International Journal of Food Science and Technology, 2025, 60(2), vvaf252
https://doi.org/10.1093/ijfood/vvaf252
Advance access publication: 6 December 2025
Original Article

Effect of ora-pro-nobis (*Pereskia aculeata* Miller) improved oxidative stability of sausage

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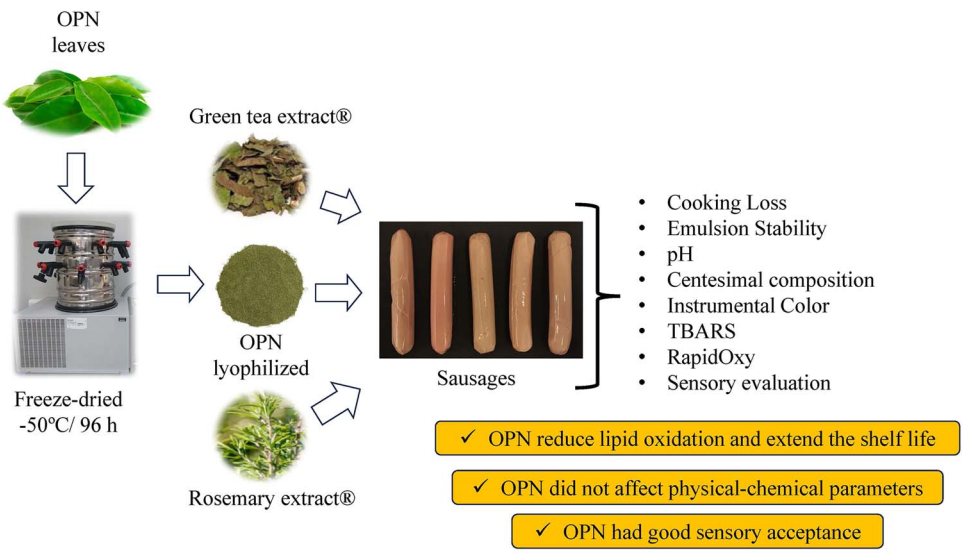
Abstract

Unconventional food plants have been widely studied because their leaves are rich in phenolic compounds, making them of great interest to the food and pharmaceutical industries. This study aimed to evaluate the effects of adding freeze-dried ora-pro-nobis (OPN) leaves to sausages on lipid oxidation and physicochemical attributes during refrigerated storage, and to compare them with other natural commercial antioxidants (rosemary [ROS] and green tea [GT] extracts) and a synthetic antioxidant (sodium erythorbate [ERY]). Five treatments were prepared: a control without antioxidants (CONs); 0.05 g/kg ERY; 0.05 g/kg freeze-dried OPN leaves; 0.05 g/kg ROS extract; and 0.05 g/kg GT extract. The ΔE^* analysis showed that OPN and ROS were more effective in maintaining colour stability of sausages during storage compared to GT and ERY, which exhibited greater colour variation. After 60 days, OPN showed the lowest thiobarbituric acid reactive substances values (0.134 mg of malondialdehyde [MDA]/kg sample), compared to CON (0.256 mg of MDA/kg sample) and ERY (0.196 mg of MDA/kg sample), indicating a greater inhibition of lipid oxidation, likely due to its high content of phenolic compounds and carotenoids, known for their radical scavenging. Induction time measured by RapidOxy was higher for ROS (67.80 min), GT (67.27 min), and OPN (52.94 min) than for CON (40.69 min) and ERY (40.33 min); that reflects greater oxidative stability and resistance to lipid degradation under accelerated oxidation conditions. Overall, OPN demonstrated promising potential as a natural antioxidant ingredient capable of enhancing colour stability and reducing lipid oxidation in sausages, aligning with the growing consumer demand for clean-label and plant-based formulations.

Keywords: sausage, lipid oxidation, ora-pro-nobis, natural antioxidant, shelf life,

Graphical abstract



Received: 14 August 2025. **Accepted:** 2 December 2025.

Introduction

The search for natural sources as alternatives to synthetic ingredients used by the food industry -combined with emerging consumer trends favouring healthier and more sustainably produced foods- has led to a growing interest in Unconventional Food Plants (UFPs). The term "UFP" refers to plant species whose edible parts (such as leaves, roots, fruits, and flowers) are not commonly included in most people's diets (Milião et al., 2022) The incorporation of these plants or their parts into food matrices has gained momentum alongside the advancement of phytochemical studies, which have highlighted their excellent nutritional value, as well as their biological and technological properties. These characteristics enable a wide range of applications in the food, pharmaceutical, and cosmetic industries. The high concentration of phenolic compounds found in the leaves of these plants has particularly encouraged researchers to explore their antioxidant potential, given the known role of these compounds in preserving meat and meat products (Munekata et al., 2021; Munir et al., 2025).

In the pursuit of developing healthier, safe, and clean label foods, the use of antioxidants derived from fruits and vegetables as replacements for synthetic additives in meat products has been considered an effective strategy to delay lipid oxidation and preserve the sensory characteristics (such as colour and flavour) of these products (Bellucci et al., 2022; Inguglia et al., 2023).

In addition to providing health benefits and consequently extending product shelf life, natural antioxidants tend to have greater consumer acceptance (Lorenzo et al., 2018). In contrast, the prolonged use of synthetic antioxidants may pose potential health risks, such as food allergies and chronic diseases (Kamala Kumari et al., 2019). In this context, scientific efforts have focused on obtaining antioxidants from various plant sources, which contain bioactive compounds capable of preventing oxidative chain reactions, prolonging shelf life, and sustainably meeting the growing consumer demand for healthier products (Munekata et al., 2020; Pateiro et al., 2021).

Plants are the primary natural source of phenolic compounds, with the polyphenols—such as phenolic acids, flavonoids, stilbenes, lignans, and tannins—being the most extensively studied group of natural antioxidants in recent years (Kumar et al., 2024).

Several studies have demonstrated that native Brazilian plants are rich sources of bioactive compounds, highlighting their antioxidant potential for the development of various food products, including meat products (Bellucci et al., 2021; da Cruz Nascimento et al., 2025; Fiorucci da Silva et al., 2025; Paglarini et al., 2025).

In recent years, the species *Pereskia aculeata* Miller—popularly known as "*ora-pro-nobis*" in Brazil and classified as an UFP, has gained prominence within the scientific community due to its excellent nutritional profile. It is a source of proteins, vitamins, fibres, and minerals, and also exhibits antioxidant, antimicrobial, antifungal and anti-inflammatory properties (Garcia et al., 2019; Nogueira Silva et al., 2023a; Silveira et al., 2020; Souza et al., 2016). Despite these beneficial properties and its potential applications in a variety of food products, ora-pro-nobis (OPN) is still considered an underutilised species, and few studies have evaluated its antioxidant and technological potential as a functional ingredient in emulsified meat products.

In this context, the objective of the present study was to characterise the freeze-dried leaves of OPN and to evaluate their effects as a natural antioxidant in sausages made with Mechanically Separated Chicken Meat. In addition, the study compared OPN with other commercial natural antioxidants (rosemary [ROS] and green tea [GT]) and with a synthetic antioxidant (sodium erythorbate [ERY]), which is commonly used in the meat industry. The evaluation included proximate composition, cooking loss, pH, emulsion stability (ES), instrumental colour, lipid oxidation, and sensory attributes during refrigerated storage.

Materials and methods

Preparation of leaves of OPN

Leaves of the OPN were harvested from a rural property in São Jose do Rio Preto, located in the northwest region of São Paulo State, Brazil, during the autumn/winter seasons of 2023. The leaves were transported to the Meat Laboratory at São Paulo State University (UNESP), São José do Rio Preto, campus. Upon arrival, the leaves were sanitised using a chlorinated solution (100 ppm), rinsed under running water, dried with absorbent paper, manually selected, and packed into sealed and labelled polyethylene bags. The samples were then stored in a freezer at −18 °C. Subsequently, the leaves were frozen at −70 °C in an ultra-freezer and subjected to freeze-drying. After freeze-dried, the dried leaves were ground using a knife mill to obtain a fine flour. The resulting powder was vacuum-packed and stored at −18 °C until further analysis. The use of freeze-dried *ora-pro-nóbis* leaves was selected to better concentrate the plant's bioactive compounds, simplify logistics, and avoid the use of chemical solvents, aligned with the clean-label trend. The chosen concentration (0.05%) corresponds to the ERY level commonly used in the Brazilian meat industry and was also supported by preliminary laboratory tests.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed according to the method described by de Carvalho et al. (2020), with some modifications. For extraction, 0.5 g of freeze-dried OPN leaves was diluted in a solution of 5 ml distilled water and 5 ml methanol. The mixture was vortexed and filtered. A total of 100 μl of the supernatant was mixed with 3,900 μl of the DPPH solution (60 μM in methanol). After incubating for 10 min at 37 °C, absorbance was measured at 515 nm. Results were expressed using Trolox as standard and reported as mg Trolox equivalents (TE) per gramme of sample.

Determination of total phenolic content of freeze-dried OPN leaves

Total phenolic content (TPC) was determined according to the method described by de Carvalho et al. (2020), with some modifications. Appropriate dilutions of freeze-dried OPN leaves (0.5 ml) were prepared with distilled water and mixed 2.5 ml of diluted Folin–Ciocalteu reagent (1:10, vol/vol with water), followed by the addition of 2 ml of 7.5% Na_2CO_3 solution. The mixture was incubated at 50 °C for 10 min, and the absorbance was measured at 760 nm. Results were expressed as mg of gallic acid equivalent (GAE) per 100 g of sample.

Sausages preparation

Two independent replicates (on different days with different raw materials and the same ingredients) were produced for each treatment. Sausage samples (batch size = 10 kg) were produced at the pilot plant of International Flavours & Fragrances (Barueri, Brazil), following the formulation presented in Table 1. The treatments were: CON (control without antioxidant), ERY (0.05 g/kg), freeze-dried OPN leaves (OPN, 0.05 g/kg), commercial ROS extract

Table 1. Sausages formulations.

Ingredients (%)	CON	ERY	OPN
MSCM	60.00	60.00	60.00
Lean pork retail	10.00	10.00	10.00
Chicken skin	7.00	7.00	7.00
Ice	13.56	13.52	13.52
Soy protein	4.00	4.00	4.00
Cassava starch	1.70	1.70	1.70
Sodium chloride	1.08	1.08	1.08
Sodium tripolyphosphate	0.25	0.25	0.25
Sodium pyrophosphate	0.100	0.100	0.100
Sodium nitrite	0.015	0.015	0.015
Sodium lactate	1.50	1.50	1.50
Carrageenan	0.20	0.20	0.20
†Condiment IFF	0.60	0.60	0.60
ERY	-	0.05	-
Freeze-dried leaves of OPN	-	-	0.05
ROS extract	-	-	-
GT extract	-	-	-
Total (%)	100	100	100

Note. †Condiment IFF: refined salt, soy fibre, natural onion flavour, natural cinnamon flavour, natural garlic flavour, natural bay leaf flavour, natural nutmeg flavour. CON = control without antioxidant; ERY = sodium erythorbate; OPN = ora-pro-nobis; ROS = rosemary; GT = green tea; MSCM = Mechanically Separated Chicken Meat.

(0.05 g/kg), and GT extract (0.05 g/kg). Commercial extracts were kindly provided by the company.

Initially, all raw materials were ground using a MEW 613 Grinder (Mado, Dornhan, Germany) with a 6 mm plate. Lean pork retail and Mechanically Separated Chicken Meat were then transferred to an MTK 662 *cutter* (Mado, Dornhan, Germany), where they were mixed with salt, sodium nitrite, phosphate, and part of the crushed ice to promote myofibrillar protein extraction. When the mixture reached a temperature of 7–8 °C, the remaining ingredients were gradually added to ensure that the final temperature of the batter did not exceed 10 °C. Fat (chicken skin) and the rest of the ice were incorporated at the end of the process. Cassava flour and the natural antioxidants (OPN, ROS, and GT) or artificial antioxidant (ERY) were added during the final stage of batter preparation. The sausage batter was then stuffed into 24 mm diameter cellophane casings using an EM20 Sausage Filler (Mainca, Barcelona, Spain). The sausages were cooked in a Unimatic 2,200 steam oven (Eller SRL, Lagundo, Italy) using the following temperature sequence: 65 °C for 25 min, followed by 70 °C for 25 min, and 80 °C until reaching an internal temperature of 72 °C (monitored with a thermocouple), which took approximately 8 min (Pinton et al., 2019). After cooking, the sausages were cooled under a cold-water shower. Casings were then removed, and the sausages were vacuum-packed using a 200S vacuum sealer (Selovac, São Paulo, Brazil) and stored under refrigeration at 4 °C until transport to the Meat Laboratory at UNESP (São José do Rio Preto campus, Brazil) until their use (analysis and preparation of the sausages).

Emulsion stability

The ES was determined according to the method of Schmiele et al. (2015), with some modifications. The samples were then cooked in a water bath (Cientec, Brazil) at 70 °C for 1 h. After cooking, the exuded liquid was removed. The percentage of weight loss was calculated based on the initial weight of the sample. ES (%) was calculated using *Equation 1*, which expresses the percentage of liquid loss (exudate) released during cooking in relation to the initial weight of raw emulsion sample.

$$\text{ES}\ (\%) = (100\text{–g}/100\text{g loss}) \quad (1)$$

Cooking loss

Cooking loss was calculated by measuring the weight of the sausage before and after cooking, according to *Equation 2*:

$$\text{Cooking loss}\ (\%) = \frac{(\text{Inicial weight} - \textit{Final weight})}{\text{Inicial weight}\ (\text{g})} \times 100 \quad (2)$$

pH

The pH values were measured directly in the sausages using a digital pH metre (mPA210, Tecnopon, São Paulo, Brazil) equipped with a penetration glass electrode, previously calibrated with two standard buffer solutions (pH 4 and 7) at room temperature. Three replicates were performed for each treatment.

Proximate composition

Proximate composition of sausages was determined according to the official methods of the Association of Official Analytical Chemistry (AOAC, 2007) for moisture, protein, and ash contents. Fat content was determined using the method proposed by Bligh and Dyer (1959). All analyses were performed in triplicate and the results were expressed as g/100 g of sample.

Instrumental colour

Instrumental colour measurements were taken on the internal surface of fresh sausages. A ColorFlex 45/0 colorimeter (Hunter-Lab, Reston, VA, USA) was used, previously calibrated and operated with D65 illuminant, a 0.75-inch aperture, 10° standard observer geometry, and a 2.5-inch glass sample cup, using Universal Software version 4.10. The CIELab colour system was used, and the parameters obtained were L^* (lightness), a^* (redness), and b^* (yellowness).

Two measurements were taken per sausage, using three sausage samples per treatment. The total colour difference (ΔE^*) for each treatment was used to determine the perceptible colour difference between samples and control samples and it was classified as small difference ($\Delta E < 1.5$), distinct ($1.5 < \Delta E < 3$), and very distinct ($\Delta E > 3$) (Pathare et al., 2013). In this study, it

was measured between CON and other treatments using the *Equation* 3.

$$\Delta E^* = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (3)$$

Lipid oxidation analysis

Thiobarbituric acid reactive substances (TBARS) values were determined using the method described by Vyncke (1975), with modifications. Briefly, 2 g of sausage sample was homogenised with 10 ml of 7.5% trichloroacetic acid for 2 min using a digital ultra-turrax homogeniser (IKA T18, IKA-Werke GmbH & Co. KG, Staufen, Germany), followed by centrifugation at 2.264 ×*g* for 10 min. The resulting supernatant was filtered, and 5 ml of thiobarbituric acid solution was added to 5 ml of the filtrate. The mixture was then incubated in a water bath (Nova Técnica, Piracicaba, Brazil) at 96 °C for 40 min. Absorbance was measured at 532 nm using a spectrophotometer. TBARS values were expressed as mg of malondialdehyde (MDA) per kg of sample. 1,1,3,3-Tetraethoxypropane was used as the standard.

Test of oxidation stability (RapidOxy)

The oxidative stability of the sample was assessed using RapidOxy 100 (Anton Paar, Blankenfelde-Mahlow, Germany), a microprocessor-controlled, automatic device designed to rapidly measure the oxidative stability of lipid-containing matrices under accelerated oxidation conditions. The RapidOxy test were determined using the method described by Difonzo et al. (2022), with modifications. Sausage samples were finely ground and approximately 5 g of sample were placed directly into the RapidOxy test vessel. Each sample was analysed in triplicate. The chamber was pressurised with oxygen to 700 kPa at the start of the test, and constant temperature of 140 °C. The induction time (expressed in minutes) was defined as the time required for a 5% drop in oxygen pressure.

Sensorial evaluation

Sensory acceptance of the sausages with 15 days after production was evaluated by 80 untrained panellists (students and staff) from UNESP, São José do Rio Preto, Brazil. The assessed attributes included colour, texture, flavour, and overall acceptability. Samples were cut into uniform pieces (1.5 cm thick) and served warm (~60 °C). Participants were provided with room-temperature water and salted crackers for palate cleansing. Each sample was coded, and the presentation was randomised using a sequential monadic approach, following a balanced complete block design, as proposed by MacFie et al. (1989). A nine-point hedonic scale was used (9—liked extremely; 8—liked very much; 7—liked moderately; 6—liked slightly; 5—neither liked nor disliked; 4—disliked slightly; 3—disliked moderately; 2—disliked very much; 1—disliked extremely). The sensory test was conducted in a single session. The samples used in the sensory evaluation met the microbiological standard required by Brazilian legislation (data not shown) (Brasil, 2022) and all panellists agreed to participate in the research and signed the Free and Informed Consent Term. The research was approved by the Research Ethics Committee of the Institute of Biosciences, Humanities and Exact Sciences, UNESP, São José do Rio Preto campus (Approval No. 6.687.525/2024).

Statistical analysis

Five treatments of sausage (CON, ERY, OPN, ROS, and GT) were produced and analysed during processing and refrigerated storage (1, 30, and 60 days). Sausage preparation and storage were repeated on two separate production days, with each treatment analysed in triplicate. Statistical analysis was performed using SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA). A general linear model was applied to analyse the data using analysis of variance. Treatment and storage time were considered fixed effects, while production replicate (batch) was treated as a random effect. The interaction between treatment and storage time was also evaluated. When significant differences were observed, Tukey's post-hoc test was used for mean comparison at a 95% confidence level ($p < .05$).

Chemical composition, ES, pH, and cooking loss were analysed only for treatment effects, as these parameters were measured once, on day 1 of storage. In contrast, colour, lipid oxidation, and sensory analysis were analysed considering both fixed effects (treatment and storage time). Specifically, colour and lipid oxidation were evaluated at 1, 30, and 60 days.

Results and discussion

Antioxidant activity (DPPH) and total phenolic content (TPC) of "OPN" leaves

In the present study, the antioxidant capacity of freeze-dried OPN leaves was 21.27 μmol TE/g sample (equivalent to 5322.69 μg TE/g), as determined by the DPPH assay, and the TPC was 19.76 mg GAE/g sample. The latter value is in close agreement with that reported by de Almeida et al. (2014), who obtained 19.34 mg GAE/g in OPN leaves dried at 60 °C. Other authors have also described OPN as a rich source of phenolic compounds with high antioxidant capacity. However, these properties can vary substantially depending on several factors, including the extraction method, solvent polarity, and sample characteristics such as geographic origin or leaf state, as highlighted by Baldin et al. (2016) and Naczk and Shahidi (2006). For instance, Cruz et al. (2021) reported that the extract obtained using 100% water showed the highest DPPH radical scavenging activity (27 mg Ascorbic Acid (AA)/g sample), compared to those extracted with 100% ethanol (14 mg AA/g) and 100% acetone (11 mg AA/g). Moreover, their most efficient extracts (OP (60% water +40% ethanol +0% acetone) and E4 (50% water +50% ethanol +0% acetone) reached 44 and 39 mg AA/g, respectively. Regarding TPC, the same authors reported 52 mg GAE/g for the aqueous extract, clearly higher than the values obtained with ethanol (40 mg GAE/g) and acetone (26 mg GAE/g).

Similarly, the concentration of individual phenolic compounds, directly associated with antioxidant capacity (da S Agostini-Costa et al., 2012; Pinto et al., 2012), has been shown to vary considerably with the geographic origin of the plant material, reflecting the influence of environmental and agronomic conditions. Thus, Souza (2014) identified chlorogenic, caffeic, p-coumaric, and ferulic acids in aqueous extracts of OPN leaves at varying concentrations, although chlorogenic acid consistently appeared as the most abundant. The phenolic compounds present in OPN, particularly flavonoids and phenolic acids, possess antioxidant properties that help neutralise free radicals and thereby reduce oxidative stress (Garcia et al., 2019). These mechanisms contribute to lipid stabilisation and highlight the potential of OPN as a natural antioxidant in food systems.

Physicochemical and nutritional properties of sausages

The results for ES, cooking loss, pH, and proximate composition of all treatments are presented in Table 2. No significant differences ($p > .05$) were observed among the treatments for the

Table 2. Evaluation of chemical composition, pH, ES, and cooking loss of sausages.

Parameters	CON	ERY	OPN	ROS	GT
Chemical composition (%)					
Moisture	65.35^{ab}	65.62^{a}	64.63^{bc}	63.82^{cd}	63.73^{d}
Protein	13.44	13.59	13.59	13.81	13.47
Lipid	12.84	12.31	12.35	13.69	12.84
Ash	3.70	3.59	3.72	3.66	3.68
pH	6.31	6.31	6.30	6.31	6.31
Emulsion Stability (%)	94.17	93.83	93.95	96.37	96.39
Cooking loss (%)	2.86	2.77	3.27	2.77	3.00

Note. Mean values in the same row (different treatment in the same day) with different superscript letters ($^{a–d}$) indicate significant difference ($p < .05$); SEM = standard error of the mean; T.: significance of treatment; n.s.: not significant; $p > .05$. ***$p < .001$. Treatments—CON: sausages prepared without antioxidant; ERY: sausage prepared with sodium erythorbate at 500 mg kg^{-1}; OPN: sausages prepared with OPN leaves at 500 mg kg^{-1}; ROS: sausages prepared with rosemary extract at 500 mg kg^{-1}; GT: sausage prepared with green tea extract at 500 mg kg^{-1}. (Tukey test, $p < .05$).

Table 3. Colour parameters (L*) of sausages during refrigerated storage.

Treatments					Days			
CON	ERY	OPN	ROS	GT	1	30	60	SE
59.89^{a}	60.09^{a}	58.36^{b}	59.03^{ab}	60.05^{a}	60.62^{a}	59.18^{b}	58.56^{b}	0.1

Note. Mean values in the same row with different superscript letters ($^{a–b}$) indicate significant difference ($p < .05$); SEM: standard error of the mean; T.: significance of treatment; n.s.: not significant; $p > .05$; D: significance of storage days; T × D: interaction of treatment and storage days effects; *$p < .05$; **$p < .01$; ***$p < .001$.; Treatments—CON: sausages prepared without antioxidant; ERY: sausage prepared with sodium erythorbate at 500 mg kg^{-1}; OPN: sausages prepared with OPN leaves at 500 mg kg^{-1}; ROS: sausages prepared with rosemary extract at 500 mg kg^{-1}; GT: sausage prepared with green tea extract at 500 mg kg^{-1}. (Tukey test, $p < .05$).

protein, lipid, ash, pH, ES, and cooking loss. Nevertheless, the addition of natural extracts (ROS and GT) resulted in a significant ($p < .001$) reduction in moisture content, compared to CON and the treatment with ERY. Despite the reduction, the sausages were in compliance with Brazilian legislation, which establishes the following limits: moisture (max) 65%, fat (max) 30%, and protein (min) 12% (Brasil, 2000).

Contrasting findings were reported by Purnamayanti et al. (2020), who observed changes in the protein content of lamb sausages incorporated with GT leaf powder at different concentrations (0%, 1%, 1.5%, and 2%). These discrepancies could be attributed to key differences in processing and formulation. In our study, ~~the sausages were cooked, not fresh, which could have limited the interaction between the antioxidants and the meat matrix, as thermal processing can reduce the reactivity or functionality of phenolic compounds. Moreover,~~ the extracts were added at low levels (0.05%), typical of additive use, which could also restrict their impact on some physicochemical parameters (Moraga-Babiano et al., 2025; Nawaz et al., 2024; Sirini et al., 2025).

Instrumental colour

The results (Table 3) showed that treatment significantly affected L*, resulting in clear differences among the treatments. Storage time also significantly affected L*, suggesting that lightness changed throughout the storage period. However, the interaction between treatment and time was not significant ($p > .05$), implying that the impact of treatments on L* remained consistent over time. The OPN and ROS treatments exhibited the lowest L* values, indicating a darker colour compared to the CON, ERY, and GT formulations. This darker appearance could be attributed to the inherent coloration of the plant-based ingredients used. OPN leaves are rich in chlorophyll, the green pigment responsible for the plant's colour, while the commercial ROS extract used exhibits an amber-yellow, viscous appearance, as described in the manufacturer's technical documentation. These characteristics likely contributed to the overall darker colour observed in the OPN and ROS formulation. Similar findings were reported by Lise et al. (2021), who observed decreased L* values in mortadella formulations containing 0.05% and 0.10% OPN compared to the control without OPN. Estévez et al. (2005) found that frankfurter sausages with various levels of ROS essential oil (100, 300, and 600 ppm) exhibited significantly lower L* values than the control after 60 days of refrigerated storage.

ΔE* reflects the total colour differences between CON and samples containing antioxidants (ERY, OPN, ROS, and GT) during refrigerated storage (Figure 1). The ΔE* values from day 1 were significantly higher for the ERY (4.53), which were classified as "very distinct." OPN and ROS presented ΔE* values of 2.04 and 2.12, respectively, both categorised as "distinct." In contrast, GT exhibited the lowest ΔE* value (1.08), which was significantly different ($p < .05$) from all other treatments and classified as a "small difference."

As shown in Figure 1, up to 30 days of storage, the ΔE* values of samples containing natural antioxidants differed significantly ($p < .05$) from those of the ERY-treated samples; however, after 60 days, this difference was no longer significant ($p > .05$). At the end of storage (60 days), the treatments showed values ranging from 1.5 to 3, classified as "distinct," indicating that the colour differences between the antioxidant-treated samples and the control (CON) were visually noticeable.

Despite this perceptibility, the sensory evaluation results (Table 6) showed no significant difference ($p > .05$) in consumer acceptability scores, indicating that the observed colour changes did not negatively affect the sensory perception on the products.

Table 4 presented the results for the colour parameters a* and b*. As can be seen a* values were significantly affected ($p < .05$) by both treatment and storage time, indicating that this colour attribute (representing the red-green axis) changed depending on the type of antioxidant used and the duration of storage. Additionally, a significant ($p < .001$) interaction was observed between treatment and storage time, suggesting that the influence of treatments on redness varied over the storage period. Overall, from day 30 of refrigerated storage onward, the a* value remained relatively stable across all treatments. Borella et al. (2019)

Table 4. Colour parameters (a^* and b^*) of sausages during refrigerated storage.

Parameters	Days	Treatments					SEM	T	D	TxD
		CON	ERY	OPN	ROS	GT				
	1	6.15^{bB}	9.56^{a}	5.76^{cB}	5.80^{cB}	5.27^{dB}	0.154	***	***	***
a^*	30	9.06^{aA}	9.50^{a}	7.59^{bA}	7.96^{bA}	7.09^{cA}				
	60	8.84^{aA}	9.38^{a}	7.68^{bcA}	8.13^{bA}	7.15^{cA}				
	1	15.58^{aA}	12.83^{bAB}	15.61^{aA}	15.85^{aA}	15.84^{aA}	0.125	***	***	***
b^*	30	12.85^{bB}	13.08^{bA}	14.14^{aB}	14.35^{aB}	14.26^{aB}				
	60	12.70^{bB}	12.55^{bB}	13.71^{aC}	13.82^{aB}	13.45^{aC}				

Note. Mean values in the same row (different treatment in the same day) with different superscript lowercase letters ($^{a–c}$) indicate significant difference ($p < .05$); Mean values in the same column (same treatment in different days) with different superscript capital letters ($^{A–C}$) indicate significant difference ($p < .05$); SEM: standard error of the mean; T.: significance of treatment; D: significance of storage days; T × D: interaction of treatment and storage days effects; *$p < .05$; **$p < .01$; ***$p < .001$. Treatments—CON: sausages prepared without antioxidant; ERY: sausage prepared with sodium erythorbate at 500 mg kg^{-1}; OPN: sausages prepared with OPN leaves at 500 mg kg^{-1}; ROS: sausages prepared with rosemary extract at 500 mg kg^{-1}; GT: sausage prepared with green tea extract at 500 mg kg^{-1}. (Tukey test, $p < .05$).

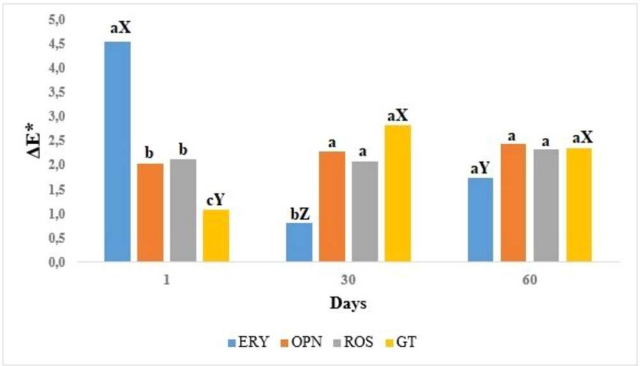


Figure 1. Total colour difference (ΔE*) in sausages during refrigerated storage. Means values followed by different superscript lowercase ($^{a–c}$) letters (different treatment in the same day), indicate significant difference ($p < .05$); Mean values followed by different capital ($^{X–Z}$) letters (same treatment in different days) indicate significant difference ($p < .05$); Treatments—CON: sausages prepared without antioxidant; ERY: sausage prepared with sodium erythorbate at 500 mg kg^{-1}; OPN: sausages prepared with OPN leaves at 500 mg kg^{-1}; ROS: sausages prepared with rosemary extract at 500 mg kg^{-1}; GT: sausage prepared with green tea extract at 500 mg kg^{-1}. (Tukey test, $p < .05$).

evaluated the effect of addition various concentrations and forms of ROS (liquid and powder forms) to mixed hamburgers during 120 days of frozen storage. They reported a decrease in the a^* value beginning at Day 60 for samples contain 0.03% powdered ROS extract, at Day 90 for samples with ROS extract concentrations ranging from 0.015% to 0.06% in different forms, and only at day 120 for those with the lowest extract concentration (0.015%). Similarly, Lise et al. (2021) and Sobrinho et al. (2015)reported findings consistent with the present study when evaluating the use of OPN mucilage and flour in emulsified meat products. They observed reduced a^* values in formulations containing OPN, compared to control formulation without its addition.

Regarding b^* values, the results showed that this parameter was significantly ($p < .001$) affected by both treatment and storage time. In general, this colour parameter decreased over time, with higher values observed in treatments containing OPN and commercial extracts (ROS and GT), and lower values in CON and ERY treatments. A significant interaction between treatment and time was also observed, indicating that the natural antioxidants used did not effectively prevent the oxidation of myoglobin, the pigment responsible for the red colour of meat. According to Chen et al. (2008), for an extract to protect myoglobin from oxidation, it must penetrate the cell membrane, as the pigment is located intracellularly.

Among the formulations, only CON and ERY remained stable after 30 days of storage, while all other treatments exhibited a reduction in yellowness (b^*). When analysing the colour of fresh pork sausages, Schilling et al. (2018) reported a significant decrease in redness (a^*) and a significant increase in yellowness (b^*) at 14 and 21 days of storage—results that contrast with those of the present study. The authors concluded that while the addition of ROS and GT extracts delayed sausage discoloration, it did not prevent colour oxidation.

Oxidative stability (RapidOxy and TBARS)

The RapidOxy method involves accelerated oxidation under oxidative stress conditions, allowing the assessment of a sample's oxidative stability. It monitors oxygen pressure over time while subjecting the sample to high pressure and elevated temperature (Caruso et al., 2017). The longer the induction period, the greater the oxidative stability (Sharma et al., 2019). The results (Table 5) showed that the OPN, ROS, and GT treatments demonstrated good stability throughout the storage period, as confirmed by RapidOxy and TBARS analyses.

The RapidOxy data indicate that all treatments with natural antioxidants exhibited significantly ($p < .001$) higher induction times (in min) compared to the control (CON) and the erythorbate-treated sample (ERY). Throughout the shelf life, the natural extract treatments remained significantly different from CON and ERY. By Day 60, the ROS and GT treatments showed the highest induction times—68 and 67 min, respectively—indicating superior oxidative stability. These findings are consistent with those of Difonzo et al. (2022), who evaluated the oxidative stability of salami using the RapidOxy method and olive leaf extract. The authors reported significantly higher induction times (73.68 and 63.37 min) in treatments with higher olive leaf extract concentrations (800 mg/kg) compared to the control (no extract added).

Across the storage periods analysed (1, 30, and 60 days), a reduction in induction time was observed in all treatments except GT. The GT treatment maintained stable induction times between 67 and 69 min, showing no significant decline over time.

On day 1, no significant differences in TBARS values were observed among treatments. However, at 30 and 60 days, significant differences ($p < .05$) were detected (Table 4). After 30 days of storage, the control (CON) showed the highest TBARS value (0.290 mg MDA/kg sample), whereas the other treatments (ERY,

Table 5. Oxidative stability (*RapidOxy* and *TBARS*) of sausage during refrigerated storage.

Parameters	Days	Treatments					SEM	T	D	TxD
		CON	ERY	OPN	ROS	GT				
RapidOxy	1	55.83^{dA}	56.60^{dA}	62.40^{cA}	70.37^{aA}	68.58^{b}		***	***	***
Induction time *(min)*	30	48.11^{cB}	47.98^{cB}	58.23^{bB}	67.76^{aB}	67.52^{a}	0.922			
	60	40.69^{cC}	40.33^{cC}	52.94^{bC}	67.80^{aB}	67.27^{a}				
TBARS	1	0.163^{B}	0.153^{B}	0.139^{B}	0.134^{B}	0.134^{B}		***	***	**
(mg of MDA/kg	30	0.290^{aA}	0.225^{bA}	0.198^{bA}	0.224^{bA}	0.219^{bA}	0.007			
sample)	60	0.256^{aA}	0.196^{bAB}	0.134^{cB}	0.177^{bcAB}	0.166^{bcB}				

Note. Mean values in the same row (different treatment in the same day) with different superscript lowercase letters ($^{a–d}$) indicate significant difference ($p < .05$); Mean values in the same column (same treatment in different days) with different superscript capital letters ($^{A–C}$) indicate significant difference ($p < .05$); SEM: standard error of the mean; T.: significance of treatment; n.s.: not significant; $p > .05$; D: significance of storage days; T × D: interaction of treatment and storage days effects; $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. Treatments—CON: sausages prepared without antioxidant; ERY: sausage prepared with sodium erythorbate at 500 mg kg^{-1}; OPN: sausages prepared with OPN leaves at 500 mg kg^{-1}; ROS: sausages prepared with rosemary extract at 500 mg kg^{-1}; GT: sausage prepared with green tea extract at 500 mg kg^{-1}. (Tukey test, $p < .05$).

OPN, ROS, GT), showed lower values: 0.224, 0.199, 0.224, and 0.219 mg MDA/kg sample, respectively. After 60 days, the OPN treatment exhibited the lowest TBARS value (0.134 mg MDA/kg sample), which was significantly lower than those of the CON (0.256 mg MDA/kg sample) and ERY (0.224 mg MDA/kg sample) treatments. However, it did not significantly differ from the ROS and GT treatments, which recorded values of 0.178 and 0.165 mg MDA/kg sample, respectively.

The TBARS values of all treatments increased up to the 30th day of storage, followed by a slight decrease by the end of the storage period (60 days). This reduction was statistically significant ($p < .05$) only for the OPN and GT treatments, which reached values of 0.134 and 0.165 mg MDA/kg sample, respectively. Although a decrease in MDA values over time is uncommon, similar trends have been reported in the literature. Schilling et al. (2018) evaluated the effects of combining ROS and GT extracts, in various proportions, with a synthetic antioxidant (control) in fresh pork sausages. The study demonstrated a reduction in TBARS values during refrigerated storage in treatments containing the combined plant extracts (ROS + GT), similar the trend observed in the present study. According to Georgantelis et al. (2007), this phenomenon may result from the decomposition of MDA by bacteria capable of selectively metabolising carbonyl compounds, including MDA. Additionally, MDA could undergo further oxidation into secondary products such as alcohols and acids, which are not detected by the TBARS assay.

The positive results obtained for OPN in both oxidative stability assays (RapidOxy and TBARS) can be attributed to its remarkable antioxidant capacity, evidenced by its higher activity compared to Trolox in the DPPH and ABTS assays, as well as by its high concentrations of various classes of antioxidants, such as carotenoids (α- and β-carotene, lutein, zeaxanthin, and violaxanthin), phenolic compounds—particularly phenolic acids (caffeic acid derivatives)—and flavonoids (quercetin, kaempferol). These compounds are well known for their ability to donate hydrogen atoms, chelate pro-oxidant metals, and scavenge free radicals (Garcia et al., 2019; Nogueira Silva et al., 2023b). These mechanisms can be compared to those reported for ROS extract, whose antioxidant activity is mainly associated with rosmarinic acid, carnosol, and carnosic acid, compounds that effectively scavenge radicals and prevent lipid oxidation in meat systems (Aziz et al., 2022; Mohamed et al., 2016). One of the mechanisms by which GT inhibits lipid oxidation involves the interaction between the active hydroxyl groups of its polyphenols and free radicals (Yang et al., 2009). Its antioxidant activity is primarily attributed to catechin gallates, particularly epigallocatechin gallate (Miao et al., 2022). Owing to these properties, GT extract has been widely applied as a natural preservative in foods—especially meat products—where it helps to prevent lipid oxidation, preserve colour and flavour, and extend shelf life (Pateiro et al., 2014). In this context, the phenolic profile and antioxidant activity observed in OPN suggest that it may represent a promising alternative to these well-established plant-based antioxidants for improving oxidative stability in meat products.

Although TBARS and RapidOxy are well-established assays for evaluating lipid oxidation in meat products, relying solely on these methods represents a limitation. Lipid oxidation is a complex, multi-step process, and a more comprehensive evaluation including additional markers such as peroxide value (primary oxidation products), conjugated dienes, p-anisidine value (secondary aldehydes), and volatile compounds is strongly recommended in future studies in order to provide a more robust and detailed understanding of the antioxidant action of *OPN* in meat matrice.

Sensory evaluation

The sensory evaluation results are presented in Table 6. No significant differences ($p > .05$) were observed among treatments for the attributes of colour, flavour, texture, and overall acceptance.

Regarding colour, although instrumental analysis revealed significant differences ($p < .05$) in L^*, a^*, and b^* among treatments (Table 4), the sensory analysis showed that evaluators did not perceive these differences visually. This suggests that the addition of OPN and the commercial natural extracts (ROS and GT), at a concentration of 0.05%, did not negatively impact the perceived colour of the sausages. It is important to note that no natural or artificial colourants were added to the sausage formulations in this study. Sobrinho et al. (2015) reported similar findings when evaluating the instrumental and sensory colour of emulsified meat products containing 1% and 2% OPN flour. Although instrumental measurements showed significance differences ($p < .05$) among treatments, panellists did not report significant differences in perceived colour between OPN-enriched treatments and the control (without OPN flour).

In the present study, only the odour attribute showed a significant difference ($p < .05$) between the OPN (5.56) and ROS (6.12) treatments. This difference may be attributed to the presence of volatile compounds in both ingredients. Rosemary extract contains compounds such as 1,8-cineol (with a refreshing aroma), α-pinene (pine-like aroma), and camphor (minty aroma), which contribute to its characteristic scent Svoboda and Deans (1992). According to Brewer (2011), rosemary extract is rich

Table 6. Acceptance scores according to consumers.

Attributes	Treatments					SEM	Sig.
	CON	ERY	OPN	ROS	GT		
Colour	5.85	6.12	5.88	6.07	5.77	0.091	n.s.
Odour	5.95^{ab}	6.07^{ab}	5.56^{b}	6.12^{a}	5.72^{ab}	0.091	*
Flavour	6.90	7.28	6.81	7.06	7.12	0.081	n.s.
Texture	7.17	7.27	6.87	7.16	6.82	0.080	n.s.
Overall acceptability	6.68	7.01	6.52	6.82	6.81	0.074	n.s.

Note. Mean values in the same row (different treatment) with different superscript lowercase letters (a–b) indicate significant difference ($p < .05$).
Treatments—CON: sausages prepared without antioxidant; ERY: sausage prepared with sodium erythorbate at 500 mg kg^{-1}; OPN: sausages prepared with OPN leaves at 500 mg kg^{-1}; ROS: sausages prepared with rosemary extract at 500 mg kg^{-1}; GT: sausage prepared with green tea extract at 500 mg kg^{-1}. (Tukey test, $p < .05$). SEM = standard error of the mean; n.s. = not significant; *$p < .05$.

in diterpenes, volatile oils, and phenolic acids, which can influence consumer's sensory perception. Similarly, the bioactive compounds in *P. aculeata* include phenolic acids, alkaloids, terpenes, and flavonoids (Torres et al., 2022), which, in addition to their antioxidant and anti-inflammatory properties, also possess aromatic characteristics. These compounds may act as precursors to substances that affect aroma and colour (Habschied et al., 2020). Therefore, the interaction of these volatiles with other compounds in the cooked meat matrix may have influenced the perceived odour by the evaluators.

Although no significant difference was found in the attributes of colour, flavour, texture, and overall acceptance, the ERY formulation consistently received the highest numerical scores. Scores for this treatment ranged from 6.07 to 7.28, corresponding to "slightly liked" (6) and "moderately liked" (7) on the 9-point hedonic scale. Based on evaluators comments in the sensory questionnaires, it is likely that these higher scores are associated with the external appearance of the sausages. The ERY formulation exhibited a pinker colour, likely due to the presence of the synthetic additive ERY. In addition to its antioxidant function, ERY promotes colour development in meat products, contributing to the curing process. Comments such as "pale" or "very light color" were frequently associated with samples that received lower scores for the colour attribute.

It is important to highlight that in this study only one concentration of OPN was evaluated, and a trained sensory panel was not used, which could have provided more in-depth insights into the use of the plant in meat products.

Conclusion

Freeze-dried OPN leaves proved effective in controlling lipid oxidation and maintaining colour stability in sausages. Their use represents a promising strategy to replace synthetic antioxidants, meeting the growing demand for natural ingredients in meat formulations. Both TBARS and RapidOxy results confirmed that natural extracts effectively contributed to oxidative stability, showing comparable performance to ERY. From an industrial perspective, the use of freeze-dried material offers potential benefits but may require formulation adjustments and increase processing costs. Overall, OPN stands out as a promising natural additive capable of extending shelf life and supporting the development of clean-label meat products.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Jenifer Mayara Monari Henck (Conceptualisation, Data curation, Writing—original draft), Miriam Gonçalves Marquezini (Formal analysis, Writing—review & editing), Noemí Echegaray (Formal analysis, Investigation) José Manuel Lorenzo (Formal analysis, Investigation), and Andrea Carla da Silva Barretto (Conceptualisation, Writing—review & editing

Funding

Funding for open access charge: Universidade de Vigo/CISUG.

Conflicts of interest

None declared.

Acknowledgements

Jenifer Henck would like to thank the financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES –88887.680131/2022-00). Noemí Echegaray acknowledges to Axencia Galega de Innovación (GAIN) for granting with a postdoctoral scholarship (grant number IN606B-2022/006). The authors Noemí Echegaray and José M.Lorenzo are members of the Competitive Reference Group "FunFood" (Functional Food), funded by GAIN (Grant number IN607A2023/01).

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International Journal of Food Science and Technology, 2025, **60(2)**, vvaf252
https://doi.org/10.1093/ijfood/vvaf252
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