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Use of natural preservatives to increase the shelf life of seasoned pork loin (dry and wet)

Uso de conservantes naturais para aumento na vida útil de lombo suíno temperado (a seco e a úmido)

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The consumption of synthetic additives is associated with health risks to consumers, and the meat industry seeks natural substitutes to extend the shelf life of products. The study aimed to evaluate the viability of natural preservatives and stabilizers in seasoned chilled pork products, on yield, physicochemical, microbiological, and sensory quality. Eight formulations (F) of lean pork cut (loin) were prepared, four prepared with dry curing (D) and four with 15% wet curing (W). Formulations F1D, F1W, F2D, and F2W contained 1.5% of organic acids, their salts, and peptides. F2D and F2W, in addition, acerola (0.3%) and beetroot (0.20%) extracts, and F2W added yeast and citrus (0.7%) extracts. Controls C1S and C1U contained synthetic preservatives, and C2S and C2U, no preservatives. The following were evaluated: centesimal composition, pH, color, water activity (Aw), oxidative stability by the percentage of metmyoglobin and Thiobarbituric Acid Reactive Substances (TBARS) index, microbiological stability, weight loss after cooking, and sensory acceptance. The formulations complied with the legislation for centesimal composition, with higher moisture content for samples with wet curing. The natural ingredients would provide Aw and pH close to those of synthetic additives. F2D and F2W stood out in color. The samples presented microbiological and oxidative stability, and the metmyoglobin formation rates were lower for F1D and F1W. The mixture of yeast and citrus extracts provided lower cooking loss. Sensorially, the natural ingredients proved to be promising alternatives. Therefore, the natural additives provided positive effects on yield, oxidative stability, and product acceptance.
Keywords: natural antioxidants, natural antimicrobials, clean label.

O consumo de aditivos sintéticos é associado a riscos à saúde dos consumidores e a indústria cárnea busca substitutos naturais para estender a vida útil dos produtos. O estudo objetivou avaliar a viabilidade de conservantes e estabilizantes naturais em produtos cárneos suínos resfriados temperados, sobre o rendimento, qualidade físico-química, microbiológica e sensorial. Foram elaboradas oito formulações (F) de corte suíno magro (lombo), quatro preparados com cura seca (S) e quatro com 15% de cura úmida (U). As formulações F1S, F1U, F2S, F2U continham 1,5% de ácidos orgânicos seus sais e peptídeos, F2S e F2U com adição de extratos de acerola (0,3%) e beterraba (0,20%), F2U com adição de extratos de levedura e cítrico (0,7%). Controles C1S e C1U continham conservantes sintéticos, e C2S e C2U, nenhum conservante. Foram avaliadas composição centesimal, pH, cor, atividade de água (Aw), estabilidade oxidativa pelo percentual de metamioglobina e índice de TBARS, estabilidade microbiológica, perda de peso após cozimento e aceitação sensorial. As formulações atenderam a legislação para composição centesimal, com teor de umidade superior para amostras com cura úmida. Os ingredientes naturais proporcionariam Aw e pH próximos aos aditivos sintéticos. F2S e F2U se destacaram na cor. As amostras apresentaram estabilidade microbiológica e oxidativa e os índices de formação de metamioglobina foram inferiores para F1S e F1U. A mistura dos extratos de levedura e cítrico proporcionou menor perda de cozimento. Sensorialmente, os ingredientes naturais se revelaram alternativas promissoras. Portanto, os aditivos naturais propiciaram efeitos positivos sobre rendimento, estabilidade oxidativa e a aceitação dos produtos.
Palavras-chave: antioxidantes naturais, antimicrobianos naturais, clean label.

1. INTRODUCTION

Pork is widely consumed in Brazil and worldwide, standing out for its nutritional value and accessibility. However, it is a highly perishable food, with rapid deterioration caused by physicochemical and microbiological reactions, such as lipid oxidation and contamination by microorganisms [1, 2]. To extend its shelf life, the meat industry traditionally uses synthetic preservatives, such as nitrates, nitrites, phosphates, and antioxidants. However, the continued use of these additives has been associated with potential health risks, including toxic and carcinogenic effects [3-5].

The growing consumer demand for healthier, more natural, and clean-label foods has driven the search for alternatives to synthetic additives. In response, agribusinesses have been investing in the development of seasoned and chilled pork cuts with natural ingredients, such as organic acids and their salts, recognized for their antimicrobial and acidity-regulating action [6, 7]. In addition, ingredients such as yeast extract contribute to water retention and oxidative stability [8], form strong gels, improving the texture of meat products [9]. Another alternative, as a natural ingredient that has shown interest and grown in recent years, is acerola extract, due to the presence of bioactive substances with antioxidant, anti-inflammatory, and antimicrobial effects [10].

Despite the benefits in conservation provided using these natural compounds, synthetic chemical preservatives also give a characteristic color to products, a color that is not achieved with the application of natural compounds, and maintaining attractive colors is also important for food quality, as it affects consumer acceptance and for this reason, the application of natural pigments can be an alternative to improve the visual appearance of products. Among the pigments, with aesthetic characteristics like those expected in pork meat products, betalain stands out, a nitrogenous pigment that is highly soluble in water and abundant in beets [11]. The additive industry already offers ingredients based on concentrated powdered beet extract, soluble in water, which, in addition to betalain, are standardized with at least 30,000 ppm of natural nitrite [12], being an alternative cure for the development of more natural products.

Although the individual benefits of these compounds are well documented, integrated studies evaluating the combined effect of these ingredients in seasoned pork products are still scarce. Therefore, it is essential to investigate their practical applicability, aiming to develop safer products with longer shelf life and sensory acceptance, increasing the competitiveness of natural products in the meat market. The present study aims to evaluate the feasibility of applying natural curing ingredients, preservatives, stabilizers, and antioxidants in dry and wet seasoned chilled pork products, to extend shelf life and maintain microbiological, physicochemical, and sensory quality characteristics like conventional products.

2. MATERIAL AND METHODS

2.1 Material

The raw meat material, chilled boneless pork loin (*longissimus dorsi*), was supplied by Industries of meat products Pepinão Ltda (Céu Azul, Paraná, Brazil). The natural ingredients used as preservatives were organic acids and their salts and peptides derived from natural fermentation (SAFE PLATE 621), natural curing powdered beetroot extract (SAFE PLATE 300), natural antioxidant powdered acerola extract (SAFE PLATE 34) and the stabilizer yeast extract and citrus extract (PHA 700, all from the brand Wenda Ingredients (São Paulo, Brazil), supplied by BClarking Ingredients Technology (São Paulo, Brazil). The other ingredients for product development were supplied by Conditec Indústria de Aditivos (Medianeira, Paraná, Brazil). The reagents used in the analyses were of analytical grade.

2.2 Processing of seasoned pork loin product

Eight seasoned pork loin formulations were prepared, divided equally between dry curing and wet curing methods (with 15% brine injection). Four formulations were made for each curing type,

two with natural preservatives in different combinations, and two controls (one with conventional synthetic additives and the other without preservatives) (Table 1). The formulations were defined based on pre-tests and prepared under standardized conditions of good manufacturing practices.

Whole chilled boneless loin pieces from the same production batch were separated by formulation and processed separately. For the dry curing process, a mix of ingredients (Table 1) was prepared and then mixed with the cuts by hand, rubbing the mix into the pieces. In the wet process, brines (Table 1) were prepared and injected into the pieces with the aid of a syringe at a proportion of 15% of the final product. Afterwards, the formulations were tumbled for 30 min (Tambler Dorit, VV-20, Belgium). Subsequently, the 8 formulations were vacuum-packed and stored under refrigeration in a BOD incubator at 7 ± 1 °C for physicochemical, instrumental, microbiological, and sensory evaluations. In addition to this temperature, samples were also stored at a temperature of 2 ± 1 °C to evaluate microbiological stability during shelf life.

Tabela 1: Formulations (F) for pork loin seasoned by dry cure (D) and wet cure (W) (15% injection) with natural with natural preservatives (F1D and F1W - Organic acids, their salts and peptides, and F2D - Organic acids, their salts and peptides, Acerola, and Beetroot extracts, and F2W - Organic acids, their salts and peptides, Acerola, Beetroot, and yeast extracts), synthetic preservatives (C1D and C1W)) and preservative-free (C2D and C2W).

Ingredients (%)	Dry cure				Wet cure			
	F1D	F2D	C1D	C2D	F1W	F2W	C1W	C2W
Pork loin	96.3	95.8	97.3	97.8	85.0	85.0	85.0	85.0
Salt	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60
Seasoning (parsley, rosemary, oregano)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Dehydrated garlic	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
White pepper	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Organic acids, their salts, and peptides	1.50	1.50	0.00	0.00	1.50	1.50	0.00	0.00
Acerola extract	0.00	0.20	0.00	0.00	0.00	0.20	0.00	0.00
Beetroot extract powder	0.00	0.30	0.00	0.00	0.00	0.30	0.00	0.00
Curing salt	0.00	0.00	0.25	0.00	0.00	0.00	0.25	0.00
Sodium erythorbate	0.00	0.00	0.25	0.00	0.00	0.00	0.25	0.00
Water	-	-	-	-	12.3	10.3	11.1	10.1
Polyphosphates	-	-	-	-	0.00	0.00	0.50	0.00
Yeast extract and citrus extract	-	-	-	-	0.70	0.70	0.00	0.00

2.3 Centesimal composition and physicochemical analyses

The analyses for centesimal composition were performed according to Adolfo Lutz Institute (2008) [13]. Total lipids analyze was performed by the direct extraction method in Soxhlet (method 032/IV), the moisture content by drying in an oven at 105 °C until constant weight (method 012/IV), proteins were determined by the Kjeldahl method with multiplication of the total nitrogen by factor 6.25 (method 037/IV), and ash by incineration in a muffle furnace at 550 °C (method 018/IV). All samples were evaluated in triplicate.

The cooking weight loss (CWL) of the standardized 400 g samples (in triplicate) was determined by cooking in an industrial oven at a temperature of 180 °C, to an internal temperature of 74 °C. The percentage weight loss that occurred in the process was calculated by the difference in the weight of the samples before and after cooking [14].

The physicochemical properties of color, pH, and water activity (Aw) were determined in raw samples after preparation (time 0) and during shelf life at 15, 30, and 45 days, in triplicate. The instrumental measurement of color was obtained by a colorimeter (Konica Minolta, CR 400, Osaka, Japan) with D65 illuminant and a viewing angle of 10°. The data were expressed according to the CIELAB system, evaluating the values of L* (luminosity), a* (red-green component), b* (yellow-blue component), C* defined as saturation, and hº, which represents the hue [15]. The pH determination was performed by a contact pH meter (HI 99163, Hanna Instruments Brasil, Barueri, SP, Brazil), inserting the electrode inside the loin pieces until the reading stabilized. And Aw was

determined using a water activity meter (4TE, Aqualab®, São Paulo, Brazil), at a temperature of 25 °C.

2.4 Oxidative stability

Oxidative stability of the samples was evaluated in triplicate by analyzing the TBARS (Thiobarbituric Acid Reactive Substances) index and percentage of metmyoglobin formation during the storage time at 7°C (0, 15, 30, and 45 days). The TBARS index was performed according to the methodology of Tarladgis et al. (1964) [16] and modified by Honda et al. (1988) [17]. After distillation with thiobarbituric acid 0.02 mol L^{-1}, the samples were heated in a water bath at 85 °C for 35 min, followed by cooling and reading in a UV-Vis spectrophotometer (Lambda XLS, PerkinElmer, Beaconsfield, United Kingdom) at 530 nm. The calibration curve was performed using a 1,1,3,3-tetraethoxypropane (TEP) solution at a concentration of 0.004 to 0.4 mol L^{-1} ($R^2 = 0.9994$).

The percentage of metmyoglobin formation was estimated according to Krzywicki (1982) [18] with adaptations. The samples (10 g) were solubilized in 50 mL of phosphate buffer (40 mMol L^{-1}) at pH 6.8. The mixture was homogenized using an Ultra Turrax (IKA, T 18, Staulfen, Germany) at 6500 RPM for 30 min in an ultra-thermostatic bath (7lab, SSD-10L, Rio de Janeiro, Brazil) at 4 °C. Subsequently, the homogenate was centrifuged in a refrigerated centrifuge (Cientec, CT 5000R, Belo Horizonte, Brazil) at 5000 g for 30 min at 4 °C. The supernatant was filtered with Whatman filter paper No. 1, and the absorbance was read in a UV-Vis spectrophotometer (Lambda XLS, PerkinElmer, Beaconsfield, United Kingdom) at 525, 572, and 700 nm.

2.5 Microbiological stability

The microbiological quality of the samples was evaluated after processing (time 0) and during storage at 7 °C (15, 30, and 45 days) or while there was no deterioration, stored at temperatures of 2 °C and 7 °C. The samples were evaluated in triplicate for the presence of *Salmonella spp*. in 25 g and mesophilic aerobes/g, as required by Normative Instruction No. 161 [19] for vacuum-packed chilled seasoned pork, and thermotolerant coliforms. The procedures adopted were in accordance with the Bacteriological Analytical Manual [20].

2.6 Sensory acceptance

Sensory analysis after sample preparation was performed with approval from the Human Research Ethics Committee of UTFPR – Campus Medianeira (CAAE No. 67194923.0.0000.0165) and after microbiological analyses that attested to the safety of the products. The samples were evaluated by the hedonic scale test using a 9-point scale (1 = I disliked it very much and 9 = I liked it very much) to analyze overall acceptance and the attributes, color, appearance, aroma, flavor and texture, and by the attitude scale test to evaluate purchase intention using a 5-point scale (1 = I would definitely not buy it and 5 = I would definitely buy it), by 120 untrained tasters in two sessions with the same tasters, using a complete block design. The sensory tests were conducted in individual booths with white fluorescent lamps, 7 days after sample processing. The samples were baked in an oven until an internal temperature of 74 °C before being served, cut into standardized slices of approximately 30 g, and served to the tasters in a random and balanced form, in disposable containers coded with three random digits.

2.7 Data analysis Assessments of centesimal and physicochemical composition of samples

The data obtained were evaluated using analysis of variance (ANOVA, one-way) for data from the physicochemical and instrumental analyses and ANOVA (two-way) for data from the sensory analysis and the Tukey mean comparison test (significance $p \leq 0.05$). The results were expressed as mean ± standard deviation of the mean (SMD). All statistical analyses were developed with the support of Excel for Windows software.

3. RESULTS AND DISCUSSION

3.1 Assessments of centesimal composition and physicochemical properties of samples

The seasoned pork loin samples showed average protein contents between 20.48% and 21.39%, with no significant differences ($p > 0.05$) between the treatments with natural preservatives (dry and wet curing) and the controls (Table 2). The results are in accordance with Bertol et al. (2019) [21], which indicate protein contents above 18% for lean cuts such as loin, and with the minimum requirements of Brazilian legislation (≥ 16%) [22].

Table 2: Centesimal composition and cooking weight loss (CWL) of pork loin formulations (F) prepared by dry (D) and wet (W) curing methods, with natural preservatives (F1D and F1W - Organic acids, their salts and peptides, and F2D - Organic acids, their salts and peptides, Acerola, and Beetroot extracts, and F2W - Organic acids, their salts and peptides, Acerola, Beetroot, and yeast extracts), synthetic preservatives (C1) and preservative-free (C2)

Samples	Protein (%)	Lipids (%)	Moisture (%)	Ash (%)	CWL (%)
F1D	$20.70^{a} \pm 0.53$	$0.74^{b} \pm 0.01$	$70.64^{c} \pm 0.29$	$2.43^{bcd} \pm 0.05$	$34.19^{ab} \pm 2.62$
F2D	$21.39^{a} \pm 0.12$	$0.25^{d} \pm 0.02$	$71.95^{bc} \pm 0.31$	$2.63^{bc} \pm 0.03$	$30.11^{bc} \pm 1.55$
C1D	$20.21^{a} \pm 0.76$	$0.36^{c} \pm 0.01$	$70.01^{c} \pm 0.72$	$2.75^{b} \pm 0.03$	$33.64^{ab} \pm 1.80$
C2D	$20.76^{a} \pm 1.22$	$0.82^{a} \pm 0.01$	$70.84^{c} \pm 0.65$	$2.13^{d} \pm 0.02$	$33.60^{ab} \pm 1.44$
F1W	$20.48^{a} \pm 0.41$	$0.29^{d} \pm 0.15$	$73.41^{a} \pm 0.40$	$2.37^{cd} \pm 0.05$	$26.69^{c} \pm 0.71$
F2W	$20.53^{a} \pm 0.68$	$0.12^{e} \pm 0.02$	$73.08^{ab} \pm 0.66$	$3.24^{a} \pm 0.05$	$25.62^{c} \pm 1.13$
C1W	$20.30^{a} \pm 0.63$	$0.28^{d} \pm 0.04$	$74.04^{a} \pm 0.36$	$2.76^{b} \pm 0.05$	$26.01^{c} \pm 1.43$
C2W	$20.42^{a} \pm 0.17$	$0.29^{d} \pm 0.02$	$73.81^{a} \pm 0.18$	$2.21^{d} \pm 0.32$	$34.52^{a} \pm 0.23$

*Mean ± standard deviation (n=3) followed by different letters indicates a significant difference by Tukey's test ($p < 0.05$).

As for lipids, a significant variation was observed between the formulations ($p < 0.05$) (Table 2). The samples with brine showed a lower lipid content, which is expected due to the increase in moisture. The lack of a pattern between the treatments can be attributed to anatomical variations or in the preparation of the cuts [23]. In all cases, the fat content was less than 0.8%, below the recommended 3% lipids for pork loins. It should be noted that Brazilian legislation does not establish a maximum fat limit for the seasoned product [22].

Moisture contents were significantly higher ($p < 0.05$) in the wet-cured samples, ranging up to 74.1% (Table 2), reflecting the addition of brine. All formulations remained below the 75% limit established by law [22]. Finally, ash contents ranged from 2.13% to 3.24%. The control samples without additives (C2D and C2W) and those containing only organic acids and their salts (F1D and F1W) presented lower mineral contents ($p < 0.05$), which can be attributed to the absence of mineral-rich ingredients.

Weight losses during cooking were higher in dry-cured pork loin samples (30.11–34.19%) (Table 2), except for F2D, which presented the lowest loss among these ($p < 0.05$). On the other hand, wet-cured formulations with 15% brine injection and natural ingredients showed significantly lower losses ($p < 0.05$), like samples with synthetic additives containing polyphosphates (C1W; $p > 0.05$). These results demonstrate that the combination of yeast extract and citrus extract can promote water retention comparable to synthetic phosphates. Yeast extract has a high protein content [24], and therefore, it can act as an ingredient with high water-binding capacity [8, 25] and formation of strong gels in meat products, having already shown promising effects in meat products such as ham [9], improving yield and reducing cooked purge. The citrus extract enhances this effect [26]. Considering the growing search for “clean label” products, this performance reinforces the potential of the formulation as a natural substitute for polyphosphates, whose adverse health effects have been reported [5].

Table 3 shows the pH values of the eight seasoned pork loin formulations over 45 days of storage. The formulations with natural preservatives maintained a stable pH between 5.6 and 5.8 in the first

30 days, a range considered adequate for fresh pork, according to Bertol et al. (2019) [21]. During storage at 7 °C, a greater variation in pH was observed between the 30th and 45th day, possibly related to microbial growth. Lactic acid bacteria can reduce pH via fermentation [27], while Gram-negative microorganisms such as *Pseudomonas* and Enterobacteriaceae can increase it through protein degradation and ammonia release [28]. Despite the statistical differences, all formulations remained within the expected range for fresh pork. The drop in pH in samples F2D and F2W may be associated with the acidifying action of acerola, rich in vitamin C [29].

Table 3: pH and water activity of pork loin formulations (F) prepared by dry (D) and wet (W) curing methods, with natural preservatives (F1D and F1W - Organic acids, their salts and peptides, and F2D - Organic acids, their salts and peptides, Acerola, and Beetroot extracts, and F2W - Organic acids, their salts and peptides, Acerola, Beetroot, and yeast extracts), synthetic preservatives (C1) and preservative-free (C2), for 45 days of storage at 7 °C

Samples	0 day	15 days	30 days	45 days
		pH*		
F1D	5.70 ^{bcA} ± 0.07	5.63 ^{fAB} ± 0.02	5.69 ^{cdA} ± 0.03	5.53 ^{acB} ± 0.02
F2D	5.60 ^{cdB} ± 0.01	5.60 ^{efB} ± 0.02	5.76 ^{bcA} ± 0.01	5.50 ^{eC} ± 0.02
C1D	5.67 ^{bcdA} ± 0.01	5.75 ^{cdA} ± 0.02	5.65 ^{dA} ± 0.02	5.28 ^{dB} ± 0.09
C2D	5.55 ^{dB} ± 0.02	6.17 ^{aA} ± 0.07	5.55 ^{eB} ± 0.01	5.45 ^{cdB} ± 0.02
F1W	5.86 ^{aA} ± 0.09	5.86 ^{bcA} ± 0.07	5.65 ^{dB} ± 0.01	5.62 ^{abB} ± 0.01
F2W	5.85 ^{aA} ± 0.02	5.88 ^{bA} ± 0.03	5.85 ^{aA} ± 0.04	5.49 ^{cB} ± 1.05
C1W	5.77 ^{abA} ± 0.07	5.72 ^{dfA} ± 0.03	5.82 ^{abA} ± 0.04	5.74 ^{aA} ± 0.02
C2W	5.62 ^{bcA} ± 0.01	5.49 ^{eB} ± 0.01	5.53 ^{eB} ± 0.01	5.35 ^{deC} ± 0.04
		Atividade de água*		
F1D	0.9782 ^{aA} ± 0.001	0.9732 ^{abA} ± 0.002	0.9775 ^{aA} ± 0.002	0.9731 ^{bA} ± 0.000
F2D	0.9781 ^{aA} ± 0.001	0.9712 ^{bC} ± 0.000	0.9737 ^{abBC} ± 0.001	0.9756 ^{bAB} ± 0.001
C1D	0.9765 ^{abA} ± 0.000	0.9705 ^{bB} ± 0.002	0.9786 ^{aA} ± 0.000	0.9751 ^{bA} ± 0.001
C2D	0.9729 ^{bB} ± 0.002	0.9732 ^{abB} ± 0.000	0.9746 ^{abB} ± 0.004	0.9816 ^{aA} ± 0.001
F1W	0.9771 ^{abA} ± 0.001	0.9772 ^{aA} ± 0.002	0.9784 ^{aA} ± 0.000	0.9749 ^{bA} ± 0.002
F2W	0.9727 ^{bA} ± 0.002	0.9737 ^{abA} ± 0.002	0.9716 ^{bA} ± 0.001	0.9737 ^{bA} ± 0.001
C1W	0.9794 ^{aA} ± 0.000	0.9755 ^{abB} ± 0.001	0.9761 ^{abAB} ± 0.001	0.9721 ^{bC} ± 0.001
C2W	0.9765 ^{abAB} ± 0.003	0.9726 ^{abAB} ± 0.002	0.9764 ^{abB} ± 0.001	0.9812 ^{aA} ± 0.001

*Mean ± standard deviation (n=3) followed by different letters indicate significant difference by Tukey's test ($p < 0.05$); lowercase, between formulations (rows); uppercase, between storage times (columns).

Regarding water activity, dry-cured samples showed variations between 0.9705 and 0.9816, and wet-cured samples, between 0.9716 and 0.9812 (Table 4), therefore showing similarity in relation to the Aw of fresh pork, which is in the range of 0.97 and 0.98, depending on the cut [30]. Up to 30 days of storage, statistical differences ($p < 0.05$) were observed between times and between formulations, but without a relevant pattern. However, at 45 days, the control formulations without preservatives in both types of curing (C2D and C2W) presented higher Aw than the samples with natural ingredients and synthetic additives. This change can be justified by the fact that these samples exuded brine or endogenous water from the muscle during storage, and this water, in free form, contributed to the increase in Aw, since according to Damodaran and Parkin (2017) [23], Aw indicates the intensity with which water associates with non-aqueous constituents. Therefore, considering this parameter, natural ingredients would provide stability close to that obtained with synthetic additives.

Another parameter evaluated was the color. Table 4 presents the parameters L*, a*, b*, C*, and hº for the eight formulations of dry and wet seasoned pork loin.

Table 4: Color parameters of pork loin formulations (F) prepared by dry (D) and wet (W) curing methods, with natural preservatives (F1D and F1W - Organic acids, their salts and peptides, and F2D - Organic acids, their salts and peptides, Acerola, and Beetroot extracts, and F2W - Organic acids, their salts and peptides, Acerola, Beetroot, and yeast extracts), synthetic preservatives (C1) and preservative-free (C2), for 45 days of storage at 7 °C

Parameter (time - days)	Samples[1]							
	F1D	F2D	C1D	C2D	F1W	F2W	C1W	C2W
L* (0)	34.34 ^{dB} ± 1.07	41.73 ^{bB} ± 0.51	36.74 ^{cdC} ± 1.50	51.22^{aA} ± 0.78	37.62 ^{cdB} ± 0.77	37.23^{cdB} ± 2.18	40.11 ^{bcC} ± 1.27	39.54 ^{bcB} ± 1.46
L* (15)	53.53 ^{abA} ± 2.24	48.82 ^{bA} ± 1.13	50.40 ^{bB} ± 1.14	53.00 ^{abA} ± 3.96	53.07 ^{abA} ± 0.49	51.87^{abA} ± 2.08	50.86 ^{bA} ± 1.13	57.65 ^{aA} ± 3.05
L* (30)	51.77 ^{abA} ± 2.22	51.37 ^{abA} ± 1.35	55.73 ^{aA} ± 1.09	56.26 ^{aA} ± 0.80	55.18 ^{bA} ± 1.73	47.71^{bcA} ± 2.89	45.67 ^{cB} ± 1.28	54.73 ^{aA} ± 1.84
L* (45)	52.08 ^{bcA} ± 1.48	49.08 ^{cdA} ± 1.31	58.05 ^{aA} ± 0.96	44.12 ^{eB} ± 0.65	54.51 ^{cA} ± 1.66	47.46 ^{dA} ± 0.72	50.03 ^{cdA} ± 1.22	58.49 ^{aA} ± 0.48
a* (0)	1.82 ^{bB} ± 0.26	2.09 ^{abB} ± 0.25	4.55 ^{aAB} ± 1.90	2.81^{abAB} ± 0.64	1.31 ^{bB} ± 0.77	1.86 ^{bA} ± 1.01	2.77 ^{abAB} ± 1.19	0.73 ^{bB} ± 0.21
a* (15)	4.19 ^{bcA} ± 0.50	3.96 ^{bcB} ± 0.37	7.50 ^{aA} ± 0.94	4.72 ^{bAB} ± 1.21	3.83 ^{bcA} ± 0.46	2.30 ^{cA} ± 1.15	3.83 ^{bcA} ± 0.89	0.44 ^{dB} ± 0.19
a* (30)	4.27 ^{bA} ± 1.21	9.00 ^{aA} ± 2.69	5.87 ^{abAB} ± 0.22	2.25 ^{bB} ± 1.07	2.96 ^{bAB} ± 0.63	3.23 ^{bA} ± 1.80	2.55 ^{bAB} ± 0.18	2.19 ^{bA} ± 0.92
a* (45)	0.85 ^{bB} ± 0.53	3.26 ^{bcdB} ±0.38	3.95 ^{bC} ± 0.77	7.42 ^{aA} ± 1.38	2.60 ^{bcdAB}± 1.24	3.51 ^{bcA} ± 1.47	1.07 ^{cdB} ± 0.44	2.67 ^{bcdA} ± 0.42
b* (0)	2.51 ^{dB} ± 0.29	5.38 ^{abA} ± 0.29	5.17 ^{abcB} ± 1.10	6.07 ^{aA} ± 0.19	3.51 ^{bcdA}± 0.32	5.74 ^{aB} ± 1.35	5.16 ^{abcB} ± 0.47	3.16 ^{cdB} ± 0.73
b* (15)	6.96 ^{aAB} ± 0.27	7.53 ^{aAB} ± 0.85	7.87 ^{aA} ± 0.39	7.71 ^{aA} ± 1.52	5.82 ^{aA} ± 0.14	6.16 ^{aB} ± 0.19	6.63 ^{aA} ± 0.59	6.22 ^{aA} ± 3.01
b* (30)	7.75 ^{abA} ± 0.32	9.00 ^{abA} ± 2.09	9.27 ^{aA} ± 0.82	7.21 ^{abcA}± 0.94	6.25 ^{abcA}± 0.90	8.13 ^{abA} ± 1.43	5.90 ^{bcAB} ± 1.36	4.18 ^{cB} ± 0.58
b* (45)	4.39 ^{bAB} ± 0.38	6.81^{abAB}± 0.20	8.14 ^{aA} ± 0.31	6.97 ^{abA} ± 1.37	4.78 ^{bA} ± 0.52	6.24 ^{abB} ± 0.98	4.74 ^{bC} ± 0.26	6.22 ^{abA} ± 0.56
hº (0)	54.03 ^{bcB} ± 4.65	68.84^{abcA}±1.39	49.66 ^{cBC} ± 6.01	65.19^{abcAB}±5.63	69.93^{abcA}±10.62	71.50^{abA}± 9.91	62.46^{abcAB}± 8.55	75.95^{aA} ± 7.42
hº (15)	58.97^{abcB} ± 3.93	62.21^{cdB}± 2.57	46.53 ^{cC} ± 2.22	58.73^{abcB}±1.56	56.77 ^{bcA}± 2.62	70.03 ^{cdA} ± 9.12	60.17 ^{abB}±6.27	85.75^{dB} ± 1.46
hº (30)	61.50 ^{abB}± 5.96	45.40^{bC} ± 2.22	57.54 ^{abAB} ± 3.21	73.12 ^{aA} ±5.87	64.42 ^{abA} ± 5.98	68.24 ^{aA} ±12.38	65.97 ^{abAB}±5.58	62.23 ^{abA} ± 12.63
hº (45)	79.40 ^{aA} ± 6.26	64.46^{aAB}± 2.98	64.28 ^{aA} ± 3.54	43.18 ^{bC} ± 1.31	55.72 ^{aA} ±8.62	61.71 ^{abA} ± 7.47	77.35 ^{aA} ± 4.94	68.87 ^{aA} ± 1.97
C* (0)	3.10^{dC} ± 0.29	5.77^{abcdB}± 0.36	6.92 ^{aB} ± 2.05	6.71 ^{aA} ± 0.10	3.79 ^{bcdB} ± 0.43	6.10 ^{abB} ± 1.27	5.90 ^{abcAB}± 0.90	3.26 ^{cdA} ± 0.65
C* (15)	8.14 ^{abA} ± 0.12	8.52^{abAB}± 0.84	10.88 ^{aA} ± 0.93	9.05 ^{abA} ± 1.92	6.97^{bA} ± 0.36	6.63 ^{bA} ± 0.54	7.68 ^{abA} ± 0.69	6.23 ^{bA} ± 3.01
C* (30)	8.88 ^{abcA} ± 0.84	12.73^{aA} ± 3.37	10.99 ^{abA} ± 0.59	7.58 ^{bcA} ± 1.17	6.94^{bcA} ± 0.81	8.89 ^{abcA} ± 1.21	6.44 ^{cAB} ± 1.21	4.80 ^{cA} ± 0.30
C* (45)	4.49 ^{dB} ± 0.46	7.56^{abcB}± 0.18	9.06 ^{abAB} ± 0.61	10.18 ^{aA} ± 1.93	5.82^{cdA} ± 0.04	7.19 ^{abcdA} ± 1.53	4.88 ^{cdB} ± 0.30	6.77 ^{bcdA} ± 0.66

[1]Mean ± standard deviation (n=3) followed by different letters indicate significant difference by Tukey's test ($p < 0.05$); lowercase, between formulations (columns); uppercase, between storage times (lines).

Analyzing the L* parameter, which represents the brightness of the samples on a scale ranging from black (0) to white (100) [15], variations in the range of 34.34 to 58.49 can be observed (Table 6). The L* values in the samples ranged from 34.34 to 51.22; lower L* values were found in the F1D and C1D, F1W, F2W formulations, with no effect related to the ingredients used. During storage, after 15 days, there was a significant increase in the L* values for all samples with the addition of natural preservatives, which had a similar behavior to the control samples with synthetic preservatives and to the C2W samples, without preservatives, for both types of curing, with values in the range of 44.12 to 58.49. Fresh pork presents L* values that vary greatly according to the literature, but the American Meat Science Association (AMSA) considers L* values between 49 and 60 within the quality standards for pork. Brewer et al. (2001) [31] obtained L* of 51.31 for *longissimus thoracis et lumborum* (10th rib), while for *triceps brachii*, L* was 39.93. Santos et al. (2012) [32] evaluated the effect of marination on L* values of pork with different pH values and obtained L* values in the range of 45.5, and found no significant variation in relation to the L* values for fresh meat. The wide range of variation obtained in the present work can be attributed to intrinsic factors that alter the amount of sarcoplasmic proteins such as race, muscle fiber type, genetics, among others [23] or operational factors, such as sample standardization [32], because the sample is a whole cut and despite being the same muscle, according to Lawrie and Ledward (2006) [33] and Bertol et al. (2019) [21], the amount of myoglobin in a muscle can vary hundreds of times in a space of one centimeter away due to changes in fiber types in the same muscle.

Regarding the parameter a* for the dry-cured formulations, F2D and C1D, as well as for the wet-cured formulations, F2W and C1W obtained higher values ($p < 0.05$), probably due to the reddish coloration conferred by the beetroot extract in F2 and the curing salts in C1. However, the values obtained (Table 4) were lower than those reported by Santos et al. (2012) [32] for marinated pork, with an average value of a* of 12.67. A similar behavior was observed among the samples for the parameter b*. However, in the dry-cured samples, the control without preservatives presented the highest value of b*, followed by the formulations F2 and C1 of both cures. The results obtained in the present study for the parameter b* were close to those obtained by Santos et al. (2012) [32] (b*=3.14).

The values of a* and b* (Table 4) were used to calculate the Hue angle (h°), considered the qualitative color attribute. The Hue angle allows comparing samples and is calculated as $\tan^{-1}$ b*/a*. The 0º angle represents the red color and the 90º angle, the yellow color [15]. The values obtained with marination can be observed in Table 4. In dry curing, the F2D formulation obtained the highest value, with no significant difference from the control without preservatives (C2D). For the wet-cured formulations, there was no significant difference between the samples with natural preservatives (F1W and F2W) in relation to the controls, with and without additives (C1W and C2W). With the storage time for the samples cured via the wet method, the control with additives had an increased hº value with the increase in the storage period, while for F1W and F2W, there were no significant changes ($p > 0.05$). In the dry cure, F1s had a significant increase on the 45th day, but remained stable until 30 days, and F2D, despite significant changes over the course of 45 days, maintained values close to or below those obtained on day 0. These results demonstrate a positive effect of natural ingredients on the product's hue in both curing processes.

Similar behavior to that observed in hº at time 0 was observed for color saturation (C*). C* is directly linked to the concentration of the coloring element and represents a quantitative attribute for intensity. A higher value chroma indicates a greater saturation of the colors perceptible to humans. Neutral colors have low saturation, while pure colors have high saturation and, therefore, are brighter in human perception [15]. Regarding storage time, in general, an increase in C* values can be observed on the 15th and 30th day, with a reduction on the 45th day, suggesting a decrease in the amount of pigment that may have occurred together with water losses due to exudation, considering that myoglobin is a water-soluble pigment [21, 23]. It is noteworthy that at time 0, the F2 formulations presented C* values equal to the control with the addition of synthetic preservatives (C1) for both cures. Meanwhile, at 45 days, for dry curing, the same behavior was observed, and in the wet curing, the C* value obtained for F2W was even higher than C1W.

3.2 Oxidative stability of samples

The results of the oxidation analysis indicated that all seasoned pork loin formulations presented low TBARS levels during the 45 days of storage (Table 5), remaining below the sensorially perceptible limit. According to Olivo and Shimokomaki (2001) [34], values lower than 1 mg of MDA kg^{-1} do not confer residual rancid flavors or odors associated with lipid oxidation. Formulations F2D and F2W stood out, as they presented the lowest malonaldehyde levels at the end of the evaluated period, with no significant increase over time. This result may be associated with the presence of acerola extract, known for its antioxidant capacity. Previous studies, such as those by Realini et al. (2015) [35], also observed similar effects, suggesting its effectiveness in the oxidative stabilization of meat products. The mechanisms of action of acerola bioactive compounds include hydrogen atom donation by phenolic compounds, free radical stabilization by tocopherols, oxygen scavenging, and synergistic action of ascorbic acid [36]. These findings confirm the potential of acerola extract as an effective natural antioxidant for application in seasoned and refrigerated meat products.

Table 5: Oxidative stability of pork loin formulations (F) prepared by dry (D) and wet (W) curing methods, with natural preservatives (F1D and F1W - Organic acids, their salts and peptides, and F2D - Organic acids, their salts and peptides, Acerola, and Beetroot extracts, and F2W - Organic acids, their salts and peptides, Acerola, Beetroot, and yeast extracts), synthetic preservatives (C1) and preservative-free (C2), for 45 days of storage at 7 °C

Samples	0 day	15 days	30 days	45 days
	TBARS Index* (mg of malonaldehyde. kg^{-1})			
F1D	$0.19^{abB} \pm 0.0032$	$0.03^{bcC} \pm 0.0011$	$0.08^{acBC} \pm 0.0042$	$0.47^{aA} \pm 0.0097$
F2D	$0.11^{bcA} \pm 0.0045$	$0.02^{cB} \pm 0.0005$	$0.06^{abcAB} \pm 0.0040$	$0.01^{dB} \pm 0.0004$
C1D	$0.06^{cB} \pm 0.0063$	$0.04^{abcB} \pm 0.0007$	$0.05^{abcB} \pm 0.0017$	$0.32^{bA} \pm 0.0099$
C2D	$0.11^{bcB} \pm 0.0053$	$0.04^{abcC} \pm 0.0007$	$0.06^{abcBC} \pm 0.0009$	$0.21^{cA} \pm 0.0027$
F1W	$0.21^{aA} \pm 0.0062$	$0.03^{bcB} \pm 0.0014$	$0.03^{bcB} \pm 0.0016$	$0.05^{dB} \pm 0.0005$
F2W	$0.15^{abcA} \pm 0.0026$	$0.03^{bcB} \pm 0.0015$	$0.02^{cB} \pm 0.0008$	$0.03^{dB} \pm 0.0004$
C1W	$0.15^{abcA} \pm 0.0036$	$0.05^{abA} \pm 0.0007$	$0.09^{aA} \pm 0.0033$	$0.06^{dA} \pm 0.0007$
C2W	$0.15^{abcA} \pm 0.0036$	$0.06^{aB} \pm 0.0024$	$0.02^{cB} \pm 0.0008$	$0.11^{cdA} \pm 0.0013$
	Metamyogobin formation (%)			
F1D	$66.20^{dC} \pm 1.30$	$74.68^{abA} \pm 0.35$	$65.26^{cC} \pm 0.55$	$68.76^{bB} \pm 1.54$
F2D	$76.35^{aA} \pm 0.12$	$73.10^{bB} \pm 0.51$	$71.05^{abcB} \pm 0.90$	$71.96^{aC} \pm 0.79$
C1D	$70.45^{cA} \pm 0.61$	$69.37^{cAB} \pm 0.54$	$67.96^{abcB} \pm 0.47$	$68.68^{bAB} \pm 2.13$
C2D	$74.41^{bA} \pm 0.84$	$75.31^{aA} \pm 0.58$	$71.35^{abcB} \pm 0.65$	$68.35^{bC} \pm 2.59$
F1W	$67.64^{dB} \pm 0.98$	$67.17^{dB} \pm 1.86$	$70.36^{abcA} \pm 2.33$	$65.01^{cB} \pm 1.06$
F2W	$73.96^{bB} \pm 1.17$	$75.27^{aA} \pm 0.48$	$73.10^{abB} \pm 0.41$	$73.90^{aB} \pm 0.67$
C1W	$71.35^{cA} \pm 0.86$	$68.63^{cdB} \pm 0.67$	$67.54^{bcB} \pm 8.09$	$67.02^{bcB} \pm 0.53$
C2W	$70.98^{cC} \pm 0.46$	$73.18^{bAB} \pm 0.29$	$74.49^{aA} \pm 1.84$	$71.99^{aBC} \pm 0.81$

*Mean ± standard deviation (n=3) followed by different letters indicate significant difference by Tukey's test ($p < 0.05$); lowercase, between formulations (rows); uppercase, between storage times (columns).

Metmyoglobin is formed due to the oxidative process of the myoglobin pigment, causing a brown coloration in the meat, indicating its oxidation. This coloration is undesirable and may therefore cause rejection by consumers [21]. At the initial time (day 0), the average metmyoglobin percentages in the seasoned pork loin samples ranged from 66.20% to 76.35% (Table 5). Formulations F1D and F1W, prepared with organic acids and their salts, presented metmyoglobin levels equal to or lower than the respective control samples (C1D and C1W) with synthetic antioxidants, during the 45 days of storage. Formulations F2D and F2W — containing acerola extract and beetroot extract — presented higher metmyoglobin values, possibly influenced by the presence of the natural colorant, with no correlation with greater lipid oxidation (Table 9) or microbial growth (Table 5). According to Bertol et al. (2019) [21], microbial activity reduces oxygen tension in the tissue, favoring the formation of deoxymyoglobin, which can subsequently be oxidized to metmyoglobin in the presence of peroxides.

In the wet-cured samples, except for C1W, there was an increase in metmyoglobin formation up to 15 or 30 days, followed by a decline at 45 days ($p < 0.05$). For the dry-cured samples, a continuous reduction trend was observed, which may be related to the loss of water and, consequently, of myoglobin, a water-soluble pigment [23]. This behavior highlights the influence of natural ingredients and storage conditions on the color stability of refrigerated seasoned meat products.

3.3 Quality and microbiological stability of seasoned loin samples during shelf life

All seasoned pork loin formulations met the microbiological criteria established by Normative Instruction No. 161 of the Ministry of Agriculture and Livestock [19], with a total absence of *Salmonella spp.* (25 g) in all treatments, regardless of the storage temperature or time evaluated (Table 6). Regarding thermotolerant coliforms, including Escherichia coli, the results remained below the legal limit of 10^3 CFU g^{-1} [19] until the 30th day of storage at 7 °C for all formulations. An increase in microbial levels was observed between the 30th and 45th day, especially in the wet-cured formulations, possibly due to the greater availability of free water resulting from the injection of brine. Reducing the temperature to 2 °C was effective in slowing microbial growth, keeping levels within regulatory limits up to day 37 for all formulations tested. These findings demonstrate that the use of natural ingredients, combined with temperature control, can maintain the microbiological safety of the product throughout most of its shelf life.

The results for the aerobic mesophilic count (CFU g^{-1}) in pork loin samples with synthetic additives replaced by natural sources, subjected to temperatures of 2 °C and 7 °C, can be seen in Table 6. In chilled products, the presence of aerobic mesophilic microorganisms indicates the hygiene conditions of the process, which, when not carried out properly, results in an increase in the count [37]. By law, the aerobic mesophilic count must not exceed 106 CFU g^{-1} [19]; values above this indicate the product is outside the ideal hygienic-sanitary conditions for pork.

For aerobic mesophilic bacteria, the highest values were verified when the samples were stored at 7 °C. At this temperature, for the time intervals tested, the samples were suitable for consumption up to the 15th day of storage, regardless of the use of chemical or synthetic preservatives. However, when subjected to storage at a temperature of 2 °C, the F2W formulation was suitable for consumption up to the 30th day, while F2D up to 45 days of storage, as established in legislation [19].

It is noteworthy that both samples, F2D and F2W, presented in their formulation natural ingredients, organic acids, their salts and peptides that exhibit antimicrobial activity, in addition to regulating acidity and controlling water activity [6, 7], yeast extract with the ability to reduce microbial growth performance, in addition to its antioxidant capacity [8], acerola extract with the presence of bioactive substances with antioxidant, anti-inflammatory and antimicrobial effects [10], and beetroot extract powder with at least 30,000 ppm of natural nitrite [12], indicating superiority in shelf life even in relation to samples made with synthetic additives.

Table 6: Count of thermotolerant coliforms (NMP g^{-1}) and aerobic mesophilic count in pork loin formulations (F) prepared by dry (D) and wet (W) curing methods, with natural preservatives (F1D and F1W - Organic acids, their salts and peptides, and F2D - Organic acids, their salts and peptides, Acerola, and Beetroot extracts, and F2W - Organic acids, their salts and peptides, Acerola, Beetroot, and yeast extracts), synthetic preservatives (C1) and preservative-free (C2), for 45 days of storage at 2 and 7 °C

Sample	Storage time (days) at 7 °C					
	0	**15**	**22**	**30**	**37**	**45**
	Count of thermotolerant coliforms (NMP g^{-1})					
F1D	< 3.0	< 3.0	n.d.	< 3.0	n.d.	< 3.0
F2D	< 3.0	< 3.0	n.d.	< 3.0	n.d.	< 3.0
C1D	< 3.0	< 3.0	n.d.	< 3.0	n.d.	3.4
C2D	< 3.0	< 3.0	n.d.	< 3.0	n.d.	3.2
F1W	3.4	< 3.0	n.d.	23.0	n.d.	> 1100
F2W	3.2	< 3.0	n.d.	21.7	n.d.	230.0
C1W	13.7	< 3.0	n.d.	< 3.0	n.d.	86.3
C2W	4.4	< 3.0	n.d.	< 3.0	n.d.	386.7
	Mesophilic aerobic count (CFU g^{-1})					
F1D	2.3×10^2	2.8×10^5	n.d.	5.6×10^6	n.d.	3.1×10^7
F2D	7.0×10^2	6.8×10^3	n.d.	2.7×10^6	n.d.	3.6×10^7
C1D	1.3×10^2	6.8×10^5	n.d.	2.4×10^7	n.d.	3.8×10^7
C2D	6.0×10^2	2.7×10^5	n.d.	1.5×10^7	n.d.	4.8×10^7
F1W	6.7×10^3	3.2×10^6	n.d.	3.5×10^7	n.d.	7.4×10^7
F2W	6.8×10^3	1.0×10^5	n.d.	5.7×10^7	n.d.	7.3×10^7
C1W	1.6×10^3	3.7×10^5	n.d.	6.6×10^7	n.d.	6.4×10^7
C2W	4.9×10^2	2.3×10^5	n.d.	3.6×10^6	n.d.	2.1×10^7
	Storage time (days) at 2 °C					
	0	**15**	**22**	**30**	**37**	**45**
	Count of thermotolerant coliforms (NMP g^{-1})					
F1D	9.2	<3.0	3.2	<3.0	<3.0	<3.0
F2D	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
C1D	<3.0	3.0	<3.0	<3.0	<3.0	<3.0
C2D	3.9	3.4	<3.0	3.0	5.0	3.0
F1W	3.4	3.0	9.3	<3.0	<3.0	<3.0
F2W	9.3	<3.0	<3.0	<3.0	<3.0	<3.0
C1W	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
C2W	7.3	3.4	1.1 x 10	3.0	3.2	3.0
	Mesophilic aerobic count (CFU g^{-1})					
F1D	2.1×10^4	3.3×10^4	4.0×10^4	3.9×10^6	2.1×10^7	2.2×10^7
F2D	2.5×10^4	2.6×10^4	6.1×10^4	3.4×10^4	2.0×10^4	4.2×10^4
C1D	2.4×10^4	3.3×10^4	4.6×10^4	7.4×10^4	1.9×10^7	2.8×10^6
C2D	2.7×10^4	4.4×10^4	6.3×10^5	3.9×10^6	2.2×10^7	2.1×10^6
F1W	3.0×10^4	1.1×10^6	1.5×10^6	8.2×10^6	2.9×10^7	7.5×10^7
F2W	1.3×10^4	3.1×10^4	2.2×10^5	3.2×10^5	1.1×10^7	1.1×10^7
C1W	1.4×10^4	1.1×10^5	4.1×10^6	8.3×10^6	1.6×10^7	2.2×10^7
C2W	4.7×10^4	2.1×10^5	6.4×10^6	1.3×10^7	2.3×10^7	1.2×10^8

n.d.: not determined.

3.4 Sensory acceptance of samples

The tasting team was composed mostly of young people (83.9% up to 25 years old), with a gender balance (51.7% female) and high levels of education (91.5% studying or having completed higher education). All participants were pork consumers, with 51.7% reporting weekly or daily consumption. The results (Table 7) indicated greater overall acceptance for the F2D and F2W formulations ($p < 0.05$), with averages close to 8 ("I liked it a lot") for the attributes color, appearance, odor, flavor, and texture. The presence of beetroot extract in these formulations contributed to a more intense reddish color, as shown by the high C* and hº values observed (Table 4), which favored visual acceptance. Beetroot, rich in natural nitrite and betalains, has been indicated as an alternative to synthetic curing salts for color stabilization in meat products [38, 39]. In addition, the distribution of overall acceptance scores was concentrated between 7 and 9 for F2D and F2W (Figure 1), revealing homogeneity in the evaluations and highlighting the sensory potential of the natural ingredients used.

Table 7: Sensory acceptance of pork loin formulations (F) prepared by dry (D) and wet (W) curing methods, with natural preservatives (F1D and F1W - Organic acids, their salts, and peptides, and F2D - Organic acids, their salts and peptides, Acerola, and Beetroot extracts, and F2W - Organic acids, their salts and peptides, Acerola, Beetroot, and yeast extracts), synthetic preservatives (C1) and preservative-free (C2)

Sample	Attributes					Overall acceptance
	Color	Appearance	Smell	Taste	Texture	
F1D	6.83 ^{b} ± 1.79	6.86 ^{c} ± 1.83	7.14 ^{bc} ± 1.64	7.46 ^{bc} ± 1.64	7.30 ^{bc} ± 1.61	7.22 ^{d} ± 1.60
F2D	7.92 ^{ab} ± 1.08	7.98 ^{a} ± 1.02	8.14 ^{a} ± 1.02	8.26 ^{a} ± 0.92	8.17 ^{a} ± 1.01	8.16 ^{ab} ± 0.83
C1D	7.13 ^{b} ± 1.23	6.93 ^{bc} ± 1.36	7.22 ^{bc} ± 1.39	7.18 ^{c} ± 1.41	7.34 ^{bc} ± 1.32	7.41 ^{cd} ± 1.14
C2D	6.97 ^{b} ± 1.59	6.98 ^{bc} ± 1.52	7.14 ^{bc} ± 1.48	7.16 ^{c} ± 1.54	7.37 ^{bc} ± 1.49	7.27 ^{d} ± 1.34
F1W	6.70 ^{b} ± 1.65	6.87 ^{c} ± 1.70	6.93 ^{c} ± 1.74	7.08 ^{c} ± 1.75	7.01 ^{c} ± 1.64	7.00 ^{d} ± 1.58
F2W	8.20 ^{a} ± 0.91	8.18 ^{a} ± 0.98	8.12 ^{a} ± 1.08	8.20 ^{a} ± 0.97	8.32 ^{a} ± 0.98	8.28 ^{a} ± 0.80
C1W	7.48 ^{b} ± 1.38	7.41 ^{b} ± 1.35	7.52 ^{b} ± 1.34	7.79 ^{ab} ± 1.47	7.54 ^{b} ± 1.65	7.77 ^{bc} ± 1.17
C2W	6.72 ^{b} ± 1.56	6.86 ^{c} ± 1.54	7.06 ^{bc} ± 1.56	6.98 ^{c} ± 1.82	7.09 ^{bc} ± 1.69	7.06 ^{d} ± 1.41

*Mean ± standard deviation (n=120) followed by different lowercase letters indicate significant difference by Tukey's test ($p < 0.05$) between formulations (rows).

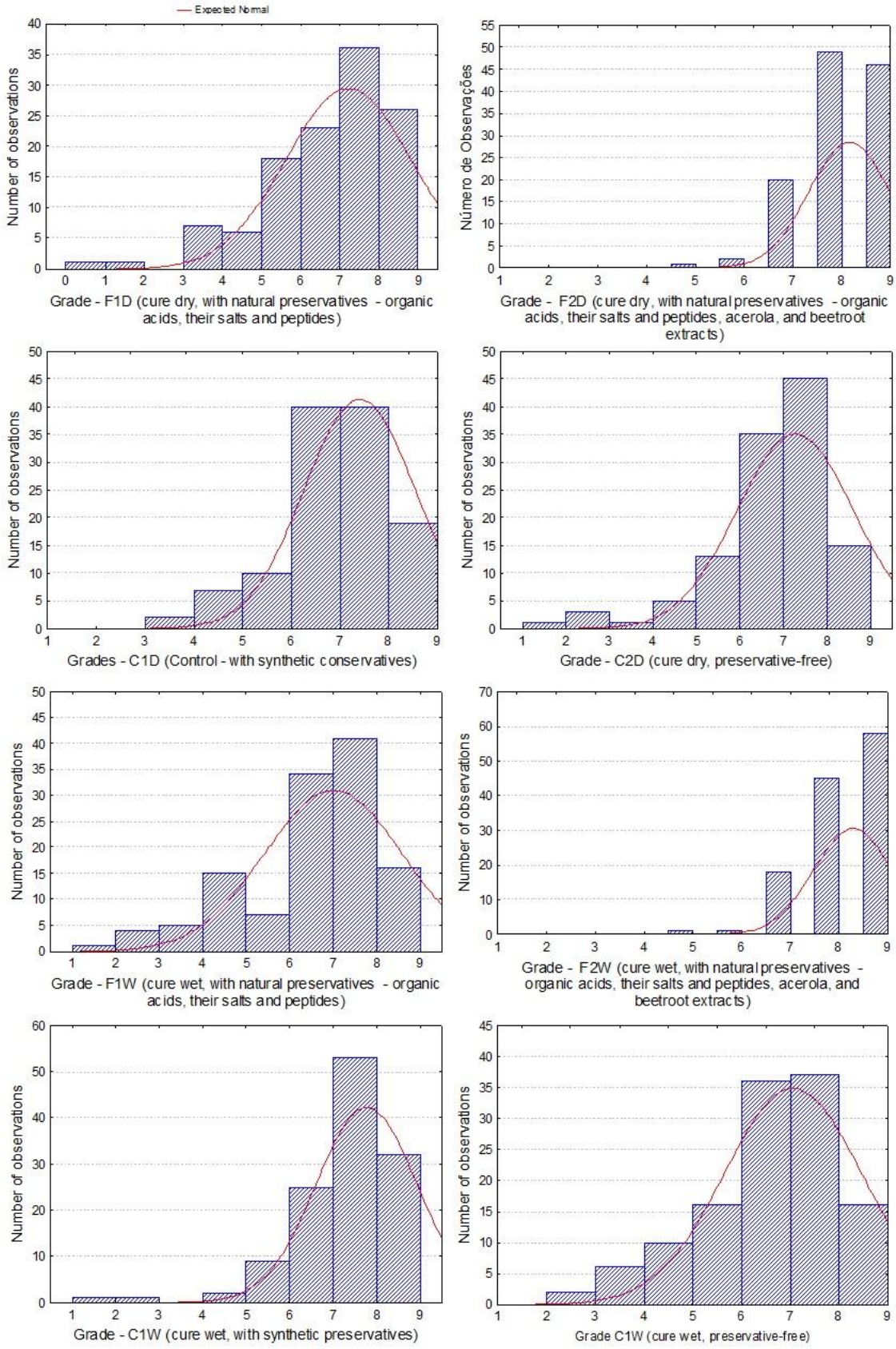

Figure 1: Distribution of scores given by tasters for the overall acceptance of samples of pork loin seasoned by dry and wet methods.

The purchase intention of the samples was also assessed, with scores from 1 to 5 (1 - certainly would not buy, and 5 - certainly would buy), as shown in Figure 2.

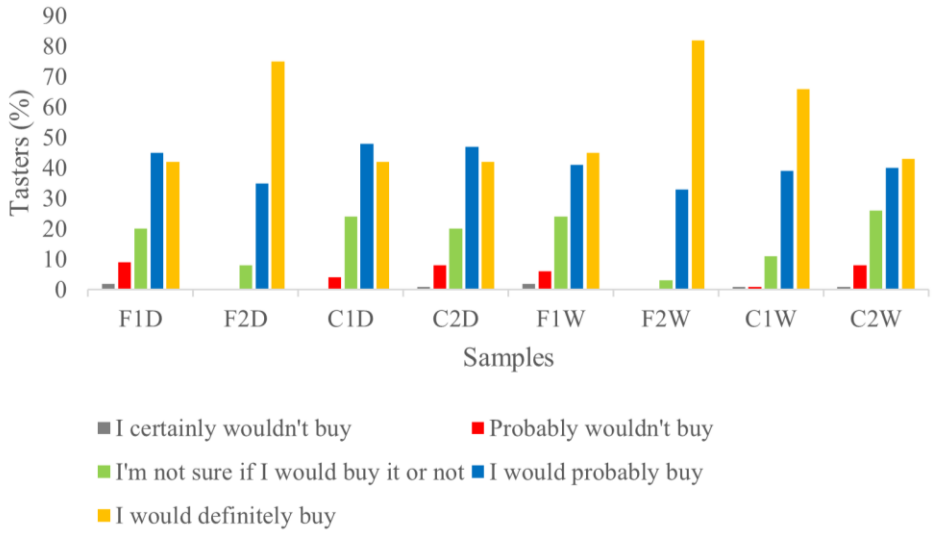

Figure 2: Scores obtained for the purchase intention of pork loin formulations (F) prepared by dry (D) and wet (W) curing methods, with natural preservatives (F1D and F1W - Organic acids, their salts and peptides, and F2D - Organic acids, their salts and peptides, Acerola, and Beetroot extracts, and F2W - Organic acids, their salts and peptides, Acerola, Beetroot, and yeast extracts), synthetic preservatives (C1) and preservative-free (C2).

The results of the purchase intent scale confirmed a higher purchase intention for the F2D and F2W samples ($p < 0.05$), comparable to the sample with synthetic additives (C1W). This shows that the combination of natural ingredients (organic acids, salts, peptides, acerola extract, beetroot, and yeast) guarantees sensory acceptance equivalent to or superior to that of products with synthetic additives.

4. CONCLUSION

The use of natural ingredients, including yeast extract. citrus extract. acerola extract. beetroot extract. and organic acids with their salts and peptides, proved to be a viable alternative to synthetic additives in the preparation of chilled and seasoned pork loins. by dry and wet curing. The formulations proposed complied with current legislation. with a protein content of over 20%. moisture below 74.4%. and low lipid levels (<0.82%). The pH and water activity (Aw) behavior indicated stability like that obtained with synthetic preservatives. The F2 samples (with beetroot extract) stood out positively in terms of color (C* and h°). with values close to the synthetic controls and good visual stability during storage. The addition of the yeast and citrus extract blend contributed to higher yield and lower cooking losses. even without the use of polyphosphates. increasing the competitive potential of natural products in the market. All formulations maintained low levels of lipid oxidation (TBARS) and metmyoglobin up to 45 days. with emphasis on F2 and F1. in both cure types. with performance like controls with synthetic antioxidants. Although the natural ingredients did not significantly inhibit microbial growth at 7 °C after 15 days. But refrigeration at 2 °C ensured microbiological stability for up to 45 days (dry curing) and 30 days (wet curing). Sensorially. the products made with natural compounds showed high acceptance for all attributes evaluated. confirming their potential as promising technological alternatives for the preservation and valorization of seasoned pork cuts with clean label appeal.

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6. REFERENCES

1. Wu X, Fu H, Tian X, Wu B, Sun J. Prediction of pork storage time using Fourier transform near infrared spectroscopy and Adaboost-ULDA. J Food Process Eng. 2017 Dec;40(6):e12566.3. doi: 10.1111/jfpe.12566
2. Duran A, Kahve HI. The effect of chitosan coating and vacuum packaging on the microbiological and chemical properties of beef. Meat Sci. 2020 Apr;162:107961. doi: 10.1016/j.meatsci.2019.107961
3. Kim T-K, Hwang K-E, Lee M-A, Paik H-D, Kim Y-B, Choi Y-S. Quality characteristics of pork loin cured with green nitrite source and some organic acids. Meat Sci. 2019 Jun;152:141-5. doi: 10.1016/j.meatsci.2019.02.015
4. Boeira CP, Piovesan N, Flores DCB, Soquetta MB, Lucas BN, Heck RT, et al. Phytochemical characterization and antimicrobial activity of *Cymbopogon citratus* extract for application as natural antioxidant in fresh sausage. Food Chem. 2020 Jul;319:126553. doi: 10.1016/j.foodchem.2020.12655
5. Molina RE, Bohrer M, Mejia MV. Phosphate alternatives for meat processing and challenges for the industry: A critical review. Food Res Int. 2024 Apr;166:112624. doi: 10.1016/j.foodres.2023.112624
6. Almeida ALF. Conservantes químicos para alimentos. Food Ing. 2011;18:43-9.
7. Fell EL, Brum LM, Nicoletti E, Sartoreto L, Hoelscher H, Colet R, et al. Nisin or organic acid salt mixtures for the Calabrese-type sausages in industrial-scale production. Braz J Food Technol. 2024;27:e2023061. doi: 10.1590/1981-6723.06123
8. Tao Z, Yuan H, Liu M, Liu Q, Zhang S, Liu H, et al. Yeast extract: Characteristics, production, applications and future perspectives. J Microbiol Biotechnol. 2023 Feb;33(2):151-66. doi: 10.4014/jmb.2207.07057
9. Pancrazio C, Cunha SC, Pinho PG, Loureiro M, Meireles SS, Ferreira IMPLVO, et al. Spent brewer's yeast extract as an ingredient in cooked hams. Meat Sci. 2016 Nov;121:382-9. doi: 10.1016/j.meatsci.2016.07.009
10. Li Y, Zhang J-J, Xu D-P, Zhou T, Zhou Y, Li S, et al. Bioactivities and health benefits of wild fruits. Int J Mol Sci. 2016 Aug;17(8):1258. doi: 10.3390/ijms17081258
11. Rahimi P, Abedimanesh S, Mesbah-Namin SA, Ostadrahimi A. Betalains, the nature-inspired pigments, in health and diseases. Crit Rev Food Sci Nutr. 2019 Nov;59(18):2949-78. doi: 10.1080/10408398.2018.1479830
12. Wenda Ingredients [Internet]. SafePlate® Beet Powder; [date unknown] [cited in 2025 Dec 22]. Available from: https://wendaingredients.com/products/beet-powder/.
13. Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. 4. ed. São Paulo: Instituto Adolfo Lutz; 2008. Available from: http://www.ial.sp.gov.br/resources/editorinplace/ial/2016_3_19/analisedealimentosial_2008.pdf.
14. Francisco NS, Altemio ADC, Santos LNB, Garcia RG, Sapaterro GA. Características físico-químicas de linguiça de frango elaborada com fibra de trigo e colágeno bovino. Encicl Bios. 2013 Dez;9(17):551-8.
15. Pathare PB, Opara UL, Al-Said FA-J. Colour measurement and analysis in fresh and processed foods: A Review. Food Bioproc Tech. 2013 Jan;6(1):36-60. doi: 10.1007/s11947-012-0867-9
16. Tarladgis BG, Pearson AM, Jun LRD. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II - formation of the tba-malonaldehyde complex without acid-heat treatment. J Sci Food Agric. 1964 Sep;15(9):602-7. doi: 10.1002/jsfa.2740150904
17. Honda R, Crackel RHL, Gray JI, Booren AM, Pearson AM, Buckley DJ. Effect of antioxidants on lipid stability in restructured beef steaks. J Food Sci. 1988 Mar;53(2):656-7. doi: 10.1111/j.1365-2621.1988.tb07780.x
18. Krzywicki K. The determination of haem pigment in meat. Meat Sci. 1982 Jul;7(1):29-36. doi: 10.1016/0309-1740(82)90095-X
19. Brasil. Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Instrução Normativa - IN n° 161, de 01 de julho de 2022. Estabelece os padrões microbiológicos dos alimentos. Brasília (DF): Diário Oficial da União; 6 jul 2022. Available from: https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-161-de-1-de-julho-de-2022-413366880.

20. Andrews WH, Wang H, Jacobson A, Beilei G, Zhang G, Hammack T. Manual Analítico Bacteriológico (BAM). Silver Spring: Food and Drug Administration; 2023.
21. Bertol T, Oliveira EA, Santos Filho JI. Composição e aspectos de qualidade da carne suína. In: Bertol T, editor. Estratégias nutricionais para melhoria da qualidade da carne suína. Brasília (DF): Embrapa; 2019. p. 13-38.
22. Brasil. Ministério da Agricultura, Pecuária e Abastecimento, Secretaria de Defesa Agropecuária. Instrução Normativa SDA/MAA n° 21, de 31 de jul de 2000. Aprova os regulamentos técnicos de identidade e qualidade de patê, de bacon ou barriga defumada e de lombo suíno. Diário Oficial da União. 03 ago 2000. Available from: https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-animal/legislacao.
23. Damodaran S, Parkin KL. Fennema's food chemistry. 5. ed. Boca Raton (FL): CRC Press; 2017.
24. Caballero-Córdoba GM, Sgarbieri VC. Nutritional and toxicological evaluation of yeast (*Saccharomyces cerevisiae*) biomass and a yeast protein concentrate. Sci Food Agric. 2000 Feb;80(3):341-51. doi: 10.1002/1097-0010(200002)80:3<341::AID-JSFA533>3.0.CO;2-M
25. Christ JJ, Blank LM. *Saccharomyces cerevisiae* containing 28% polyphosphate and production of a polyphosphate-rich yeast extract thereof. FEMS Yeast Res. 2019 May;19(3):foz011. doi: 10.1093/femsyr/foz011
26. Wenda Ingredients [Internet]. NatureBind® phosphate alternative; [date unknown] [cited in 2025 Dec 22]. Available from: https://wendaingredients.com/products/phosphate-alternative/.
27. Beck PHB, Matiucci MA, Monge Neto AA, Feihrmann AC. Sodium chloride reduction in fresh sausages using salt encapsulated in carnauba wax. Meat Sci. 2021 May;175:108462. doi: 10.1016/j.meatsci.2021.108462
28. Verma SP, Sahoo J. Improvement in the quality of ground chevon during refrigerated storage by tocopherol acetate preblending. Meat Sci. 2000 Dec;56(4):403-13. doi: 10.1016/s0309-1740(00)00072-3
29. Simão S. Cereja das Antilhas. In: Simão S, editor. Manual de fruticultura. São Paulo: Agronomia Ceres; 1971. p. 477-85.
30. Terra NN, Cichoski AJ, Freitas RJSD. Microbiological and physical chemical aspects in the internal parts pig palette cured, matured and fermented during processing and storage. Cienc Rural. 2008 Aug;38(4):1118-24. doi: 10.1590/S0103-84782008000400033
31. Brewer MS, Zhu LG, Bidner B, Meisinger DJ, McKeith FK. Measuring pork color: effects of bloom time, muscle, pH and relationship to instrumental parameters. Meat Sci. 2001 Feb;57(2):169-76. doi: 10.1016/s0309-1740(00)00089-9
32. Santos VMO, Caldara FR, Seno LO, Feijó GLD, Paz ICLA, Garcia RG, et al. Marinade with alkaline solutions for the improvement of pork quality. Pesqui Agropecu Bras. 2012 Nov;47(11):1655-62.
33. Lawrie RA, Ledward DA. Lawrie's meat science. 7 ed. Boca Raton: CRC; 2006.
34. Olivo R, Shimokomaki M. Carnes no caminho da pesquisa. Cocal do Sul (BR): Imprint; 2001.
35. Realini CE, Guàrdia MD, Díaz I, García-Regueiro JA, Arnau J. Effects of acerola fruit extract on sensory and shelf-life of salted beef patties from grinds differing in fatty acid composition. Meat Sci. 2015 Jan;99:18-24. doi: 10.1016/j.meatsci.2014.08.008
36. Shah MA, Bosco SJ, Mir SA. Plant extracts as natural antioxidants in meat and meat products. Meat Sci. 2014 Sep;98(1):21-33. doi: 10.1016/j.meatsci.2014.03.020
37. Galarz L, Fonseca G, Prentice-Hernández C. Crescimento microbiano em produtos à base de peito de frango durante simulação da cadeia de abastecimento Microbial growth in chicken breast products during supply chain simulation. Ci Tecnol Aliment. 2010 Dez;30(4):870-7. doi: 10.1590/S0101-20612010000400006
38. Majou D, Christieans S. Mechanisms of the bactericidal effects of nitrate and nitrite in cured meats. Meat Sci. 2018 Nov;145:273-84. doi: 10.1016/j.meatsci.2018.06.013
39. Jin SK, Choi JS, Yang HS, Park TS, Yim DG. Natural curing agents as nitrite alternatives and their effects on the physicochemical, microbiological properties and sensory evaluation of sausages during storage. Meat Sci. 2018 Dec;146:34-40. doi: 10.1016/j.meatsci.2018.07.032