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Extraction of Bioactive Compounds from Prickly Pear and Incorporation into Fresh Chicken Sausages: Physicochemical, Microbiological and Sensory Evaluation

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Abstract: Fresh sausage is a popular meat product, but its ingredients and processing can reduce stability. Natural extracts rich in bioactive compounds, such as those from *Opuntia ficus-indica*, could function as antioxidants, though their use in meat products remains limited. This study aimed to evaluate the effects of prickly pear extract (PPE) on the physicochemical and microbiological stability of fresh chicken sausages, and to characterize the products and assess sensory acceptance. Extracts obtained through five different methods were analyzed for their antioxidant capacity to identify the best option for incorporation into the products. Four sausage treatments were produced: control (CON, without antioxidants), sodium erythorbate (ERY, 500 ppm), and two different concentrations of extracts (1.88% and 3.75%). Shelf life was analyzed on days 0, 5, 10, and 15 of refrigerated storage (2 °C). PPE use did not affect proximate composition, pH, or cooking losses ($p > 0.05$); however, it slightly increased hardness, decreased lightness (L*), and increased yellowness (b*) ($p < 0.05$). All the fresh sausage treatments were positively received from a sensory perspective, achieving scores close to or above 7 on a 9-point hedonic scale. The extract obtained from whole prickly pear fruit showed potential as a multifunctional ingredient for fresh chicken sausages, particularly for reducing psychrotrophic counts and protein oxidation. However, its effect on lipid oxidation remains inconclusive, and further optimization of the extract concentration and analytical methodology is required before its potential to replace synthetic antioxidants can be established.

Keywords: *Opuntia ficus-indica*; natural antioxidants; natural antimicrobials; lipid oxidation; protein oxidation

1. Introduction

Meat is widely consumed globally, and its industrialization provides convenient, diverse, and generally more affordable products like meatballs, hamburgers, breaded items, and sausages [1]. Chicken fresh sausages, for example, are appreciated for their distinctive flavor and high nutritional value, providing high-quality proteins, amino acids, essential fatty acids, vitamins, and minerals [2]. However, due to the nature of the raw materials used

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in their manufacture, their high fat content, and the absence of pasteurization, fresh sausages are highly perishable. Furthermore, grinding exposes the meat to environmental factors that promote microbial growth and accelerate oxidation reactions, which can compromise the product's quality and lead to undesirable sensory changes, making the product highly perishable [3]. Consequently, it is essential to use substances capable of preserving properties and safety during the shelf life of fresh sausages.

The industry commonly uses synthetic antioxidants to extend shelf life by delaying or inhibiting lipid oxidation. However, their use has been increasingly questioned as consumers seek cleaner-label products with fewer and more familiar ingredients, and reduced use of synthetic additives [4]. In this sense, the search for natural alternatives has increased, with plant-derived sources showing good protective activities against damage related to oxidative stress, capable of acting in food preservation, in addition to being rich in bioactive compounds with potential health benefits [5]. While spices and aromatic herbs can also delay oxidation, their use is limited by their strong sensory impact [6]. Therefore, extracts obtained from relatively low-cost materials such as agro-industrial residues, seeds, peels, and pulps of fruits have proven to be rich in natural antioxidants and have emerged as a promising and economical alternative [7].

Cactus pears, or prickly pears, *Opuntia ficus-indica* (L.) Mill., originally cultivated in the Americas, have been widely recognized for their nutritional and medicinal properties since they are rich in beneficial components such as amino acids, magnesium, calcium, vitamins, carotenoids, betalains, betaxanthins, and polyphenols; therefore, they have a good capacity to act as antioxidants [8,9].

The growing search for natural antioxidants has spurred the development of extraction methods for obtaining bioactive compounds from different plant matrices, including emerging technologies like ultrasound, pulsed electric field, and supercritical fluids [10]; however, conventional solid–liquid extraction with GRAS solvents (e.g., ethanol–water) remains widely used, and extraction conditions must be optimized to preserve compounds and maximize yield [11,12]. Several intrinsic characteristics, as well as the matrix composition, directly influence the recovery yield and the properties of the extracted compounds. Therefore, it is essential to carefully select the extraction conditions and appropriate solvent to preserve bioactive compounds and maximize yield. This study aimed to compare various extraction techniques for obtaining prickly pear extracts, focusing on yield, total phenolic content, and *in vitro* antioxidant activity to develop a natural alternative to synthetic antioxidants. It also evaluated the effects of adding these extracts to fresh chicken sausage on oxidative stability, microbiological safety, physicochemical characteristics, and sensory acceptance.

2. Materials and Methods

2.1. Preparation of Natural Extracts

Approximately 25 kg of orange-fleshed cactus pear fruits were purchased in a single batch from a local market in Vinhedo, São Paulo, Brazil, in February 2021. The experiment was conducted at the Department of Food Engineering (ZEA), located at the Fernando Costa campus of the University of São Paulo in Pirassununga, São Paulo, Brazil. After selection, the items were stored at 4 °C, then washed with running water and sanitized using 70% ethanol-water (*v*/*v*). Five different hydroethanolic extraction methods from the literature were tested to optimize antioxidant capacity and select the best extract for later application in meat products. Both the whole fruit and its pulp and peel were tested. To obtain the pulp, fruits were manually peeled and pulped in a blender, followed by sieving to separate seeds. Peels and pulps were packed in vacuum packaging and frozen (−18 °C). Extracts were obtained according to different methodologies available in the scientific literature, namely:

A. Methodology by Sáenz et al. [13] with modifications: pulp was mixed with ethanol (1:2) and homogenized in an orbital shaker for 24 h at 120 rpm and 24 °C. Afterwards, the mixture was centrifuged for 20 min at 2000× *g*. The remaining solid was re-extracted twice under the same conditions. All collected supernatants were combined and concentrated.
B. Adapted methodology of Lourith & Kanlayavattanakul [14]: the peels were cut into 2 × 2 mm pieces, dried in an oven at 50 °C for 24 h, and then crushed to obtain a fine powder. The dry material was added to 80% ethanol (*v*/*v*) at a 1:5 ratio, homogenized in a shaker incubator for 60 min, and filtered.
C. Methodology of Wu et al. [15], with modifications: 50 g of pulp was mixed with 200 mL of 80% ethanol-water (*v*/*v*) in an Ultra-Turrax (TE 102, Tecnal, Piracicaba, Brazil) for 10 min. The mixture was filtered, and the retained solid was extracted with 100 mL of solvent for 5 min, followed by a new filtration.
D. Methodology by Cunha et al. [16], with some modifications: peels cut into small pieces were dried at 50 °C for 24 h and ground. About 15 g of this powder was mixed with an ethanol-water solvent (40:60, *v*/*v*) and extracted using microwave treatment (400 W for 30 s). The mixture was centrifuged for 15 min at 2000× *g*,

and the supernatant was transferred to an amber vial, while the solid was re-extracted with 20 mL of the solvent under the same conditions described above. The liquid supernatants were combined and concentrated.

E. Methodology by Aruwa, Amoo & Kudanga [17] with modifications: fruits were sliced and dried at 50 °C for 96 h. The material was ground, solubilized in 80% ethanol (*v*/*v*) at a 1:10 ratio, homogenized in an orbital shaker incubator for 24 h (120 rpm, 24 °C), and filtered.

Subsequently, all A–E extracts were concentrated in a rotary evaporator (model 802, Fisatom, São Paulo, Brazil) at 60 rpm, starting the process at 40 °C to remove the ethanolic portion and with a gradual increase in temperature up to 60 °C for water evaporation. Extracts were stored frozen in amber flasks at −18 °C until analysis.

2.2. Extract Yield

Yields were calculated as a percentage (% *v*/*m*), considering the amount of fruit needed to obtain the extract, according to Equation (1).

$$\text{Yield (\%)} = (\text{Volume of extract obtained (mL)})/(\text{Initial fruit mass required (g)}) \times 100 \quad (1)$$

2.3. Total Phenolic Compounds (TPC) and In Vitro *Antioxidant Activity Assessment*

2.3.1. Determination of Total Phenolic Compounds

The assessment of total phenolic compounds (TPC) was based on the Singleton & Rossi Jr. [18] method for all five extracts. The samples were properly diluted in distilled water. After mixing 0.5 mL of each dilution with 2.5 mL of 10% Folin-Ciocalteu reagent for 6 min, 2.0 mL of 7.5% (*w*/*v*) sodium carbonate was added to the test tube. Samples were stored protected from light for 2 h. Then, the absorbance at 760 nm was read in a UV/visible spectrophotometer (model SP-22, Biospectro, São Paulo, Brazil). Results were calculated in terms of mg of Gallic Acid Equivalent (GAE)/g of sample ($y = 10.148x + 0.026$; $r^2 = 0.9968$). After carrying out preliminary tests, extracts with the highest TPC were selected for the other tests to determine their antioxidant activity (FRAP and ABTS).

2.3.2. Ferric Reducing Antioxidant Power Method (FRAP)

The ferric reducing antioxidant power (FRAP) procedure was adapted from that proposed by Benzie & Strain [19]. To prepare the FRAP reagent, the following solutions were mixed in a 1:1:10 (*v*:*v*:*v*) ratio: 10 mM TPTZ solution (2,4,6-tri(2-pyridyl)-s-triazine), 20 mM ferric chloride solution ($FeCl_3 \cdot 6H_2O$), and 300 mM sodium acetate (CH_3COONa) buffer with a pH of 3.6. After preparing the appropriate aqueous dilutions of extracts and solutions, 100 µL of the sample was added to 3 mL of FRAP reagent. Tubes were incubated in the dark at 37 °C for 30 min. The absorbance at 593 nm was measured in a UV/vis spectrophotometer (model SP-22, Biospectro, São Paulo, Brazil), and the result was calculated using a linear regression curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) with concentration varying between 0 and 0.85 µmol/mL ($y = 1.3508x + 0.0543$; $r^2 = 0.9962$). Results were expressed as the concentration of Trolox equivalents (µmol Trolox equivalent (TE)/g extract).

2.3.3. ABTS Radical Scavenging Test

The ABTS radical cation decolorization method (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) is one of the most commonly used methods to estimate the total antioxidant activity of compounds of lipophilic and hydrophilic nature, applied according to the methodology of Re et al. [20]. A 7 mM ABTS stock solution was added to a 140 mM potassium persulfate aqueous solution. This mixture was stored in the dark at room temperature for about 16 h, and then diluted in ethyl alcohol until it reached an absorbance of 0.7 ± 0.05 (approximately 1 mL of ABTS-mixed solution in 100 mL of ethanol) at 734 nm. For sample analysis, 30 µL of an adequate dilution was reacted with 3 mL of previously prepared ABTS radical, homogenized by vortexing, and kept for 6 min in the absence of light before absorbance reading (734 nm). A Trolox calibration curve (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was prepared at concentrations ranging from 0 to 2.0057 µmol/mL ($y = 39.187x + 2.6885$; $r^2 = 0.9948$), and results were expressed in µmol Trolox Equivalent (TE)/g extract. The percentage of inhibition of the ABTS radical was calculated as shown in Equation (2).

$$\text{Inhibition (\%)} = [(\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}})/\text{Absorbance}_{\text{blank}}] \times 100 \quad (2)$$

2.4. Concentration of Extract to Be Added to Fresh Chicken Sausages

The concentration of prickly pear extract needed to be added to sausages was calculated to match the antioxidant activity (AA) of sodium erythorbate (500 ppm, or 0.05%). Based on antioxidant capacity results and extraction yield, the required amount of extract was estimated (Equation (3)), enabling the selection of the best extract and the definition of four sausage treatments.

$$\text{Extract concentration estimated (\%)} = (AA_{\text{sodium erythorbate}})/(AA_{\text{extract}}) \times 0.05 \quad (3)$$

2.5. Preparation of Meat Products

Four fresh chicken sausage treatments were produced: CON (control treatment, without added antioxidants), ERY (500 ppm of sodium erythorbate), PPE1.88, and PPE3.75 (addition of 1.88% and 3.75% prickly pear extract, respectively). All treatments were produced with the following ingredients: chicken breast fillet (80.0%), chicken skin (14.0%), sodium chloride (1.8%), onion powder (0.30%), garlic powder (0.15%), white pepper powder (0.05%), and sodium nitrite (150 ppm). Ice water was added as needed to adjust the formulations to 100% (CON = 3.69%; ERY = 3.64%; PPE1.88 = 1.80%; and PPE3.75 = 0%). Chicken breast and skin were respectively ground into 6 and 4 mm disks (model Picador 22, Beccaro, Rio Claro, Brazil), mixed with the other ingredients, and kept refrigerated (0 ± 1 °C) for 30 min for the condiments to set. Then, the product was filled into natural pork casing, packed in vacuum polyethylene bags, and refrigerated at 2 ± 1 °C for 15 days to carry out the analyses. The complete experiment was independently repeated three times with a 1-month interval between repetitions.

2.6. Physicochemical Characterization of Sausages

2.6.1. Proximate Composition

Proximate composition was determined following the standard AOAC methodologies for proteins (Kjeldahl method; N × 6.25 conversion factor), moisture (air oven drying), and ashes (muffle furnace) determinations [21], while lipids were assessed by the procedure of Bligh & Dyer [22].

2.6.2. Cooking Losses (CL)

Chicken sausages previously weighed were cooked in a preheated electric oven at 180 °C until they reached an internal temperature of 72 °C. Once cooled to 24 °C, the samples were weighed again, and the determination of weight loss after cooking was expressed as a percentage (Equation (4)).

$$CL\ (\%) = [(\text{Weight}_{\text{initial}} - \text{Weight}_{\text{final}})/\text{Weight}_{\text{initial}}] \times 100 \quad (4)$$

2.6.3. Instrumental Hardness Analysis

The instrumental texture (hardness) was measured using a TA.XTplus texture analyzer (Stable Micro Systems, Surrey, UK). After cooking the sausages to an internal temperature of 72 °C, 20 mm thick slices were cut and then compressed with a P-35 probe to 50% of their original height. A 30 kg load cell was employed at a test speed of 1 mm/s. The parameters were analyzed using Texture Exponent 32 software (version 1.0.0.68, Stable Micro Systems, Vienna Court, UK).

2.7. Physicochemical Stability

The physicochemical stability was evaluated during refrigerated storage on days 0, 5, 10, and 15.

2.7.1. Objective Color and pH

Samples were longitudinally sectioned for internal color reading and exposed to atmospheric air for 30 min before analysis. A portable spectrophotometer (HunterLab, model MiniScan XE, Reston, VA, USA) with D_{65} illuminant, cell opening of 30 mm, and observation angle of 10° was used to evaluate the parameters of the CIELab system: L* (lightness), a* (red-green color), b* (yellow-blue color), and ΔE* (according to Equation (5)), obtaining an average of 5 measurements taken from random positions.

$$\Delta E^* = [(L^*_{5-15} - L^*_0)^2 + (a^*_{5-15} - a^*_0)^2 + (b^*_{5-15} - b^*_0)^2]^{1/2} \quad (5)$$

The pH of samples was determined using a digital pH meter (Hanna Instruments S.R.L., model HI 99163, Woonsocket, RI, USA) equipped with a combined electrode for reading, by drilling 3 points in each sample in each repetition. The pH meter was calibrated with pH 4.01 and 7.01 solutions.

2.7.2. Lipid Oxidation

Lipid oxidation was evaluated by the 2-thiobarbituric acid reactive substances (TBARS) index, as described by Vyncke [23]. Results were calculated with a TEP calibration curve (1,1,3,3-tetraethoxypropane) and expressed in parts per million (ppm) of malondialdehyde (MDA).

2.7.3. Protein Oxidation Analysis

Protein oxidation was quantified by the technique defined by Oliver et al. [24] with modifications [25] at the beginning (t = 0) and at the end of storage (t = 15 days), performing two measurements: carbonyl and protein quantification. Contents were spectrophotometrically determined at 370 and 280 nm, respectively. Calculations were performed using a curve prepared with standard bovine serum albumin (BSA) as a basis for calibration, and the results were given in nmol/mg.

2.8. Microbiological Stability

The microbiological analyses of pathogenic agents were carried out as recommended by the Brazilian Normative Instruction, which determined the microbiological criteria and standards for food and for Group 5 (poultry meat); there is a specific category (b) that defines standards for fresh sausage [26]. In this case, the search for *Salmonella* spp. and *Escherichia coli* was determined at the beginning and at the end of storage. The verification of deteriorating microorganisms was performed by counting aerobic psychrotrophic microorganisms on days 0, 5, 10, and 15 of refrigerated storage.

Escherichia coli was determined by counting on "Compact Dry EC" plates (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), which allow the counting of *E. coli* and total coliforms after inverted incubation for 24 h at 35 ± 2 °C. In the research of the genus *Salmonella*, "Compact Dry SL" plate (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) was used, with inverted plates being incubated at 41 ± 2 °C for 24 h after plating the sample pre-enriched in broth for *Enterobacteriaceae* EE Broth Mossel (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Aerobic psychrotrophic microorganisms were determined as described by APHA 13.61:2015 standard methodology, by sowing on the surface in a Petri dish containing standard count agar (Merck, Hesse, Germany), with inverted incubation at 17 ± 1 °C for 16 h, followed by 72 h at 7 ± 1 °C for further counting [27].

2.9. Sensory Acceptance

The affective sensory acceptance test utilized a 9-point hedonic scale, ranging from 1 (Disliked Extremely) to 9 (Liked Extremely), to assess appearance, aroma, texture, flavor, and overall quality [28]. A total of 120 regular chicken and meat consumers were recruited from among the students and employees of the Faculty of Animal Science and Food Engineering (FZEA/USP). The sausages were cooked in an electric oven at 180 °C until they reached an internal temperature of 72 °C at the geometric center and then cut into 2 cm slices. The samples were presented monadically in completely randomized blocks, with each consumer evaluating all four treatments. The study was reviewed and approved by the local Research Ethics Committee of FZEA/USP under approval number 4.254.749, and informed consent was obtained from each participant before their involvement in the study.

2.10. Statistical Analysis

The physicochemical results were analyzed using analysis of variance (ANOVA), followed by Tukey's test at a 5% significance level. For the variables evaluated during storage, treatment and storage time were considered separate factors in the statistical analyses, and their main effects were evaluated independently. The treatment × storage time interaction was not included in the statistical model. Each of the three independently conducted experimental replicates was considered a separate block. For the sensory acceptance test based on the hedonic scale, treatments were considered fixed effects (independent variables), while consumers were considered random effects. Tukey's mean comparison test was also applied at a 5% significance level. Data analysis was conducted using Statistica™ software version 13.5.0.17 (TIBCO Software Inc., San Ramon, CA, USA).

3. Results and Discussion

The total phenolic compounds (TPC) are thought to play a protective role against tissue damage; their measurement in gallic acid equivalents is based on their acid stability and favorable response to the Folin-Ciocalteu reagent [29]. The TPC results for *Opuntia ficus-indica* extracts obtained by different extraction methodologies are presented in Table 1, which also shows yields (% *v*/*m*) based on the amount of fruit required to obtain the extract. Based on the aforementioned findings, the differences in TPC among prickly pear extracts may be attributed to the composition of the different fruit parts, seasonality, variety, variability in solvent polarity, and the extraction methods used to isolate polyphenolic substances.

Table 1. Yield values and determination of total phenolic compounds (TPC) of prickly pear extracts obtained by different extraction methodologies.

Extract	Yield (% *v*/*m*)	TPC (mg GAE/g Extract)
A	7.87	3.23 ± 0.57 [c]
B	0.54	18.96 ± 0.44 [a]
C	12.91	1.51 ± 0.12 [d]
D	17.06	1.16 ± 0.04 [d]
E	9.36	13.66 ± 0.06 [b]

Different lowercase letters in the same column (different extraction methodologies): values differ statistically from each other ($p < 0.05$). GAE: gallic acid equivalents. A: Pulp extracted with ethanol; B: Peels extracted with 80% ethanol; C: Pulp extracted with 80% ethanol; D: Peels extracted with ethanol-water solvent (40:60, *v*/*v*); E: Whole fruits extracted with 80% ethanol.

In this study, the preliminary tests showed that methodologies "B", applied to the peel (18.96 ± 0.44 mg GAE/g of extract), and "E", applied to the whole *O. ficus-indica* fruits (13.66 ± 0.06 mg GAE/g of extract), proved to be more effective in obtaining extracts richer in phenolic compounds. These results indicate a higher concentration of phenolic compounds than those obtained by other authors, such as Chaalal et al. [30], who analyzed the hydroethanolic extract (50% *v*/*v*) of prickly pear seeds and obtained a value of 2.30 mg GAE/g, and Ortega-Hernández et al. [31], who presented pulp and peel values for the red variety of 4.00 and 13.00 mg GAE/g, respectively. The extraction of phenolic compounds from pulp and peel with ethanol by Aruwa, Amoo & Kudanga [17] resulted in values similar to those of the present study: 14.39 and 17.59 mg GAE/g, respectively.

Since a higher polyphenol content can show a strong correlation with better antioxidant activity, playing a crucial role in scavenging free radicals, extracts B and E were selected for further testing to determine their antioxidant capacity.

The FRAP results (Table 2) were superior to those found for methanolic pulp extracts of red, orange, and yellow varieties, which were, respectively, 2.98, 3.47, and 2.06 μmol TE/g, and for extracts obtained from peel, with values of 11.47 (yellow), 19.54 (red), and 21.86 μmol TE/g (orange) [32]. Another method used to measure antioxidant activity was the ABTS radical-scavenging assay, with results expressed as the percentage of radical inhibition. The antioxidant activity measured by $ABTS^{+}$ (Table 2) was superior to that obtained by other authors for methanolic extracts of three fruit varieties (yellow, white, and red), whose values ranged from 4.20 to 5.30 μmol TE/g [33].

Table 2. Results of analyses for determining the antioxidant capacity of *Opuntia ficus-indica* extracts and the synthetic antioxidant sodium erythorbate.

Sample	Extract B	Extract E	Sodium Erythorbate
TPC (mg GAE/g sample)	18.96 ± 0.44 [b]	13.66 ± 0.06 [c]	633.39 ± 10.80 [a]
FRAP (μmol TE/g sample)	44.21 ± 0.50 [b]	37.42 ± 1.30 [c]	3700.90 ± 29.43 [a]
ABTS (μmol TE/g sample)	45.09 ± 1.82 [b]	45.01 ± 2.02 [b]	3663.68 ± 402.39 [a]

a–c Different lowercase letters in the same row (different samples): values differ statistically from each other ($p < 0.05$). GAE: gallic acid equivalents. TE: Trolox equivalents.

To determine the amount of extract concentration to be added to the fresh sausages to match the antioxidant activity of a synthetic additive, the same analyses were applied to sodium erythorbate, obtaining the results found in Table 2. Based on these findings, it was possible to estimate the amount of natural prickly pear extract needed to achieve the antioxidant activity of 500 ppm (0.05%) of the synthetic additive, a concentration usually applied in this class of product, with the data presented in Table 3.

Results in Table 2 show that sodium erythorbate exhibits significantly greater antioxidant activity than natural extracts. This is because sodium erythorbate is a synthetic antioxidant that is essentially a pure compound, whereas

the extracts derived from natural sources contain a small amount of antioxidant compounds per gram of material [34]. Nevertheless, natural antioxidants from plants are beneficial to health and food preservation, as they have the ability to neutralize reactive oxygen species (ROS), including free radicals and non-free radical species, which are associated with the aggravation of cell damage and aging processes, as well as causing oxidative deterioration of foodstuffs. All parts of the cactus plant are rich in biologically active components. In general, the most prominent polyphenols in the cactus fruit extracts are gallic acid, quercetin, syringic acid, and rutin [35].

Table 3. The amount of extract that should be incorporated into sausages to obtain equivalence to the antioxidant activity of 500 ppm (0.05%) of sodium erythorbate, according to the antioxidant capacity analyses.

Analysis	Extract B	Extract E
TPC	1.67 ^{b}	2.32 ^{a}
FRAP	4.19 ^{b}	4.94 ^{a}
ABTS	4.06 ^{a}	4.00 ^{a}
Average	3.31	3.75

a,b Different lowercase letters on the same row (different extracts): values differ statistically from each other ($p < 0.05$).

According to the results presented in Table 3, the concentration of Extract "B" (obtained from the peel of *Opuntia ficus-indica*) required to achieve an antioxidant activity of 500 ppm, equivalent to sodium erythorbate, is slightly lower than the concentration of Extract "E" (derived from the whole fruit). However, when considering the yield data for obtaining these extracts, as shown in Table 1, it becomes evident that the more suitable extract for incorporation is the one obtained from the whole fruit, since the yield from the peel alone is only 0.54%. Finally, the four treatments of fresh chicken sausage manufactured and analyzed in the next stage of the experiment were defined, namely:

- Control treatment (CON): no addition of natural or synthetic antioxidants;
- Treatment with synthetic antioxidant (ERY): formulated with the addition of 500 ppm sodium erythorbate;
- Treatment with the addition of natural extract (PPE1.88): addition of 1.88% prickly pear extract, which represents the amount necessary to reach 50% of the antioxidant activity of 500 ppm of sodium erythorbate;
- Treatment with the addition of natural extract (PPE3.75): addition of 3.75% prickly pear extract, concentration calculated to reach the antioxidant capacity of sodium erythorbate (500 ppm).

Table 4 presents the proximate composition, cooking losses (CL), and instrumental hardness values of the four fresh chicken sausage treatments. The use of natural prickly pear extract did not alter the proximate composition of any of the nutritional components under study compared to the other treatments ($p > 0.05$). The values found are in accordance with the Brazilian technical regulation of identity and quality of fresh sausages [36], which establishes a minimum protein value of 12% and maximum moisture and fat values of 70 and 30%, respectively.

The cooking process promotes the release of fluids that carry water and fat molecules, as well as nutrients, pigments, and other compounds responsible for the flavor and aroma of food. Measuring these losses during cooking is a way of estimating the capacity of the product to keep water and fat properly bound in the system after protein denaturation and aggregation [37]. According to results presented in Table 4, the incorporation of the natural extract did not affect the weight loss of sausages during cooking ($p > 0.05$), and no significant differences were observed in similar meat products, such as burgers formulated with or without the addition of açaí antioxidant compounds [38] and fresh sausages formulated with different plant extracts [37].

Table 4. Proximate composition, cooking losses (CL), and texture analysis (hardness) of fresh chicken sausages.

Parameter	CON	ERY	PPE1.88	PPE3.75
Proximate composition (g/100 g)				
Moisture	69.14 ± 0.42	70.10 ± 0.47	69.95 ± 0.24	69.26 ± 1.18
Protein	18.33 ± 0.73	18.48 ± 0.55	18.86 ± 0.64	18.81 ± 0.97
Lipids	9.48 ± 0.45	8.34 ± 0.37	8.31 ± 0.91	8.26 ± 0.63
Ashes	2.46 ± 0.05	2.50 ± 0.05	2.63 ± 0.06	2.68 ± 0.22
CL (%)	11.21 ± 1.50	11.44 ± 1.25	10.04 ± 2.18	9.75 ± 1.73
Hardness (N)	1.03 ± 0.01 ^{b}	1.08 ± 0.1 ^{ab}	1.23 ± 0.1 ^{a}	1.21 ± 0.1 ^{a}

a,b Different lowercase letters on the same row (different treatments): values differ statistically from each other ($p < 0.05$). Treatments: CON: prepared without antioxidant; ERY: prepared with 500 mg/kg sodium erythorbate; PPE1.88: prepared with 1.88% prickly pear extract; PPE3.75: prepared with 3.75% prickly pear extract.

On the other hand, formulations manufactured with the addition of antioxidants showed higher hardness values ($p < 0.05$) in the present study (Table 4). Similar behavior was observed by Sharma & Yadav [39], who found higher hardness values in chicken burger samples containing pomegranate peel natural extract (*Punica granatum*) compared to the control formulation. The increased rigidity observed may result from the solid components in the natural extract, as well as from the development of non-enzymatic browning reactions, such as caramelization and Maillard reactions, favored by the presence of fruit sugars. These reactions tend to occur more rapidly when samples are cooked at high temperatures [40,41]. Caramelization results in condensation reactions and the formation of a polymeric network that confers greater hardness to the material [42]. Furthermore, Maillard reactions alter the functional properties of proteins by forming conjugates through covalent bonds between their chains and reducing sugars, such as glucose and fructose present in prickly pear extract. These reactions also favor crosslinking between protein chains, which alters the texture of foods [43].

Regarding color parameters (Table 5), in general, storage time did not cause significant differences ($p > 0.05$) in any of the treatments for any of the analyzed parameters (L, a*, and b*). Color differences in the samples from the four fresh chicken sausage treatments at the beginning of the storage period are illustrated in Figure 1. Samples formulated with PPE showed a tendency to be darker, indicating that the presence of the extract affected the natural color of chicken sausages. The same behavior was reported in chicken burgers by Menegali et al. [44] by adding pink pepper extract, and by Selani et al. [45] with the addition of grape extracts.

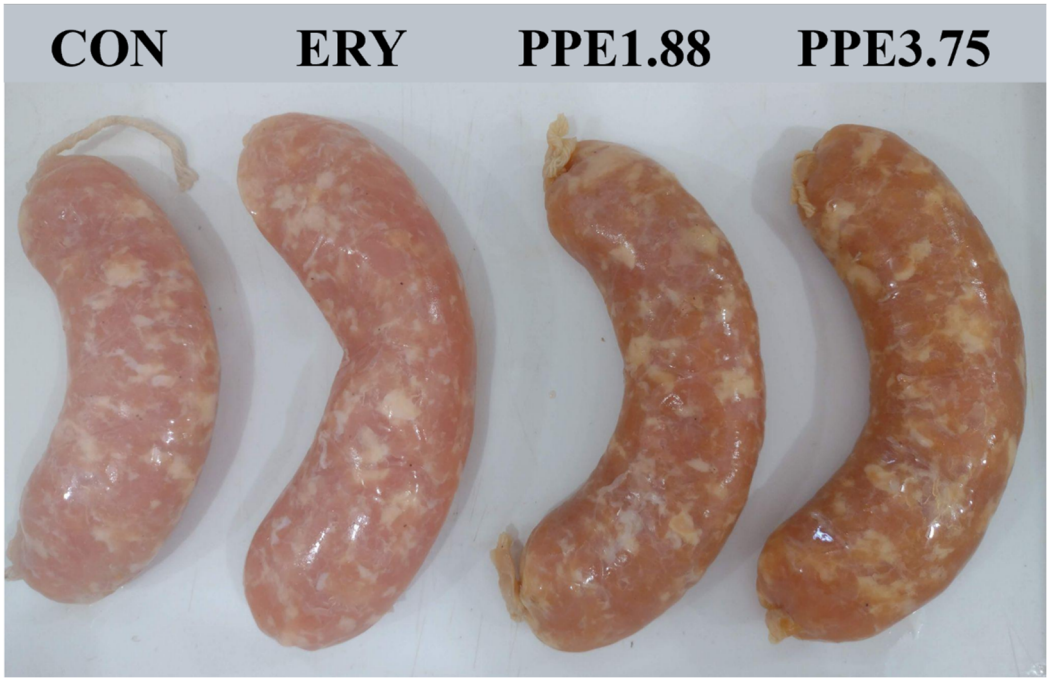

Figure 1. Samples of fresh chicken sausage at the beginning of refrigerated storage. Treatments: CON: prepared without antioxidant; ERY: prepared with 500 mg/kg erythorbate; PPE1.88: prepared with 1.88% prickly pear extract; PPE3.75: prepared with 3.75% prickly pear extract. Source: Adapted from the first author's Master's thesis (Gonçalves [46]).

The a* and b* parameters tended to be relatively higher as the natural extract concentration increased, a phenomenon even more pronounced for the b* parameter, showing that the extract obtained from the orange variety of *Opuntia ficus-indica* caused yellowing in fresh chicken sausage samples ($p < 0.05$). However, the total color variation calculated by ΔE* did not show significant differences between treatments ($p > 0.05$) or storage periods ($p > 0.05$). However, absolute values exceeded the perceptible limit considered "very clear" (with ΔE* greater than 3) starting from the 10th day of storage for the treatments CON, ERY, and PPE1.88. Meanwhile, the treatment with 3.75% added *O. ficus-indica* extract remained in the range classified as a "clear" total color difference ($1.5 < \Delta E^* < 3$) throughout the analyzed period [47].

The pH value of fresh sausages did not change either with differences in the formulations used ($p > 0.05$) or with the storage time ($p > 0.05$), remaining practically constant throughout the refrigerated storage period, as presented in Table 5. These results indicate that the addition of *Opuntia* extract had no influence on the acidity of meat products. Selani et al. [45] also did not observe a significant difference in the pH of chicken burger samples added with natural grape extracts compared to control samples, and Hossain et al. [48] observed no significant

difference in the pH values of chicken sausages added with ginger extract compared to the treatment without antioxidants during 15 days of storage.

The lipid oxidation assessment is a very important factor in food products because it is intrinsically related to sensory acceptance aspects, as its occurrence leads to the development of undesirable odors and flavors, in addition to affecting safety and nutritional value due to the formation of toxic compounds [49]. One of the ways to estimate lipid peroxidation in meat and meat products is through the spectrophotometric technique for measuring the malondialdehyde concentration (MDA), a secondary product from the hydrolysis of lipid superoxides that reacts with thiobarbituric acid (TBA), generating a complex with maximum absorbance at 532–538 nm, which can be quantified from the tetraethoxypropane calibration curve (TEP) [50].

Table 5. Color parameters and pH values of fresh chicken sausages during refrigerated storage.

Parameter	Storage Time (Days)	CON	ERY	PPE1.88	PPE3.75
L	0	31.97 ± 0.99 ^{a}	30.18 ± 0.86 ^{ab}	29.10 ± 2.39 ^{ab}	27.26 ± 1.40 ^{b}
	5	33.58 ± 2.17 ^{a}	32.48 ± 1.78 ^{ab}	28.92 ± 1.53 ^{ab}	28.67 ± 1.83 ^{b}
	10	35.09 ± 0.25 ^{a}	34.00 ± 1.43 ^{ab}	31.43 ± 0.93 ^{bc}	29.59 ± 1.32 ^{c}
	15	36.78 ± 2.33 ^{a}	34.41 ± 1.67 ^{ab}	33.85 ± 2.55 ^{ab}	29.59 ± 1.55 ^{b}
a*	0	−0.21 ± 0.10 ^{c}	0.34 ± 0.11 ^{c}	1.14 ± 0.53 ^{bB}	2.02 ± 0.12 ^{c}
	5	−0.01 ± 0.35 ^{b}	0.16 ± 0.22 ^{b}	1.66 ± 0.10 ^{aA}	2.16 ± 0.27 ^{a}
	10	−0.08 ± 0.31 ^{b}	−0.19 ± 0.61 ^{b}	1.11 ± 0.28 ^{abB}	2.08 ± 0.89 ^{a}
	15	−0.20 ± 0.44 ^{b}	0.053 ± 0.46 ^{b}	1.27 ± 0.12 ^{aB}	2.08 ± 0.36 ^{a}
b*	0	6.74 ± 1.16 ^{c}	6.91 ± 0.66 ^{c}	10.49 ± 2.01 ^{b}	15.28 ± 1.13 ^{a}
	5	7.17 ± 0.75 ^{c}	7.12 ± 0.90 ^{c}	12.12 ± 0.66 ^{b}	15.87 ± 1.13 ^{a}
	10	7.18 ± 0.97 ^{c}	6.52 ± 1.04 ^{c}	11.75 ± 1.20 ^{b}	15.31 ± 0.99 ^{a}
	15	6.55 ± 1.04 ^{c}	7.42 ± 0.66 ^{c}	11.54 ± 1.25 ^{b}	15.99 ± 0.57 ^{a}
ΔE*	0–5	1.82 ± 1.18	2.34 ± 0.95	1.96 ± 1.30	1.25 ± 0.54
	0–10	3.21 ± 0.78	3.98 ± 1.53	3.00 ± 1.75	2.44 ± 1.29
	0–15	4.84 ± 3.19	4.31 ± 2.35	5.03 ± 4.11	2.34 ± 2.70
pH	0	5.93 ± 0.09	5.94 ± 0.09	5.90 ± 0.04	5.87 ± 0.05
	5	5.92 ± 0.04	5.96 ± 0.04	5.92 ± 0.04	5.88 ± 0.02
	10	5.97 ± 0.12	5.93 ± 0.13	5.91 ± 0.08	5.90 ± 0.05
	15	5.87 ± 0.14	5.90 ± 0.10	5.86 ± 0.77	5.90 ± 0.01

a–c Different lowercase letters on the same row (different treatments): values differ statistically from each other ($p < 0.05$). A,B Different capital letters in the same column (different storage times): values differ statistically from each other ($p < 0.05$). Treatments: CON: prepared without antioxidant; ERY: prepared with 500 mg/kg erythorbate; PPE1.88: prepared with 1.88% prickly pear extract; PPE3.75: prepared with 3.75% prickly pear extract.

Despite being a simple and fast method, there are several criticisms regarding the interpretation of results, which can be considered complex, since several other substances in addition to MDA can react with TBA, interfering with the overestimation of results [51]. Several studies have pointed out that components commonly found in food matrices can contribute to the formation of TBARS, that is, substances that react with TBA but are not exactly related to lipid peroxidation, such as aldehydes, products generated by browning reactions, protein degradation, and sugars [52,53]. In addition, heating carbohydrates around 100 °C causes the formation of a colored complex in the presence of TBA, with greater sensitivity for fructose and sucrose, resulting in erroneously high values due to the overlapping of absorption spectra in the analyzed wavelength range [52,54].

This phenomenon may explain the significantly higher TBARS values ($p < 0.05$) found for treatments formulated with the addition of prickly pear extract (PPE), as shown in Figure 2, which gradually increased as the extract concentration increased, compared to the control treatment and the treatment added with 500 ppm of synthetic antioxidant. Notably, this trend was observed even on day 0, with treatments containing higher concentrations of PPE already showing proportionally higher TBARS values, indicating that the effect was present from the beginning of storage. This behavior may be related to the fact that the matrix used to obtain the extract is a fruit naturally rich in sugars, mainly fructose and glucose, according to the scientific literature [55,56].

The effectiveness of antioxidant activity may increase with higher concentrations; however, using excessively high concentrations can lead to undesirable effects. Consequently, the improvement in oxidative stability can be limited to a maximum concentration of the antioxidant substance [57]. Some authors reported that antioxidant substances may behave as pro-oxidants (chemical substances that induce oxidative stress), depending on the presence of metal ions, redox potential, and the substance concentration in the food matrix, which can exert

an antioxidant effect at small doses or a pro-oxidant effect when used at high doses, such as Vitamin C [58,59]. Phenolic compounds present in the *Opuntia ficus-indica* extract are also substances capable of exhibiting pro-oxidant effects, such as in the presence of metals (e.g., iron), which can cause oxidative stress by catalyzing the formation of phenolic radicals that damage lipid molecules, in addition to their antioxidant vs. pro-oxidant effect being directly related to the concentration used [60].

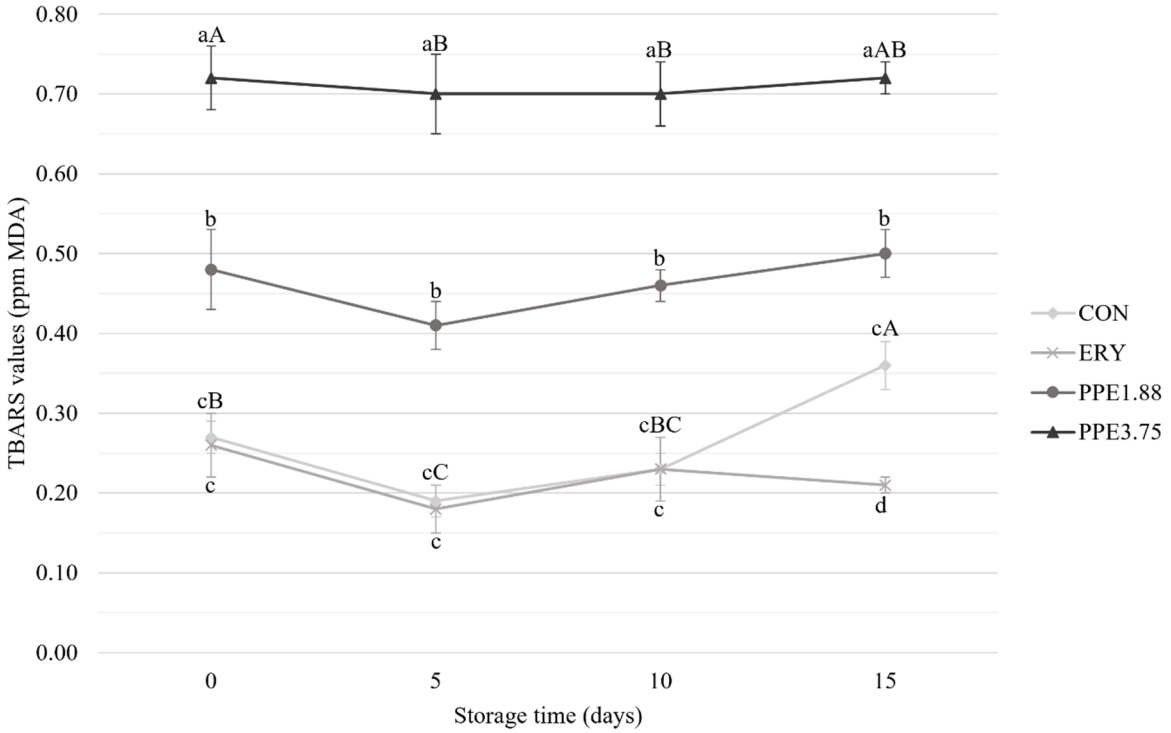

Figure 2. Evolution of TBARS values of fresh chicken sausages during refrigerated storage. A–C Different capital letters (same treatment in different storage times): values differ statistically from each other ($p < 0.05$). a–d Different lowercase letters (different treatments in the same storage time): values differ statistically from each other ($p < 0.05$). Treatments: CON: prepared without antioxidant; ERY: prepared with 500 mg/kg erythorbate; PPE1.88: prepared with 1.88% prickly pear extract; PPE3.75: prepared with 3.75% prickly pear extract.

Overall, the treatments with antioxidants, both natural and synthetic, maintained stable lipid oxidation values during shelf life, whereas the CON treatment exhibited the most significant difference between the beginning and end of the storage period. Thus, the two approaches mentioned above may have been responsible for the high TBARS rates found for PPE3.75 and PPE1.88 treatments compared to the other treatments. The first is the presence of sugars in prickly pear extract, components that can react with TBA, interfering with the overestimation of TBARS results, and the second contributor may have been the large amount of extract applied to sausages, leading to an opposite effect to what was expected and contributing to pro-oxidant activities.

The same behavior was documented by Sáyago-Ayerdi, Brenes & Goñi [61], since the use of 1.0% antioxidant grape dietary fiber resulted in lower lipid oxidation rates over 13 days of refrigerated storage of raw chicken burgers when compared to the treatment that received 2.0% of this natural antioxidant. Similar results were presented by Ryu, Shim & Shin [62]: 0.5% grape pomace extract was more effective for the oxidative stability of lipids in pork sausages than the addition of 1.0% extract.

The protein oxidation mechanisms in food systems are still difficult to explain, since they comprise a great complexity of reactions when compared to the lipid peroxidation process, leading to the generation of an even greater variety of products derived from meat degradation, which affects both hydrophilic and lipophilic components of muscle tissue [63]. The oxidation of proteins and lipids represents a significant portion of the deterioration of meat and meat products. Therefore, its control is essential to avoid reduction in quality, consumer rejection, and consequent economic losses.

Protein oxidation can be induced by the development of direct reactions with reactive oxygen species or through indirect reactions involving secondary by-products from oxidative processes, for example, from lipid oxidation, causing irreversible changes in some physical and chemical properties of protein chains and amino

acids, such as structure, conformation, solubility, and susceptibility to proteolysis, which can negatively impact the quality and functionality of the meat matrix, particularly when producing meat products, such as properties like water-holding capacity, softness, and gelling ability, while also causing nutritional losses through the degradation of essential nutrients and the formation of toxic compounds [64,65].

The formation of carbonyl groups is one of the main modifications generated by the direct or indirect oxidation of proteins, and these derivatives can react with amine groups, leading to the formation of crosslinks between protein chains [66]. The method of derivatization of carbonyl compounds with dinitrophenylhydrazine (DNPH) is one of the most adequate methods to quantify levels of protein oxidation in foods [24,67]. This was the principle of the method used in the present study; the results are presented in Figure 3.

The results show the effectiveness of the natural antioxidant used in fresh chicken sausages in reducing the propagation of protein oxidation reactions, since the carbonyl concentration of both treatments formulated with prickly pear extract was at the same level ($p > 0.05$) as that formulated with the synthetic antioxidant sodium erythorbate, in addition to the fact that there was no increase in values between the beginning and end of the storage time ($p > 0.05$), while the control formulation (without added antioxidants) showed a significant increase in the carbonyl concentration at the end of the shelf life.

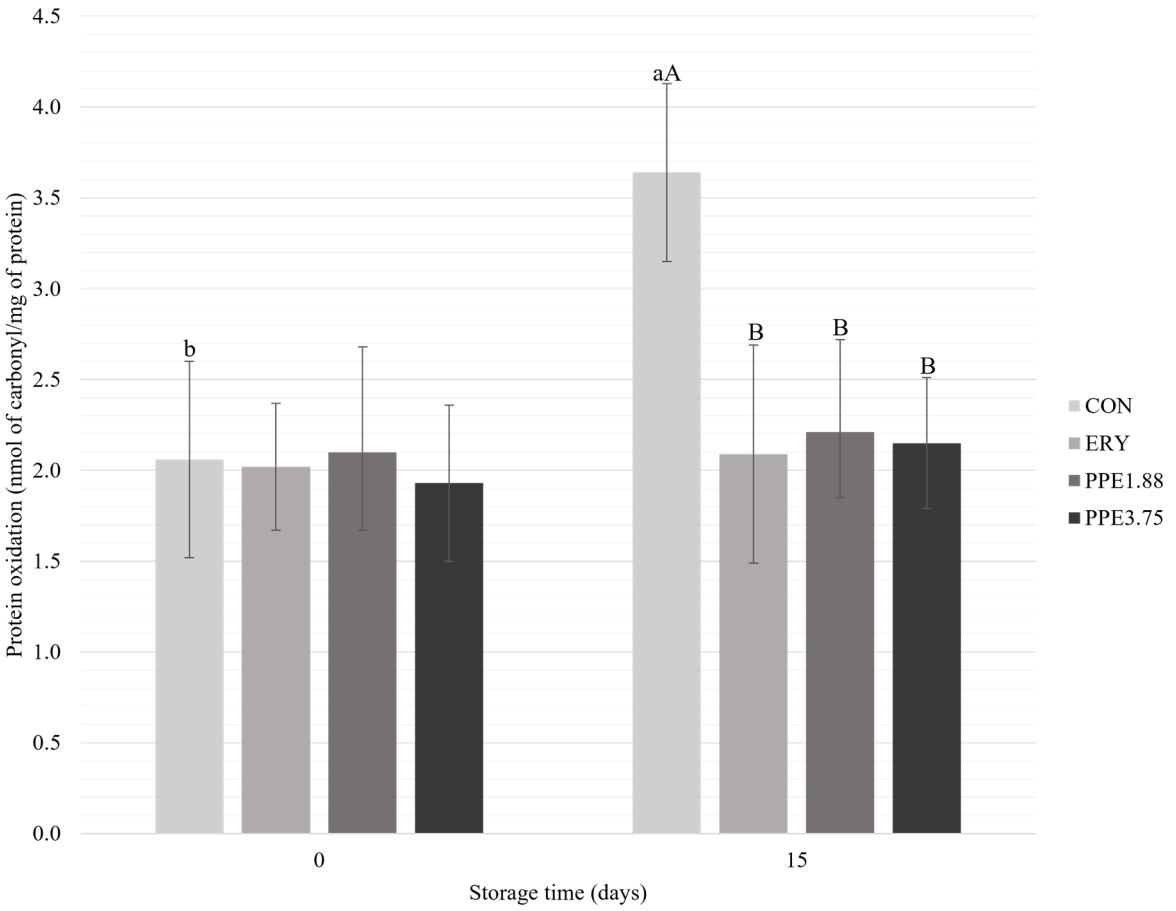

Figure 3. Evolution of protein oxidation indices of fresh chicken sausages at the beginning and end of refrigerated storage. a,b Different lowercase letters (same treatment in different storage times): values differ statistically from each other ($p < 0.05$). A,B Different capital letters (different treatments in the same storage time): values differ statistically from each other ($p < 0.05$). Treatments: CON: prepared without antioxidant; ERY: prepared with 500 mg/kg erythorbate; PPE1.88: prepared with 1.88% prickly pear extract; PPE3.75: prepared with 3.75% prickly pear extract.

It is claimed that protein oxidation is generally related to other processes catalyzed by the presence of oxygen in food, including lipid oxidation. This indicates that the formation of intermediate products from the lipid oxidation process, such as alkyl peroxyl radicals, alkoxy radicals, and hydroperoxides, can indirectly induce the oxidative denaturation of proteins; that is, the oxidation of lipids and proteins occurs simultaneously, and each process can intensify the development of the other process [64,65,68]. Thus, when observing the antioxidant effect of the natural extract on proteins, it could be assumed that this fact corroborates the Figure 2 findings, which suggest that the lipid oxidation values may have been, in fact, an overestimation of the quantification of

thiobarbituric acid reactive substances, since the development of both mechanisms can be interconnected [63]. Therefore, the use of prickly pear extract can be emphasized as a good alternative to maintain the quality and stability of fresh chicken sausages, exerting a protective activity against oxidation and contributing to extending their shelf-life.

Figure 4 shows the results of the sensory analysis of fresh chicken sausages conducted with 120 consumers. The different formulations did not differ significantly in appearance ($p > 0.05$). Treatments formulated with 3.75% and 1.88% prickly pear extract achieved relatively lower aroma, texture, flavor, and overall quality scores. Despite this, all averages were close to or higher than a score of 7 on a scale ranging from 1 (Extremely disliked) to 9 (Extremely liked), indicating that the use of the natural extract can be considered sensorially well accepted in all attributes.

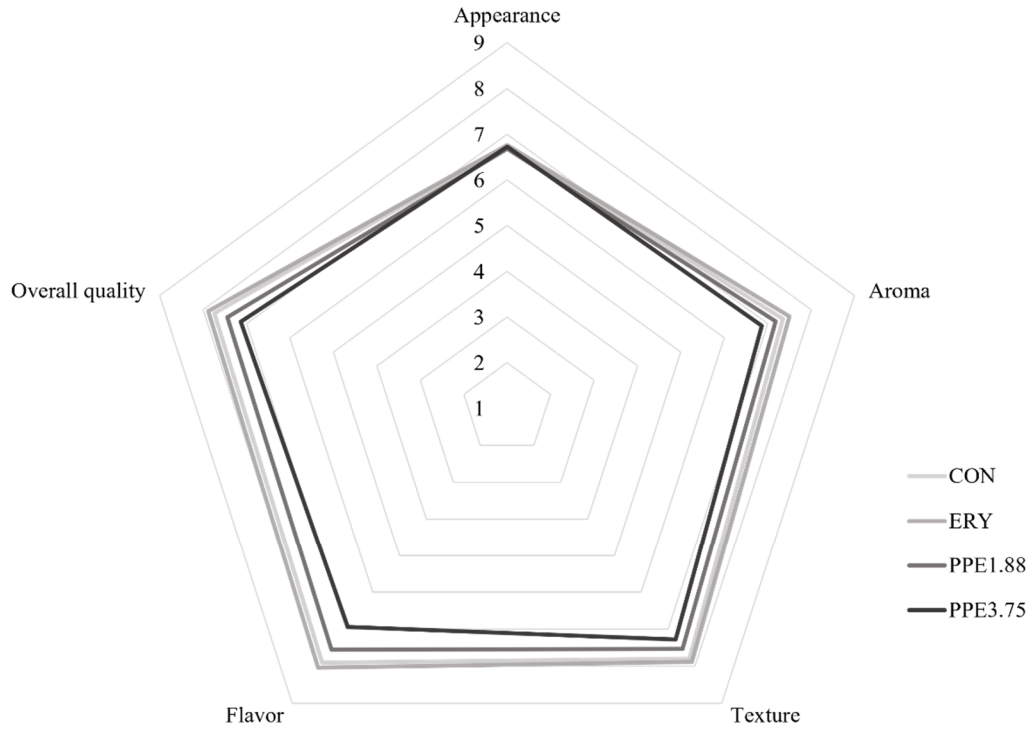

Figure 4. Results of the sensory acceptance test with 120 consumers. n.s.: no significant difference ($p > 0.05$). A–C Different capital letters: values differ statistically from each other ($p < 0.05$). Treatments: CON: prepared without antioxidant; ERY: prepared with 500 mg/kg erythorbate; PPE1.88: prepared with 1.88% prickly pear extract; PPE3.75: prepared with 3.75% prickly pear extract.

A study by Romero et al. [69] found that burger samples made with freeze-dried pulp from the red variety of *Opuntia ficus-indica* received similar overall acceptance to the control treatment. Additionally, these samples demonstrated significantly improved oxidative stability during their shelf life compared to those without added antioxidants. This improvement was linked to the beneficial effects of pulp components, such as phenolic compounds. Regarding salamis formulated with red *Opuntia* extract to replace sodium nitrite and cochineal carmine dye, acceptability increased with the amount of extract added. Specifically, samples with 2.5% extract received significantly higher acceptance than the control treatment. This heightened acceptability may be attributed to enhancements in the physicochemical and microbiological stability, which in turn improved their sensory quality characteristics [70].

Research has shown that using lower concentrations of aromatic herb essential oils can significantly affect the sensory acceptance of meat products. This poses a challenge in finding a dosage of these natural agents that effectively combats oxidative and microbiological damage while also preserving satisfactory organoleptic attributes. For example, studies indicate that the acceptable sensory levels for thyme [71] and oregano [72] essential oils are 0.3% and 0.2%, respectively. However, these concentrations may not provide sufficient antimicrobial and antioxidant properties to extend the shelf life of meat products when used as alternatives to synthetic additives. As a result, fruits present an attractive option for natural extracts, as their milder flavor profiles

allow for the use of larger quantities without causing significant changes in taste compared to aromatic herb essential oils [73].

According to Brazilian legislation [26], the microbiological limit established for *Escherichia coli* in fresh products based on chicken meat is 10^3 CFU/g, in addition to the absence of *Salmonella* in 25 g of sample. All treatments complied (Figure 5) with the legislation during shelf life and, together with total coliform count results, are indicative of good sanitary conditions adopted during processing and maintenance of adequate storage temperature.

For the count of aerobic psychrotrophic microorganisms (Figure 5III), the limit established by the International Commission on Microbiological Specifications for Foods (ICMSF) is 10^7 CFU/g for this class of products to be considered suitable for consumption without major undesirable changes and at an acceptable deterioration level [74]. In this case, all fresh chicken sausage treatments presented counts below this limit during the fifteen days of refrigerated storage, but the addition of PPE was able to reduce the count of aerobic psychrotrophic microorganisms compared to the other treatments at all points of analysis, reaching, at the end of storage, a reduction higher than 1 logarithmic cycle for treatment PPE3.75 when compared to CON and to ERY, demonstrating an advantage of prickly pear extract regarding the control of microbial proliferation.

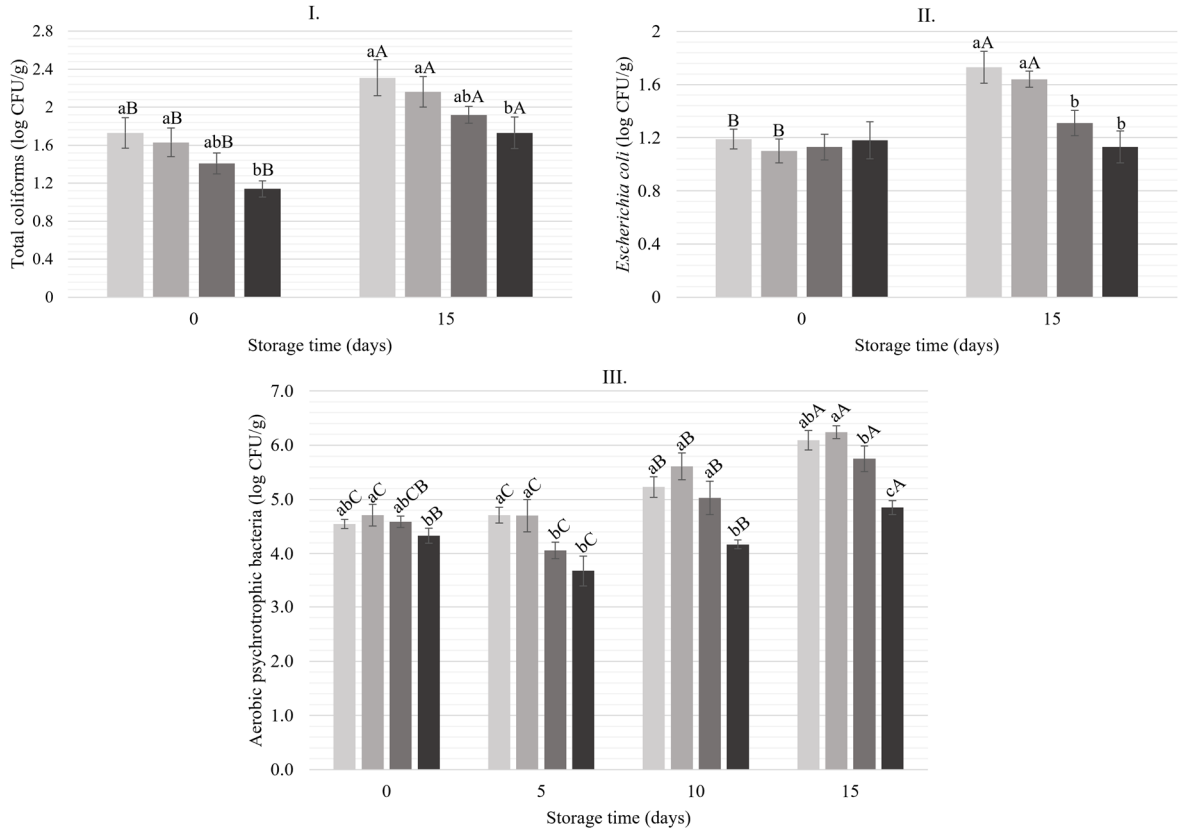

Figure 5. Microbiological stability results during refrigerated storage. Counts for (**I**) total coliforms, (**II**) *Escherichia coli*, and (**III**) aerobic psychrotrophic bacteria. A–C Different capital letters (same treatment at different storage times): values differ statistically from each other ($p < 0.05$). a–c Different lowercase letters (different treatments in the same storage time): values differ statistically from each other ($p < 0.05$). Treatments: CON: prepared without antioxidant; ERY: prepared with 500 mg/kg erythorbate; PPE1.88: prepared with 1.88% prickly pear extract; PPE3.75: prepared with 3.75% prickly pear extract. CFU/g = colony-forming units per gram.

Several other authors emphasized the antibacterial activity of extracts obtained from *Opuntia*, for example, against pathogenic microorganisms *Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus, Clostridium perfringens, Pseudomonas* sp., and *Salmonella*, demonstrating efficiency even when applied as a strategy for the conservation of meat products such as salami and burgers [7,70,75,76].

4. Conclusions

The study evaluated various extraction methods for obtaining *Opuntia ficus-indica* extracts with potential applications as antioxidant and antimicrobial agents in meat products. The incorporation of the natural extract into

fresh chicken sausages did not affect cooking losses, pH, or proximate composition, but resulted in slightly increased hardness, reduced lightness, and increased yellowness. All treatments were well-received in terms of sensory evaluation, achieving high acceptance scores despite the changes in color and the incorporation of relatively high concentrations of PPE. Regarding the oxidative stability of lipids, the samples containing PPE exhibited higher TBARS values compared to the other treatments. However, this increase may be attributed to factors leading to an overestimation of thiobarbituric acid-reactive substances, which are not necessarily associated with the progression of lipid oxidation, as suggested by the relatively higher TBARS values observed in treatments with greater PPE concentrations even at day 0. The results of protein oxidation reinforce this possibility, since treatments formulated with antioxidants, both natural and synthetic, showed lower oxidation levels than the control treatment at the end of the storage period. The extract also showed a beneficial effect by significantly reducing the counts of aerobic psychrotrophic microorganisms, contributing to the microbiological preservation of the meat products throughout their shelf life.

Therefore, the use of natural prickly pear extract represents a promising strategy for developing cleaner-label fresh chicken sausages without substantially compromising consumer acceptance. From an industrial perspective, this approach may contribute to the development of meat products with reduced reliance on synthetic additives. Nevertheless, some limitations should be considered, particularly regarding the assessment of lipid oxidation, which requires further investigation using complementary analytical methods to avoid potential interferences in TBARS determination. Additionally, further studies are needed to optimize the extract concentration and evaluate its effectiveness under different processing and storage conditions. These investigations will be important to establish its applicability and potential for broader use in the meat industry.

Author Contributions

L.A.G.: conceptualization, investigation, funding acquisition, writing—original draft, methodology, validation, visualization, software, formal analysis, data curation; L.R.M.G.: investigation, methodology, formal analysis, writing—review & editing, visualization, validation; J.M.L.: investigation, writing—review & editing, visualization, validation, project administration, supervision; M.A.T.: project administration, supervision, visualization, validation, writing—review & editing, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was approved by the local Research Ethics Committee of FZEA/USP under approval number 4.254.749 (Approved date: 2 September 2020).

Informed Consent Statement

Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Data are available upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest. Given the role as Editor-in-Chief, José Manuel Lorenzo had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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