according to Al-Kahtani et al. (1996), values lower than 3.00 mg of MDA $kg^{-1}$ of the sample can be considered ideal for conserving meat products.

Table 8 presents the results for the texture profile. The evaluated parameters were adhesiveness, cohesiveness, chewiness, elasticity, gumminess, and resilience for samples F1 and F2.

The texture results from the deformation of food when it undergoes pressing, cutting, biting, etc. In this alteration, one has knowledge of adhesiveness, elasticity, cohesiveness, gumminess, and chewability, among other studied parameters (Di Monaco et al., 2008).

As can be seen in Table 8, the parameters evaluated for the texture profile (adhesiveness, cohesiveness, chewiness, gumminess, and resilience) did not show significant differences ($p < 0.05$) between samples F1 and F2, nor individually to over the 60 days. The elasticity varied over time, both for the F1 and F2 formulations. In the Principal Component Analysis, corroborating the data in Table 8 by analysis of variance (ANOVA) and Tukey's test, the first component explains 47.04% of the variance, and the second principal component explains 31.14% of the variability between F1 and F2. Together, the two main components explain 78.18% of the variability. The cohesiveness and resilience parameters are positively correlated ($p < 0.05$), Table 9, with the first principal component and, therefore, discriminate the samples about this component. The second principal component is mainly explained by the adhesiveness, elasticity, and chewability parameters, with the F2 formulation at 45 days having the highest values for adhesiveness and elasticity. Fig. 6 suggests that formulation F1 at time 15 and F2 at time 60 present greater intensity of chewiness and gumminess parameters. The reverse is observed for the F1 formulation at 45 days.

The work carried out by Júnior et al. (2019) obtained the following results for the texture profile analysis about adhesiveness: the values ranged from 0.00 to −0.10 N $s^{-1}$; for elasticity, it was from 0.91 to 1.34 m for the chewability ranged from 253.05 to 1816.79 (N $m^{-1}$), the cohesiveness parameter ranged from 0.76 to 0.96, and finally, the resilience ranged from 0.50 to 0.62.

Literature and legislation do not present values to assign a texture profile expected by the product. Therefore, adding sodium erythorbate in the mortadella formulation did not significantly affect the texture. So, the parameters evaluated for the texture, adhesiveness, cohesiveness, elasticity, chewiness, and resilience did not present a significant difference for mortadella.

## 4. Conclusion

Based on the physical-chemical results obtained in this study, adding pigments did not significantly interfere with the pH, water holding capacity, or weight loss during cooking of the mortadella samples. The observed higher weight at the end of cooking was attributed to synthetic casing, which prevented water loss from the samples. Color analysis revealed that formulations F4 (100% cochineal carmine) and F1 (75% cochineal carmine and 25% annatto exhibited lower intensity of yellow coloring, as indicated by the chromatic coordinate b*. Specifically, formulation F4, which consisted of 100% cochineal carmine pigment, emerged as the optimized choice based on the desirability study.

Sensory analysis demonstrated a preference among assessors for formulations with intensified pink color, achieved with higher concentrations of cochineal carmine. This pigment showed excellent application potential when added to mortadella formulations containing curcumin microcrystals. Thus, it can be concluded that formulations with elevated levels of carmine exhibit appealing sensory characteristics.

Furthermore, two new formulations were developed - one with and one without sodium erythorbate - to assess shelf life over 60 days. The study revealed no significant differences in lipid oxidation between samples with or without sodium erythorbate, aligning with desirable outcomes for meat products. The addition of natural dye cochineal carmine imparted a pink hue to the samples, evident from the positive values of the a* chromatic coordinate, indicating a redder coloration.

Based on the data gathered, it is evident that in the presence of curcumin microcrystals, the synthetic antioxidant sodium erythorbate can be omitted from formulations without compromising the studied physical-chemical parameters such as pH, water retention capacity, objective color, and texture profile.

Thus, the findings underscore the potential of cochineal carmine as a natural colorant in enhancing the sensory appeal and stability of mortadella formulations, offering a viable alternative in meat processing applications.

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**Table 7**
Results of lipid oxidation analysis in samples containing curcumin microcrystals and curcumin microcrystals and sodium erythorbate.

| Variables | Samples | Tempo (dias) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 15 | 30 | 45 | 60 |
| **Lipid oxidation (mg of malonaldehyde per kg of the sample)** | F1 | $0.06^{aA} \pm 0.01$ | $0.07^{aA} \pm 0.03$ | $0.08^{aA} \pm 0.01$ | $0.09^{aA} \pm 0.02$ | $0.11^{aA} \pm 0.01$ |
| | F2 | $0.03^{aA} \pm 0.02$ | $0.04^{aA} \pm 0.01$ | $0.05^{aA} \pm 0.02$ | $0.06^{aA} \pm 0.02$ | $0.10^{aA} \pm 0.01$ |

Means on the same line, followed by distinct capital letters, differ by Tukey's test at a significance level of 5% for each sample over time 0, 15, 30, 45, and 60 days. Means in the same column, followed by different lowercase letters, differ by Tukey's test at a significance level of 5% between samples F1 and F2.

**Table 8**
Texture parameters performed on samples containing curcumin microcrystals and curcumin microcrystals with sodium erythorbate.

| | | Time (days) | | | | |
|---|---|---|---|---|---|---|
| Variables | Samples | 0 | 15 | 30 | 45 | 60 |
| adhesiveness (N $s^{-1}$) | F1 | $-0.13^{aA} \pm 0.09$ | $-0.24^{aA} \pm 0.04$ | $-0.23^{aA} \pm 0.11$ | $-0.26^{bA} \pm 0.01$ | $-0.17^{aA} \pm 0.09$ |
| | F2 | $-0.22^{aA} \pm 0.10$ | $-0.19^{aA} \pm 0.09$ | $-0.25^{aA} \pm 0.02$ | $-0.03^{aA} \pm 0.02$ | $-0.25^{aA} \pm 0.02$ |
| cohesiveness * | F1 | $0.79^{aAB} \pm 0.01$ | $0.77^{aAB} \pm 0.01$ | $0.76^{aB} \pm 0.02$ | $0.80^{aA} \pm 0.01$ | $0.80^{aA} \pm 0.01$ |
| | F2 | $0.78^{aA} \pm 0.02$ | $0.79^{aA} \pm 0.01$ | $0.78^{aA} \pm 0.01$ | $0.79^{aA} \pm 0.01$ | $0.79^{aA} \pm 0.01$ |
| chewability (N $m^{-1}$) | F1 | $3350.97^{aA} \pm 584.95$ | $3814.47^{aA} \pm 432.14$ | $3287.83^{aA} \pm 354.33$ | $2552.60^{aA} \pm 241.28$ | $3191.24^{aB} \pm 220.34$ |
| | F2 | $3289.54^{aA} \pm 471.35$ | $3569.49^{aA} \pm 582.92$ | $3351.10^{aA} \pm 307.72$ | $3433.19^{aA} \pm 330.05$ | $3751.28^{aB} \pm 216.05$ |
| elasticity (m) | F1 | $0.87^{aA} \pm 0.02$ | $0.80^{aB} \pm 0.02$ | $0.84^{aA} \pm 0.01$ | $0.79^{bB} \pm 0.01$ | $0.86^{aA} \pm 0.02$ |
| | F2 | $0.86^{aAB} \pm 0.03$ | $0.85^{aB} \pm 0.03$ | $0.80^{bC} \pm 0.01$ | $0.92^{aA} \pm 0.03$ | $0.84^{aB} \pm 0.02$ |
| gumminess (N) | F1 | $3888.13^{aA} \pm 696.05$ | $4764.35^{aA} \pm 463.18$ | $3916.96^{aA} \pm 409.84$ | $3241.90^{aA} \pm 286.70$ | $3692.87^{aA} \pm 230.00$ |
| | F2 | $3850.99^{aA} \pm 656.74$ | $4214.39^{aA} \pm 688.52$ | $4202.23^{aA} \pm 341.64$ | $3717.13^{aA} \pm 304.54$ | $4468.41^{aA} \pm 187.38$ |
| resilience * | F1 | $0.48^{aA} \pm 0.03$ | $0.49^{aA} \pm 0.02$ | $0.47^{aA} \pm 0.02$ | $0.50^{aA} \pm 0.02$ | $0.50^{aA} \pm 0.01$ |
| | F2 | $0.52^{aA} \pm 0.02$ | $0.51^{aA} \pm 0.01$ | $0.50^{aA} \pm 0.01$ | $0.51^{aA} \pm 0.01$ | $0.48^{aA} \pm 0.02$ |

Means on the same line, followed by distinct capital letters, differ by Tukey's test at a significance level of 5% for each sample over 0, 15, 30, 45, and 60 days.
Means in the same column, followed by different lowercase letters, differ by Tukey's test at a significance level of 5% between samples F1 and F2.
* does not have a unit of measurement, as it is dimensionless.

**Table 9**
Correlations between texture parameters and the first two principal components.

| Principal components | | |
|---|---|---|
| Parameters | PC1 | PC2 |
| | r | r |
| adhesiveness | 0.37115 | 0.52061 |
| cohesiveness | 0.49977 | −0.04963 |
| chewability | −0.36195 | 0.55340 |
| elasticity | 0.32971 | 0.56900 |
| gumminess | −0.50299 | 0.31063 |
| resilience | 0.34606 | 0.00150 |

r = Pearson's correlation coefficient.

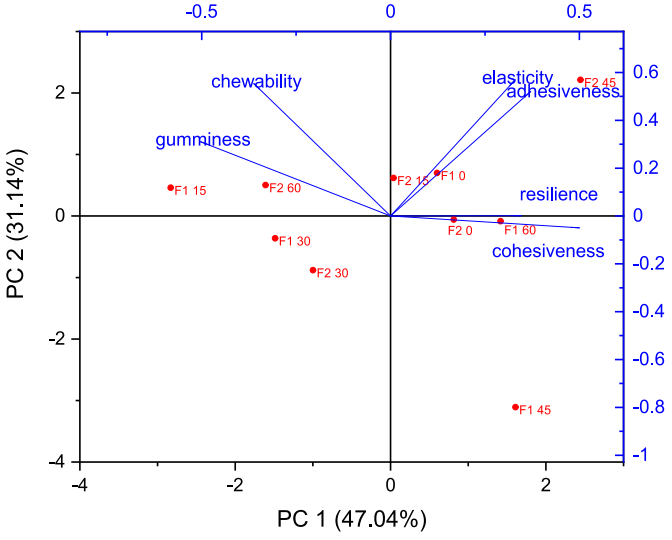


**Fig. 6.** Graphic representation of the texture parameters of the samples of the two main components.

## Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Jacqueline Thomé Henrique, Maria Victória Biason, Poliana dos Santos Mendes, Evandro Bona, Hernandez Barros Fuchs, Flávia Aparecida Reitz Cardoso, and Adriana Aparecida Droval. The first draft of the manuscript was written by Fernanda Vitória Leimann, Odinei Hess Gonçalves, Evandro Bona, Anielle de Oliveira, Leila Larisa Medeiros Marques, Renata Hernandez Barros Fuchs, Flávia Aparecida Reitz Cardoso and Adriana Aparecida Droval and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Ethics approval

The Ethics Committee of UTFPR approved the research under the CAAE opinion: 88116618.2.0000.5547.

## Consent to participate

Informed consent was obtained from all individual participants included in the study.

## Consent to publish

The authors affirm that human research participants provided informed consent for publication.

## CRediT authorship contribution statement

**Jacqueline Thomé Henrique:** Validation, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Maria Victória Biason:** Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Poliana dos Santos Mendes:** Validation, Methodology. **Flávia Aparecida Reitz Cardoso:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Fernanda Vitória Leimann:** Validation, Methodology. **Odinei Hess Gonçalves:** Validation, Methodology. **Evandro Bona:** Validation, Methodology. **Anielle de Oliveira:** Validation, Methodology. **Leila Larisa Medeiros Marques:** Validation, Methodology. **Renata Hernandez Barros Fuchs:** Validation, Supervision, Methodology. **Adriana Aparecida Droval:** Validation, Supervision, Methodology.

## Declaration of competing interest

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

## Data availability

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

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## Appendix A. Supplementary data

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