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Clean label fresh sausage: characteristics throughout its shelf life

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ABSTRACT: Consumers are increasingly demanding meat products that are natural and synthetic additive-free. This study aimed to develop a fresh pork sausage without synthetic additives and to evaluate the effects of this formulation on its physicochemical, microbiological, and sensory characteristics throughout its shelf life. Six formulations were prepared: FC = control formulation; F1 = celery extract (0.7 %) and rosemary extract with curry (0.06 %); F2 = celery extract (1 %) and rosemary extract with curry (0.06 %); F3 = celery extract (0.7 %) and rosemary extract with curry (0.1 %); F4 = celery extract (1 %) and rosemary extract with curry (0.1 %); F5 = celery extract (0.85 %) and rosemary extract with curry (0.08 %). The formulations with plant extracts exhibited a lower ($p < 0.05$) sodium content, and no differences ($p > 0.05$) were observed in the levels of lipid oxidation compared to FC. No direct relation was observed between the concentration of added celery extract and the residual nitrite content. Fresh sausage with plant extracts demonstrated a microbiological pattern like the control formulation during storage. The sensory acceptance of the formulations with plant extracts was similar to that of FC. The fresh sausages containing plant extracts showed characteristics like those of the conventional product, with these similarities becoming more pronounced throughout storage.

Keywords: healthy foods, meat products, sensory acceptance

Introduction

Demand for meat products with new clean-label concepts has recently increased (Alves Junior et al., 2023). This demand has prompted research into plant extracts, commonly used as seasonings, as potential substitutes for synthetic compounds due to their high polyphenol contents (Flores and Toldrá, 2021). Using plant extracts to prepare meat products represents an alternative approach to producing healthier foods (Martínez-Zamora et al., 2021).

Fresh sausage is one of the most popular meat products in the world and represents a way of enhancing the meat value (Ali et al., 2018). Fresh sausage is highly perishable due to its composition (high water, protein, and lipid contents) and manufacturing process. This occurs because grinding the meat increases its contact surface, enhancing lipid oxidation. Fresh sausage has a shelf life of 10 to 15 days when stored under temperature-controlled refrigeration (Torrieri et al., 2011).

The addition of nitrite and nitrate (curing salts) in sausages is related to the development of color, flavor, texture, and microbiological safety (Flores and Toldrá, 2021). Studies developed in the 1960s reported that nitrites have the potential to form carcinogenic nitrosamines under certain conditions (Alahakoon et al., 2015). Since then, numerous studies have been carried out to identify alternatives to replace these additives (Sbardelotto et al., 2023).

The use of celery extract as a substitute for synthetic additives is a promising avenue of research because of its antioxidant potential, and being a nitrate source (Flores and Toldrá, 2021). Its mild flavor and absence of pigmentation make celery extract an ideal candidate for use in food products, as it does not interfere with the sensory characteristics of the final products (Jin et al., 2018).

Meat and its derivatives have a complex composition and are highly susceptible to oxidative reactions (Lorenzo et al., 2019). Phenolic compounds, which are widely available in herbs, spices, and fruits, have great potential for application against oxidation in meat (Demarco et al., 2022). In this sense, rosemary extract exhibits high antioxidant potential due to the presence of carnosic acid, carnosol, rosmarinic acid, rosmanol, and ursolic acid (Rajeev et al., 2017). Similarly, curry leaf extract contains hydrocarbons derived from monoterpenes and alcohols with recognized antioxidant activity (Biswas et al., 2012).

Several authors have reported the use of plant extracts as a preservative and natural antioxidant in meat products, but few studies were found on their effects on the shelf life of fresh pork sausage. The objective of this study was to use an associative approach of natural sources of nitrites and antioxidants to develop a fresh pork sausage without synthetic additives (clean label) and evaluate its microbiological safety effects on its physicochemical and sensory characteristics throughout its shelf life.

Materials and Methods

Material

The study was carried out in a meat products industry in Paraná state, Brazil (–26°06′43″ S, –53°01′79″ W, altitude 650 m). The fresh sausage was produced with pork (shank) and back fat from Large White pigs, with an average live weight of 95 kg. A partner industry provided the ingredients, additives, wraps, and packaging.

The fermented celery extract (max nitrite content of 20,000 mg kg^{-1}) and the rosemary extract with curry (a condiment containing flavoring powder prepared from rosemary resin oil and curry leaf resin oil) were provided by Synthite Brasil.

Methods

Preparation of fresh sausage

Six fresh sausage formulations weighing 27 kg each were prepared using the meat products industry's standard formulation. One control formulation (FC) included synthetic preservatives and antioxidants, and five formulations were created using natural sources of nitrites and antioxidants.

Pork shank meat with a pH of 5.8 (80 %) and pork back fat (20 %) were cooled to 4 °C and ground with a 12-mm opening disc in a grinder (MADO GmbH). The resulting mixture was manually mixed with water (5 %), salt and spices (parsley, chives, garlic and black pepper = 3.85 %), and beet coloring (0.2 %). The FC received seasoning for Tuscan sausage (1.85 %), the antioxidant sodium erythorbate (0.42 %), and sodium nitrite (0.015 %) from a synthetic source. Celery extract and rosemary extracts with curry were added to the other five formulations as natural source of nitrite and antioxidants. F1 = celery extract (0.7 %) and rosemary extract with curry (0.06 %); F2 = celery extract (1 %) and rosemary extract with curry (0.06 %); F3 = celery extract (0.7 %) and rosemary extract with curry (0.1 %); F4 = celery extract (1 %) and rosemary extract with curry (0.1 %); F5 = celery extract (0.85 %) and rosemary extract with curry (0.08 %). After perfect homogenization, the mass was kept for 12 h at 4 °C.

After rest, the mass was stuffed (Handtmann VF 610 Plus) into natural pork casings previously hydrated in a saline solution, forming sections of approximately 5 cm in length. The sausages were then vacuum-packed, identified, and stored in a cold chamber at 2 °C. The concentrations for the natural preservatives and antioxidants were defined according to the manufacturer's indications for use and preliminary tests (data not shown).

Physicochemical, microbiological, and sensory characteristics of fresh sausage

The characteristics of fresh sausage formulations were evaluated through physicochemical, microbiological, and sensory analyses.

Proximate composition

The moisture content was quantified by gravimetric analysis in accordance with the AOAC (2012) guidelines. The protein content was determined by the Kjeldahl method, also in accordance with the AOAC (2012) guidelines. The lipid content was assessed by the Soxhlet method, in line with the AOAC (2012) protocol. All analyses were performed in triplicate.

Na^{+} and Ca^{2+} concentrations on a dry basis

The sodium was determined using the methodology described by the AOAC (2012), employing a flame photometer with a sodium filter (Micronal B462). The results were expressed in grams of sodium per 100 g^{-1} of the sample.

The calcium content was quantified using the permanganometry method, as proposed by the AOAC (2012). The results are expressed in grams of calcium per 100 g^{-1} of the sample. All analyses were performed in triplicate.

Water activity (a_w)

The water activity (a_w) was quantified using a LabTouch water activity meter (Novasina AG). For analysis, the sample was sectioned transversally and placed within the internal chamber of the equipment at a controlled temperature of 25 °C. The analysis was performed in triplicate.

pH value

The pH was determined in a previously calibrated MS Tecpon pH meter (mPA-210), according to the method described by AOAC (2012). The analysis was performed on days 1, 5, 10, and 15 of the storage. All analyses were performed in triplicate.

Lipid oxidation – TBARS

The extent of lipid oxidation was quantified by evaluating thiobarbituric acid reactive species (TBARS), per the methodology described by Fernández et al. (1997). The analyses were performed on days 1, 5, 10, and 15 of fresh sausage storage. The results were expressed in mg malonaldehyde (MDA) kg^{-1} of the sample. All evaluations were performed in triplicate.

Residual nitrite

Residual nitrite analysis was performed following the method described by the AOAC (2012). Samples were ground and homogenized for colorimetric measurement of nitrite concentration. The absorbance at 540 nm was quantified in a spectrophotometer (model 800XI, FEMTO). The analyses were conducted on days 1, 5, 10, and 15 of fresh sausage storage. The evaluations were performed in triplicate, with the results expressed in mg kg^{-1} of nitrite.

Color analysis

Color determination was performed using the methodology described by Fieira et al. (2018), using a colorimeter (Minolta CR-300/CIELAB System). Eight readings were performed at randomly selected points of the samples without the casing. The coordinates lightness value (L*), red/green (a*), and yellow/blue (b*) were determined on days 1, 5, 10, and 15 of storage of fresh sausage.

Texture profile

The texture profile was performed according to the methodology described by Wambui et al. (2016), with minor modifications. The analyses used a texturometer (Stable Microsystems) model TA-XTplus with a 2 kg load cell. Hardness, chewiness, adhesiveness, gumminess, and cohesiveness were determined in samples cut perpendicularly into 2 cm high slices at room temperature (23 ± 2 °C). Tests were performed with samples being compressed by 5 mm at 5 s intervals, using a P/40 probe with pre-test, test, and post-test speeds of 2, 4, and 2 mm s^{-1}, respectively. All evaluations were performed in triplicate.

Microbiological analyses

Microbiological analyses were performed on days 2 and 15 of storage to determine the presence of *Salmonella* spp., thermotolerant coliforms, total coliforms, and mesophilic aerobes (Salfinger and Tortorello, 2015).

Hedonic test

The test participants were lecturers and students randomly selected at the Universidade Tecnológica Federal do Paraná to consume fresh sausage. The participants (n = 29) were aged between 21 and 56, with 15 females and 14 males. The test used a 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; and 9 = like extremely). The respondents were asked to taste six samples and indicate their overall liking (Skubic et al., 2018).

The participants were given an informed consent form prior to the start of the questionnaire. The Ethics Committee for Human Research of the Universidade Tecnológica Federal do Paraná approved the study (CAAE N° 50340021.7.0000.0177). Sensory evaluation was performed on the 10^{th} day of storage.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), and Tukey's test application to compare means at a 5 % significance level ($p < 0.05$). The factor analysis was applied to the non-collinear physicochemical variables to select variables with high communalities, with subsequent principal component analysis (PCA). The multinomial logistic regression analysis was applied to evaluate the effect of the age and gender variables on the acceptance of fresh sausage. The data were analyzed using the Statistica 12.7 program.

Results

Proximate composition, Na^+, Ca^{+2} and water activity

The proximate composition, Na^+ and Ca^{+2} concentration on a dry basis, and water activity of fresh sausage formulations with natural substitutes for nitrites and antioxidants are presented in Table 1.

pH value, lipid oxidation, and residual nitrite

The pH value, lipid oxidation, and nitrite contents of fresh sausage formulations with natural substitutes for nitrites and antioxidants at different storage periods are presented in Table 2.

Color analysis

The color of meat greatly influences on the consumer's acceptance and is directly related to their state of conservation. The results of the color analysis of sausage formulations are shown in Table 3.

Table 1 – Proximate composition, Na^+ and Ca^{+2} concentration on a dry basis, and water activity of fresh sausage formulations with natural substitutes for nitrites and antioxidants.

Formulations	Moisture	Lipids	Protein	Na^+	Ca^{+2}	a_w
	------ % ------			------ g 100 g^{-1} ------		
FC	63.15 ± 00.73^{ab}	12.33 ± 00.04^{a}	16.27 ± 00.35^{a}	1.26 ± 0.05^{a}	0.060 ± 0.000^{ab}	0.963 ± 0.000^{a}
F1	64.24 ± 00.90^{ab}	12.57 ± 00.12^{a}	15.80 ± 00.89^{a}	0.44 ± 0.01^{e}	0.048 ± 0.000^{b}	0.963 ± 0.000^{a}
F2	62.03 ± 00.25^{ab}	13.26 ± 00.49^{a}	15.86 ± 00.82^{a}	0.63 ± 0.01^{d}	0.075 ± 0.010^{a}	0.970 ± 0.000^{a}
F3	66.20 ± 00.90^{a}	13.09 ± 00.47^{a}	15.88 ± 00.38^{a}	0.86 ± 0.13^{c}	0.056 ± 0.000^{ab}	0.966 ± 0.000^{a}
F4	66.18 ± 00.97^{a}	13.09 ± 00.45^{a}	16.09 ± 00.56^{a}	0.97 ± 0.06^{b}	0.063 ± 0.000^{ab}	0.970 ± 0.000^{a}
F5	56.79 ± 00.80^{b}	12.89 ± 00.07^{a}	16.48 ± 00.55^{a}	0.82 ± 0.00^{c}	0.062 ± 0.000^{ab}	0.970 ± 0.000^{a}

FC = control formulation = sodium nitrite (0.015 %) and antioxidant (0.42 %) from a synthetic source; F1= celery extract (0.7 %) and rosemary extract with curry (0.06 %); F2 = celery extract (1 %) and rosemary extract with curry (0.06 %); F3 = celery extract (0.7 %) and rosemary extract with curry (0.1 %); F4 = celery extract (1 %) and rosemary extract with curry (0.1 %); F5 = celery extract (0.85 %) and rosemary extract with curry (0.08 %); a_w = water activity. Mean values ± standard deviation. Means with equal lowercase letters in the same column do not differ ($p > 0.05$) by Tukey's Test.

Texture profile

Texture is an important quality parameter for meat and its derivatives, directly influencing consumers acceptance of products (Sbardelotto et al., 2023). The values for the texture profile of the fresh sausage formulations are presented in Table 4.

Principal component analysis (PCA)

To better understand the effect of replacing synthetic additives for plant extracts on the physical-chemical characteristics of fresh sausage formulations, a factor analysis was performed to identify variables with commonalities equal to or greater than 0.7, which were then subjected to further PCA. The factor analysis results indicated that the variables with the most significant influence on the formation of the groups were: adhesiveness, hardness, gumminess, chewiness, red color, and cohesion.

Microbiological analyses

Microbiological evaluations were carried out on the second and 15th day of storage to verify the sanitary quality of fresh sausages and the antimicrobial properties

Table 2 – The pH value, lipid oxidation, and nitrite contents of fresh sausage formulations with natural substitutes for nitrites and antioxidants in different storage periods.

Day	Formulations					
	pH value					
	FC	F1	F2	F3	F4	F5
1	6.00 ± 0.01^{cAB}	6.12 ± 0.01^{aA}	6.09 ± 0.03^{abA}	6.05 ± 0.01^{bcA}	6.13 ± 0.02^{aA}	6.10 ± 0.01^{abA}
5	6.11 ± 0.04^{aAB}	6.19 ± 0.03^{aA}	6.14 ± 0.03^{aA}	6.11 ± 0.05^{aA}	6.14 ± 0.01^{aA}	6.13 ± 0.01^{aA}
10	6.35 ± 0.38^{aA}	6.12 ± 0.07^{aA}	6.08 ± 0.01^{aA}	6.07 ± 0.00^{aA}	6.08 ± 0.01^{aB}	6.14 ± 0.01^{aA}
15	5.76 ± 0.05^{aC}	5.70 ± 0.16^{aB}	5.59 ± 0.02^{aB}	5.58 ± 0.03^{aB}	5.60 ± 0.01^{aC}	5.62 ± 0.03^{aB}
TBARS (mg MDA kg^{-1})						
1	0.17 ± 0.07^{aA}	0.25 ± 0.07^{aA}	0.16 ± 0.07^{aA}	0.24 ± 0.11^{aA}	0.20 ± 0.10^{aA}	0.24 ± 0.11^{aA}
5	0.18 ± 0.05^{aA}	0.23 ± 0.03^{aA}	0.19 ± 0.07^{aA}	0.24 ± 0.09^{aA}	0.13 ± 0.01^{aA}	0.13 ± 0.01^{aA}
10	0.17 ± 0.06^{aA}	0.22 ± 0.07^{aA}	0.13 ± 0.05^{aA}	0.13 ± 0.02^{aA}	0.17 ± 0.05^{aA}	0.18 ± 0.05^{aA}
15	0.23 ± 0.09^{aA}	0.29 ± 0.06^{aA}	0.31 ± 0.26^{aA}	0.16 ± 0.00^{bA}	0.17 ± 0.01^{bA}	0.28 ± 0.00^{aA}
Nitrite (mg kg^{-1})						
1	81.71 ± 0.52^{cA}	130.96 ± 0.76^{aA}	124.83 ± 0.29^{abA}	115.04 ± 0.47^{bB}	125.55 ± 0.26^{abA}	124.23 ± 0.63^{abA}
5	82.60 ± 0.26^{bA}	123.73 ± 0.32^{aB}	128.33 ± 0.90^{aA}	125.39 ± 0.44^{aA}	126.94 ± 0.81^{aA}	129.90 ± 0.10^{aA}
10	78.68 ± 0.41^{dA}	111.40 ± 0.90^{bcC}	125.00 ± 0.27^{aA}	109.52 ± 0.38^{cBC}	114.22 ± 0.37^{bB}	124.50 ± 0.02^{aA}
15	74.52 ± 0.61^{bB}	107.66 ± 0.52^{aD}	105.33 ± 0.82^{abB}	106.02 ± 0.61^{aC}	103.35 ± 0.04^{aC}	106.38 ± 0.78^{abB}

TBARS = thiobarbituric acid reactive species; FC = control formulation = sodium nitrite (0.015 %) and antioxidant (0.42 %) from a synthetic source; F1 = celery extract (0.7 %) and rosemary extract with curry (0.06 %); F2 = celery extract (1 %) and rosemary extract with curry (0.06 %); F3 = celery extract (0.7 %) and rosemary extract with curry (0.1 %); F4 = celery extract (1 %) and rosemary extract with curry (0.1 %); F5 = celery extract (0.85 %) and rosemary extract with curry (0.08 %). Mean values ± standard deviation. Means with equal lowercase letters in the same row do not differ (p > 0.05) by Tukey's Test. Means with equal capital letters in the same column do not differ (p > 0.05) by Tukey's Test.

Table 3 – Color values of fresh sausage formulations with natural substitutes for nitrites and antioxidants.

Color	Days	Formulations					
		FC	F1	F2	F3	F4	F5
L*	1	43.83 ± 1.00^{aA}	41.68 ± 1.61^{aA}	47.06 ± 1.56^{aA}	42.74 ± 1.60^{aB}	43.49 ± 1.50^{aA}	42.96 ± 1.44^{aA}
	5	43.27 ± 1.50^{aA}	41.30 ± 1.97^{aA}	46.51 ± 1.66^{aA}	43.29 ± 1.60^{aB}	43.54 ± 1.01^{aA}	46.49 ± 1.63^{aA}
	10	46.41 ± 1.90^{aA}	49.40 ± 1.66^{aA}	44.76 ± 1.69^{aA}	44.19 ± 1.50^{aAB}	46.28 ± 1.36^{aA}	49.15 ± 1.25^{aA}
	15	43.81 ± 1.44^{aA}	42.82 ± 1.89^{aA}	41.95 ± 1.48^{aA}	53.99 ± 1.85^{bA}	42.10 ± 1.85^{aA}	46.71 ± 1.67^{aA}
a*	1	16.79 ± 1.54^{aA}	17.41 ± 1.25^{aA}	13.54 ± 1.93^{aA}	16.44 ± 1.29^{aA}	16.93 ± 1.26^{aA}	14.97 ± 1.05^{aA}
	5	18.86 ± 1.53^{aA}	18.28 ± 1.93^{aA}	17.10 ± 1.21^{aA}	16.80 ± 1.55^{aA}	17.81 ± 1.30^{aA}	18.12 ± 0.35^{aA}
	10	15.35 ± 1.10^{aA}	13.39 ± 1.63^{aA}	17.90 ± 1.57^{aA}	13.76 ± 1.83^{aA}	12.27 ± 1.10^{aA}	14.17 ± 1.72^{aA}
	15	19.08 ± 1.81^{aA}	17.35 ± 1.02^{aA}	16.20 ± 1.56^{aA}	12.06 ± 1.62^{aA}	17.97 ± 1.15^{aA}	14.84 ± 1.29^{aA}
b*	1	6.98 ± 0.88^{aA}	8.90 ± 1.81^{aA}	9.01 ± 1.51^{aA}	8.80 ± 1.74^{aA}	8.80 ± 1.44^{aA}	8.47 ± 1.84^{aA}
	5	8.16 ± 1.33^{aA}	10.19 ± 1.34^{aA}	10.94 ± 0.86^{aA}	8.25 ± 1.77^{aA}	10.72 ± 0.67^{aA}	10.34 ± 0.90^{aA}
	10	7.33 ± 0.97^{aA}	9.16 ± 0.42^{aA}	9.82 ± 1.57^{aA}	7.95 ± 0.50^{aA}	7.79 ± 0.51^{aA}	8.11 ± 1.38^{aA}
	15	8.46 ± 1.36^{aA}	8.29 ± 1.17^{aA}	8.56 ± 0.10^{aA}	10.16 ± 1.62^{aA}	8.36 ± 1.03^{aA}	7.45 ± 1.39^{aA}

L* = lightness value; a* = red/green; b* = yellow/blue. FC = control formulation = sodium nitrite (0.015 %) and antioxidant (0.42 %) from a synthetic source; F1 = celery extract (0.7 %) and rosemary extract with curry (0.06 %); F2 = celery extract (1 %) and rosemary extract with curry (0.06 %); F3 = celery extract (0.7 %) and rosemary extract with curry (0.1 %); F4 = celery extract (1 %) and rosemary extract with curry (0.1 %); F5 = celery extract (0.85 %) and rosemary extract with curry (0.08 %). Mean values ± standard deviation. Means with equal lowercase letters in the same row do not differ (p > 0.05) by Tukey's Test. Means with equal capital letters in the same column do not differ (p > 0.05) by Tukey's Test.

of plant extracts. The results of the search for *Salmonella* spp., thermotolerant and total coliforms, and mesophilic aerobic count are presented in Table 5.

Hedonic test

The sensory test was applied to verify the acceptance of fresh sausages produced with plant extracts by consumers. The values attributed by the participants of the sensory panel ranged from F2 (6.00 ± 0.00) to F1 (7.17 ± 0.04). For the FC, values of 7.08 ± 0.01 were assigned.

The multinomial logistic regression was applied to the formulation that received the highest acceptance test score (F1) to explore consumers' acceptance of fresh sausage and assess the impacts of age and gender on the hedonic score. The independent variables of age and gender of the participants did not show influence (p > 0.05) on the acceptance of fresh sausage (data not shown).

Discussion

The Brazilian legislation determines a maximum moisture content of 70 %, lipids of 30 %, and a

Table 4 – Texture profile of fresh sausage formulations with natural substitutes for nitrites and antioxidants in different storage periods.

Parameter	Days	Formulations					
		FC	F1	F2	F3	F4	F5
Hardness (N)	1	0.797 ± 0.21^{aB}	0.705 ± 0.09^{aB}	0.779 ± 0.16^{aB}	0.622 ± 0.13^{bB}	0.628 ± 0.00^{bB}	0.729 ± 0.19^{aB}
	5	1.139 ± 0.00^{aAB}	0.871 ± 0.22^{abB}	0.775 ± 0.06^{bB}	0.758 ± 0.12^{bB}	0.747 ± 0.06^{bB}	0.680 ± 0.12^{bB}
	10	1.536 ± 0.33^{aA}	1.243 ± 0.31^{bA}	1.332 ± 0.17^{aA}	1.202 ± 0.13^{bA}	1.208 ± 0.18^{bA}	1.332 ± 0.28^{aA}
	15	1.581 ± 0.25^{aA}	1.290 ± 0.25^{bA}	1.094 ± 0.58^{bA}	1.182 ± 0.37^{bA}	1.081 ± 0.07^{bA}	1.148 ± 0.29^{bA}
Chewiness (N mm)	1	0.436 ± 0.09^{aB}	0.383 ± 0.04^{bB}	0.474 ± 0.17^{aB}	0.368 ± 0.08^{bB}	0.392 ± 0.03^{bB}	0.433 ± 0.12^{aB}
	5	0.629 ± 0.11^{aAB}	0.435 ± 0.10^{bB}	0.404 ± 0.02^{bB}	0.318 ± 0.05^{bB}	0.352 ± 0.04^{bB}	0.332 ± 0.11^{bB}
	10	0.843 ± 0.17^{aA}	0.547 ± 0.11^{bA}	0.352 ± 0.30^{bB}	0.431 ± 0.17^{bB}	0.560 ± 0.06^{bA}	0.511 ± 0.28^{bB}
	15	0.896 ± 0.26^{aA}	0.747 ± 0.35^{bA}	0.791 ± 0.08^{abA}	0.628 ± 0.12^{cA}	0.622 ± 0.23^{cA}	0.817 ± 0.24^{aA}
Adhesiveness (N s^{-1})	1	–0.112 ± 0.00^{aA}	–0.156 ± 0.10^{aA}	–0.227 ± 0.12^{aA}	–0.148 ± 0.08^{aA}	–0.154 ± 0.05^{aB}	–0.265 ± 0.15^{aA}
	5	–0.518 ± 0.65^{aA}	–0.525 ± 0.71^{aA}	–0.192 ± 0.08^{aA}	–0.174 ± 0.06^{aA}	–0.470 ± 0.22^{aA}	–0.540 ± 0.46^{aA}
	10	–0.153 ± 0.17^{aA}	–0.146 ± 0.05^{aA}	–0.104 ± 0.06^{aA}	–0.082 ± 0.03^{aA}	–0.086 ± 0.03^{aB}	–0.110 ± 0.05^{aA}
	15	–0.084 ± 0.01^{aA}	–0.094 ± 0.01^{aA}	–0.038 ± 0.03^{aA}	–0.066 ± 0.00^{aA}	–0.113 ± 0.01^{aB}	–0.205 ± 0.19^{aA}
Cohesiveness	1	0.645 ± 0.00^{aA}	0.643 ± 0.06^{aA}	0.667 ± 0.05^{aA}	0.653 ± 0.01^{aA}	0.637 ± 0.02^{aAB}	0.648 ± 0.01^{aA}
	5	0.680 ± 0.02^{aA}	0.678 ± 0.08^{aA}	0.682 ± 0.00^{aA}	0.669 ± 0.01^{aA}	0.697 ± 0.05^{aA}	0.707 ± 0.07^{aA}
	10	0.679 ± 0.06^{aA}	0.629 ± 0.04^{aA}	0.631 ± 0.01^{aA}	0.591 ± 0.01^{aB}	0.604 ± 0.01^{aB}	0.611 ± 0.06^{aA}
	15	0.657 ± 0.04^{aA}	0.587 ± 0.00^{aA}	0.650 ± 0.03^{aA}	0.594 ± 0.02^{aB}	0.627 ± 0.00^{aAB}	0.622 ± 0.05^{aA}
Gumminess (N)	1	0.512 ± 0.00^{aA}	0.517 ± 0.06^{aA}	0.625 ± 0.05^{aA}	0.604 ± 0.01^{aA}	0.530 ± 0.02^{aA}	0.574 ± 0.01^{aA}
	5	0.681 ± 0.02^{aA}	0.560 ± 0.08^{aA}	0.675 ± 0.00^{aA}	0.571 ± 0.01^{aA}	0.558 ± 0.05^{aA}	0.578 ± 0.07^{aA}
	10	0.593 ± 0.06^{aA}	0.547 ± 0.04^{aA}	0.588 ± 0.01^{aA}	0.482 ± 0.01^{aB}	0.512 ± 0.01^{aB}	0.653 ± 0.06^{aA}
	15	0.593 ± 0.04^{aA}	0.588 ± 0.00^{aA}	0.613 ± 0.03^{aA}	0.620 ± 0.02^{aB}	0.558 ± 0.00^{aAB}	0.623 ± 0.05^{aA}

FC = control formulation = sodium nitrite (0.015 %) and antioxidant (0.42 %) from a synthetic source; F1 = celery extract (0.7 %) and rosemary extract with curry (0.06 %); F2 = celery extract (1 %) and rosemary extract with curry (0.06 %); F3 = celery extract (0.7 %) and rosemary extract with curry (0.1 %); F4 = celery extract (1 %) and rosemary extract with curry (0.1 %); F5 = celery extract (0.85 %) and rosemary extract with curry (0.08 %). Mean values ± standard deviation. Means with equal lowercase letters in the same row do not differ (p > 0.05) by Tukey's Test. Means with equal capital letters in the same column do not differ (p > 0.05) by Tukey's Test.

Table 5 – Microbiological evaluation of fresh sausage formulations with natural substitutes for nitrites and antioxidants.

Parameters	Formulations (2nd day)					
	FC	F1	F2	F3	F4	F5
Salmonella spp.	Absence	Absence	Absence	Absence	Absence	Absence
Thermotolerant and total coliforms MLN g^{-1}	3 × 10^{0} ± 0.0^{bB}	3 × 10^{0} ± 0.0^{bB}	3.6 × 10^{0} ± 0.0^{bB}	9.2 × 10^{0} ± 0.0^{aB}	3 × 10^{0} ± 0.00^{bB}	3 × 10^{0} ± 0.0^{bB}
Mesophilic aerobic CFU g^{-1}	4.7 × 10^{3} ± 0.95^{aB}	2.8 × 10^{3} ± 0.32^{bB}	5.8 × 10^{3} ± 0.15^{aB}	2.5 × 10^{3} ± 0.00^{bB}	2.8 × 10^{3} ± 0.40^{bB}	2.6 × 10^{3} ± 0.11^{bB}
Parameters	Formulations (15th day)					
	FC	F1	F2	F3	F4	F5
Salmonella spp.	Absence	Absence	Absence	Absence	Absence	Absence
Thermotolerant and total coliforms MLN g^{-1}	2.2 × 10^{1} ± 0.05^{bA}	2.2 × 10^{1} ± 0.03^{bA}	3.6 × 10^{1} ± 0.07^{aA}	3.6 × 10^{1} ± 0.09^{aA}	3.6 × 10^{1} ± 0.01^{aA}	3.6 × 10^{1} ± 0.01^{aA}
Mesophilic aerobic CFU g^{-1}	1.58 × 10^{5} ± 0.09^{aA}	3.8 × 10^{5} ± 0.00^{aA}	1.95 × 10^{5} ± 0.03^{aA}	2.93 × 10^{5} ± 0.20^{aA}	1.87 × 10^{5} ± 0.02^{aA}	1.92 × 10^{5} ± 0.03^{aA}

FC = control formulation = sodium nitrite (0.015 %) and antioxidant (0.42 %) from a synthetic source; F1 = celery extract (0.7 %) and rosemary extract with curry (0.06 %); F2 = celery extract (1 %) and rosemary extract with curry (0.06 %); F3 = celery extract (0.7 %) and rosemary extract with curry (0.1 %); F4 = celery extract (1 %) and rosemary extract with curry (0.1 %); F5 = celery extract (0.85 %) and rosemary extract with curry (0.08 %). Mean values ± standard deviation. Means with equal lowercase letters in the same row do not differ (p > 0.05) by Tukey's Test. Means with equal capital letters in the same column do not differ (p > 0.05) by Tukey's Test. MLN = most likely number per gram of sample; CFU = colony forming unit per gram of sample.

minimum protein content of 12 % for fresh sausage (CDA, 2000). The F5 formulation exhibited significantly lower moisture content ($p < 0.05$) than formulations F3 and F4 (Table 1). The higher moisture content found in F3 and F4 could be related to the concentration of plant extracts used in these formulations. It is known that acids and phenols present in the extracts can react with proteins and improve the water-holding capacity (Zhou et al., 2020). Moisture values were like those described by Macari et al. (2021) for fresh sausage added with basil, thyme, and tarragon extract (58.10-71.48 %).

The lipid contents ranged from 12.36 to 13.26 % and exhibited no difference ($p > 0.05$) between formulations. Higher lipid contents (16.23-17.63 %) for fresh bovine sausage formulations added with pomegranate peel powder were reported by El-Nashi et al. (2015).

The fresh sausage formulations had a protein content between 15.80 and 16.48 %. No differences ($p > 0.05$) were observed between the formulations, all being above the minimum threshold established by the Brazilian legislation (12 %). Fresh sausage formulations can vary depending on the raw material availability, costs, and regional culture (Ali et al., 2018).

The FC presented a significantly higher sodium content ($p < 0.05$) than the formulations containing plant extracts (Table 1). These higher contents are possibly related to sodium in the condiment for Tuscan sausage (0.6 g Na^+ 100 g^{-1}) and sodium nitrite (0.005 g Na^+ 100 g^{-1}) added only in FC.

Formulations containing plant extracts presented different sodium contents due to the concentrations of extracts added to each formulation. Fermented celery extract and rosemary extract with curry contain 33.4 and 8 % Na^+, respectively. Thus, formulations with the addition of higher concentrations of extract (F4, F5, and F3) exhibited significantly higher sodium contents ($p < 0.05$). Nevertheless, all formulations showed a sodium reduction higher than 25 % of the conventional product (control formulation) and can be classified as a product with a reduced sodium content (ANVISA, 2012).

Brazilian legislation permits a maximum calcium content of 0.1 % on a dry basis (CDA, 2000). The calcium content in fresh sausage is established by legislation to permit the verification of the use of mechanically separated meat, which is prohibited in fresh sausage (CDA, 2000). All formulations demonstrated calcium contents below the limits established by the Brazilian legislation.

The water activity of fresh sausage formulations was similar, with no differences ($p < 0.05$) observed between formulations containing natural extracts and the control formulation. The Brazilian legislation does not establish limits for water activity in fresh sausage.

Sausage formulations containing natural extracts exhibited a higher pH value ($p < 0.05$) than the control formulation at the beginning of the storage period (day 1). The higher pH value is attributed to hydroxyl groups in the celery extract and rosemary extract with curry. Plant extracts contain several compounds, such as phenols, flavonoids, tannins, and saponins, which contain hydroxyl groups in their structure (Ferysiuk and Wójciak, 2020).

All formulations exhibited a significant reduction ($p < 0.05$) in the pH value throughout the storage period. The gradual decrease in pH is likely due to the production of organic acids by lactic acid bacteria (LAB). In this study, the development of LAB was possibly enhanced using vacuum packaging in fresh sausage formulations. Lactic acid bacteria (LAB) can thrive in various conditions, as they do not require oxygen, are resistant to inhibition by nitrite, and smoke, and can grow at relatively high salt concentrations (Demarco et al., 2022). There was no significant difference ($p > 0.05$) observed at the end of the storage period (15 days) in the pH value between formulations added with plant extracts and FC.

The results demonstrated that fresh sausages containing rosemary extract with curry showed no differences ($p > 0.05$) in lipid oxidation contents compared to the FC (Table 2). The addition of plant extract proved effective in preventing lipid oxidation of fresh sausages, thereby demonstrating its antioxidant potential. The formulations exhibited low malonaldehyde concentrations, with maximum values of 0.31 mg MDA kg^{-1}. Values below 1 mg MDA kg^{-1} in the TBARS analysis indicate that lipid oxidation did not occur (Jaberi et al., 2020).

The combination of rosemary extract with curry was effective in inhibiting lipid oxidation during storage. No significant variation ($p > 0.05$) was observed in malonaldehyde (MDA) concentration over the 15 days. The antioxidant activity of rosemary is attributed to phenolic compounds such as carnosic acid, carnosol, rosmarinic acid, rosmanol, and ursolic acid (Zhou et al., 2020). Curry leaves are a source of bioactive compounds with antioxidant and antimicrobial action (Lorenzo et al., 2019).

Formulations F3 and F4 exhibited lower MDA concentrations at the end of the storage period. The observed reduction in values may be attributed to the lower availability of MDA. Malonaldehyde can interact with proteins or undergo polymerization reactions, thereby reducing its availability to react with thiobarbituric acid (Grau et al., 2001).

Formulations containing fermented celery extract have nitrite contents that meet the requirements of Brazilian legislation (Table 2), which recommends maximum limits of 150 mg kg^{-1} of nitrite for meat products (ANVISA, 2019). The consumption of meat products has been associated with several adverse health implications, including high levels of salt and nitrites (Sbardelotto et al., 2023), which have been identified as a public health concern worldwide.

The FC exhibited a lower nitrite content ($p < 0.05$) than formulations containing plant extracts. According to the supplier, the maximum nitrite content permitted in fermented celery extract is 20 g kg^{-1} of extract. The residual nitrite values found in the fresh sausage formulations were higher than those indicated by the manufacturer, thereby demonstrating the difficulty in controlling nitrite contents when they originate from plant sources. One primary disadvantage of utilizing plant extracts is the variability in nitrate content, which can attributed to differences in the plants themselves or to variations within a single plant (Flores and Toldrá, 2021).

The formulations containing natural extracts demonstrated no direct correlation between the concentration of added celery extract and the residual nitrite content. Despite the addition of higher concentrations of celery extract in F2 and F4 (1 %), these formulations did not exhibit higher nitrite contents ($p > 0.05$) than the others. The use of reducing agents associated with herbal curing ingredients is crucial for achieving results like those traditionally cured (Posthuma et al., 2018).

All formulations gradually reduced the nitrite content over the storage period, with lower contents ($p < 0.05$) observed on the 15^{th} day. It is well established that nitrite depletion occurs during the processing and storage of meat products (Kilic et al., 2002). The reduction of nitrite in meat products is influenced by several factors, including the meat-to-water ratio, the presence of antioxidants, the pH value, the initial concentration, and the storage temperature, among others (Demarco et al., 2022).

Fresh sausage formulations containing plant extracts exhibited no differences ($p > 0.05$) regarding the L* coordinate in relation to the FC (Table 3). The formulations displayed intermediate lightness values (41.30 to 53.99) over the 15 days of storage, except for F3, which showed a significant increase ($p < 0.05$) in the L* value on the 15^{th} day of storage. The determination of the color of meat sausages through the coordinates L*, a*, and b* may present noise despite the reading at different points due to the fat cubes (Fieira et al., 2018).

The a* coordinate values for the fresh sausage formulations ranged from 12.27 to 19.08. The red color was predominant, which was expected due to the typical coloring characteristics of cured sausages. Among its numerous functions, nitrite is essential to produce the pinkish-red color characteristic of meat products (Sbardelotto et al., 2023).

The a* values did not differ significantly ($p > 0.05$) between the formulations with the addition of plant extracts and the control formulation (FC). The fermented celery extract, the source of nitrite used in this study, provided and maintained the characteristic red color of fresh sausage, similar to the synthetic source of nitrite used in the control formulation.

The b* coordinate demonstrated no significant statistical differences ($p > 0.05$) between formulations with the addition of plant extracts and the control formulation (FC). The findings differ from those by Jin et al. (2018), who observed higher b* values in pork sausages with the addition of celery powder. According to the authors, the increase in b* values was attributed to the pigments present in the plant extract.

Formulations F3 and F4 showed a reduction ($p < 0.05$) in hardness values relative to the FC on all evaluated storage days (Table 4). The lower hardness values are possibly due to the higher moisture content of these formulations (Table 1). All fresh sausage formulations added with plant extract presented hardness values significantly ($p < 0.05$) lower than FC at the end of the storage period (15 days). The reduction in the hardness of Western-style smoked sausage containing rosemary extract, grape seed extract, and green tea polyphenol was observed by Zhou et al. (2020).

The FC presented higher chewiness values than the formulations with plant extracts. Chewiness is known to be directly related to hardness. The reduction in hardness and chewiness in Spanish chorizo added with spices, fruits, and vegetables was reported by Martínez-Zamora et al. (2021).

All formulations, including FC, demonstrated a statistically significant increase ($p < 0.05$) in hardness and chewiness values over the days of storage. This increase is likely due to reduced moisture content in the fresh sausages. The prolongation of the storage time results in water loss, which subsequently leads to a gradual increase in the hardness and chewiness of meat sausages (Zhou et al., 2021).

No significant differences ($p > 0.05$) were observed between the adhesiveness values of formulations with plant extracts and the control formulation. Previous research has demonstrated an inverse correlation between fat content and adhesiveness values in fresh rabbit sausages (Wambui et al., 2016). In this study, all formulations had similar fat contents (Table 1).

All fresh sausage formulations, including the FC, showed similar ($p > 0.05$) cohesiveness and gumminess values. Gumminess is the energy required to disintegrate a semi-solid food, calculated from hardness versus cohesiveness (Rosenthal and Thompson, 2021). Cohesiveness is a crucial parameter in meat sausages, as it is directly correlated with an essential quality parameter, the slicing capacity.

The PCA of the formulations and their physicochemical characteristics are presented in Figure 1A and B, respectively. The accumulated percentage of information combined in the first two dimensions (86.4 %) demonstrates that the storage time is the main factor differentiating the samples, not the formulation types. This is evidenced by the observation that the formulations become more similar over the days. The formulations are not correlated with any of the variables of most significant impact on day 1 (lower right quadrant), as presented in Figure 1A. However, separation occurs over time. For instance, on the 5^{th} day of storage, there was a grouping of F1, F4, and F5 with the FC for the cohesion variable (Figure 1B). These observations demonstrate that

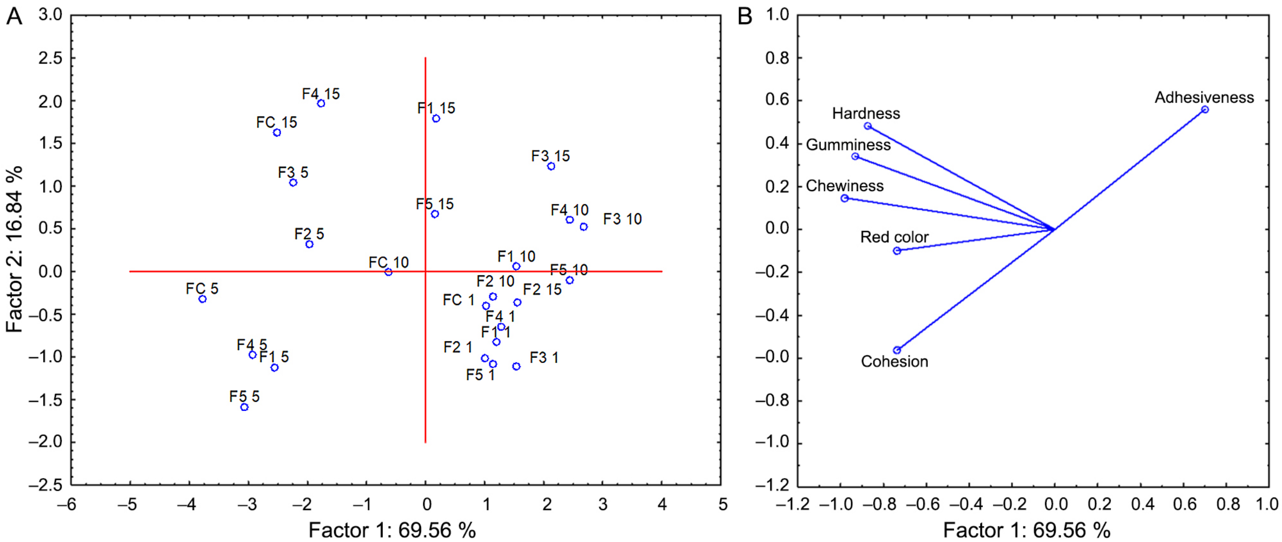

Figure 1 – Principal component analysis of fresh sausage formulations and their physicochemical characteristics. A) Fresh sausage formulations and B) physicochemical characteristics. FC = control formulation = sodium nitrite (0.015 %) and antioxidant (0.42 %) from a synthetic source; F1 = celery extract (0.7 %) and rosemary extract with curry (0.06 %); F2 = celery extract (1 %) and rosemary extract with curry (0.06 %); F3 = celery extract (0.7 %) and rosemary extract with curry (0.1 %); F4 = celery extract (1 %) and rosemary extract with curry (0.1 %); F5 = celery extract (0.85 %) and rosemary extract with curry (0.08 %). Physicochemical analysis (protein, lipids, moisture, Na^+ and Ca^{+2}, pH, water activity, lipid oxidation, residual nitrite, color and texture profile) performed on days 1, 5, 10, and 15 of storage.

the replacement of synthetic additives by plant extracts promotes similarities between the formulations over the days of storage, indicating their potential use.

The results indicate the absence of *Salmonella* spp. in FC and formulations produced with plant extracts in the two evaluated storage periods (Table 5). Regarding thermotolerant coliforms and total coliforms, an increase in the most likely number of microorganisms per gram of sample (MLN g^{-1}) was observed during the storage period. Formulations F2, F3, F4, and F5 on the 15^{th} day of storage, exhibited significantly higher counts (p < 0.05) than F1 and FC.

Over the long storage period, all fresh sausage formulations, including FC, demonstrated a significant increase (p < 0.05) in the count of mesophilic aerobic bacteria. Mesophilic bacteria are recognized as crucial microorganisms from a sanitary standpoint, as they contribute to the deterioration of meat and its derivatives during storage (Ali et al., 2018). No significant differences (p > 0.05) were observed between FC and formulations containing plant extracts at the conclusion of the storage period (15 days).

The results demonstrated that fresh sausage formulations containing plant extracts exhibited a microbiological pattern similar to that of the FC during the storage period. All formulations showed results in accordance with the microbiological standards established by Brazilian legislation (ANVISA, 2022).

The hedonic test revealed that the values assigned to formulation F1 were significantly (p < 0.05) higher than those assigned to the other formulations. No differences (p > 0.05) were observed between formulations with plant extracts (F2, F3, F4, and F5) and the control formulation (FC). The incorporation of natural additives in meat products has been demonstrated can influence their sensory characteristics and consumer acceptance (Sbardelotto et al., 2023). The findings of this study indicate that the incorporation of plant extracts did not influence the acceptability of fresh sausages, resulting in a similar outcome to that observed in sausages produced with synthetic additives.

The multinomial logistic regression indicates that age and gender do not significantly influence (p > 0.05) fresh sausage acceptance by consumers. Multivariate analysis is essential for sensory data, particularly in consumer tests, to identify which product and consumer characteristics are related to product acceptance (Symoneaux et al., 2012).

The efficacy of rosemary extract associated with curry in inhibiting lipid oxidation during storage was verified in this study. Formulations containing plant extracts demonstrated microbiological patterns similar to those of the FC throughout the storage period. Furthermore, the addition of extracts did not influence (p > 0.05) fresh sausage acceptance by consumers.

The results demonstrated that formulation F1 exhibited a significantly higher acceptance (p < 0.05) than the other formulations, including the FC. The storage time was found to be the primary factor differentiating the samples, with the formulations becoming increasingly similar over time.

Fresh sausages produced with fermented celery extract and rosemary extract with curry presented characteristics similar to the conventional product throughout the shelf life. This suggests that this technology may be an alternative to provide clean-label meat products to consumers.

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