



Role of fermented and unfermented rooibos (*Aspalathus linearis*) in wet and dry marinades on oxidative status and sensory properties of pork meat subjected to different cooking methods

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Abstract

The antioxidant potential of *Aspalathus linearis* (rooibos), either fermented (FR) or unfermented (UR), used as a natural additive in dry or wet marinades for pork loin cooked using oven, pan, sous-vide and microwave methods was investigated. Their effect on lipid peroxidation (LP), on the content and oxidative status of coenzyme Q₁₀ (CoQ₁₀), on cooking-related meat weight loss and on sensorial properties were evaluated. All cooking methods increased LP endpoints, however pan-cooking lead to lower oxidation in terms of hydroperoxides (PV) (0.020 ± 0.003 mEq O₂/100 g in wet-marinated and 0.025 ± 0.000 mEq O₂/100 g in dry-marinated meat) and malondialdehyde (MDA) (0.124 ± 0.015 mg/100 g in wet-marinated and 0.163 ± 0.002 mg/100 g in dry-marinated meat). FR limited the increase in PV in dry-marinated samples cooked using oven (0.031 ± 0.007 mEq O₂/100 g), sous-vide (0.023 ± 0.007 mEq O₂/100 g) and microwave (0.260 ± 0.006 mEq O₂/100 g), compared to the control (C) (oven = 0.045 ± 0.004 mEq O₂/100 g sous-vide = 0.042 ± 0.003 mEq O₂/100 g, microwave = 0.046 ± 0.005 mEq O₂/100 g). For MDA levels, FR was effective in dry marinated and microwaved samples (C = 0.265 ± 0.061 mg/100 g, FR = 0.106 ± 0.026 mg/100 g) and in oven-cooked samples previously wet-marinated (C = 0.211 ± 0.022 mg/100 g, FR = 0.128 ± 0.005 mg/100 g) or dry-marinated (C = 0.247 ± 0.019 mg/100 g; FR = 0.159 ± 0.005 mg/100 g). Conversely, UR was able to significantly counteract both PV and MDA in oven, sous-vide and microwave cooked meat, regardless of the marinating technique used, proving even more effective in counteracting LP. On the other hand, FR seems to provide a more favourable sensory profile, particularly in samples cooked in the microwave with a wet marinade and in samples cooked in the oven with a dry marinade. The use of rooibos influenced CoQ₁₀ oxidative status under certain conditions. This study demonstrates the ability of rooibos extracts to modulate early oxidative changes occurring during meat cooking, indicating its potential use as a natural alternative to synthetic preservatives.

Keywords Fermented rooibos · Unfermented rooibos · Lipid peroxidation · Cooking methods · Marinade · Meat

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Introduction

Throughout human history, meat preservation from spoilage has been a major concern, and lipid peroxidation is recognized as one of the most impacting chemical processes on meat quality, in particular following cooking [1]. Lipid peroxidation begins with unsaturated fatty acids autooxidation in presence of oxygen, leading to the formation of lipid hydroperoxides [2]. These compounds self-decompose rapidly into secondary products such as alkanes, alkadienyls, hydroxyalkenals and aldehydes such as hexanal and malondialdehyde (MDA). These compounds lead to the development of unpleasant taste, color degradation and loss

of meat product consistency [3–5]. Importantly, these secondary products of lipid peroxidation do not simply affect the sensorial quality of the meat, they also react with proteins lowering the nutritional value of the meat [6]. Despite a minor content in oxidized lipid in raw meat, associated with processing and storage, cooking remarkably amplifies oxidation. Heating damages cellular membranes hence contributing to the release of pro-oxidant metal ions such as iron from hemoglobin, myoglobin, ferritin and transferrin, that contribute to the formation of reactive oxygen species (ROS) accelerating peroxidation [5]. The extent of lipid peroxidation depends on several factors, including cooking duration and temperature, heating rate, the lipid composition of the meat, and the presence of antioxidants and transition metals. During cooking, protein denaturation, moisture evaporation, membrane disruption and the release of catalytic iron promote oxidative reactions that can compromise lipid stability, degrade endogenous antioxidants, and affect product quality. The magnitude of these changes varies depending on the cooking method, as different heat transfer mechanisms, temperatures, cooking times, and oxygen exposure create distinct oxidative environments within the meat, which ultimately determine its nutritional, technological, and sensory quality. Among several cooking methods, roasting leads to the highest levels of oxidation due to the prolonged exposure to high temperatures. Conversely microwave cooking, although to a lower extent, can give variable response depending on the marination technique used [6–8].

The oxidative status of cooked meat is receiving increasing interest, not only for its impact on sensorial properties and shelf-life stability, but also for its health implications. Studies on animal models and humans have shown that cooking-derived oxidized lipids are absorbed after the meal and they are carried in circulation within chylomicrons and low-density lipoproteins (LDL) concurring to systemic oxidative stress. This process has been also associated with a higher risk of chronic clinical conditions, including cardiovascular disease, colon-rectal cancer, and inflammatory diseases [9, 10]. To contrast lipid peroxidation during cooking, natural antioxidants play an essential role, in particular those of vegetal origin such as aromatic plants, spices, essential oils and polyphenol-rich extracts, that are efficient in reducing oxidation and in prolonging meat shelf-life without interfering with its sensorial properties [11, 12]. Consumers interest toward natural additives has also grown as a response to safety concerns associated with synthetic preservatives such as sodium nitrate, benzoate, BHT and BHA for their toxicological profiles [13]. In this direction, different botanical compounds have been successfully exploited: oregano oil and tannic acid in chicken cooking [14], ginger essential oil and basil in beef patties [15], green tea derived catechins

in poultry and fish [16, 17], apple polyphenols with pork [18]. Further examples include curcuma, pomegranate, and *Nigella sativa* extract, all effective in lowering thiobarbituric acid reactive substances (TBARS) levels in cooked meat [19]. Moreover, mixing beef ground meat with spices such as oregano, rosemary, and thyme significantly lowers lipid oxidation during the cooking process [20–22].

In this context, *Aspalathus linearis*, a South African leguminous shrub also known as rooibos, represents a promising candidate for meat conservation. Rooibos tea prepared from the water extraction of fermented leaves (red) or unfermented leaves (green), is a traditional beverage consumed in South Africa since the eighteenth century, but it is only in the last two decades that rooibos' health properties have been globally recognized. While green rooibos leaves are dried directly or quickly over a heat source to halt the oxidation process (thus preserving the original color and polyphenols), the preparation of red rooibos is based on a traditional method of crushing the shoots into small pieces, crushing them with wooden hammers to initiate browning, "sweating" (fermentation), and drying to develop the aroma [23]. Antioxidant, anti-inflammatory and anti-hyperglycemic properties of rooibos have all been demonstrated [23–25]. Rooibos health benefits are linked to its distinctive polyphenolic composition, in particular to aspalathin dihydrochalcone (which is uniquely found in this plant) and nothofagin, and their oxidation derivatives orientin and isoorientin. Other relevant flavonoids found in *Aspalathus linearis* include vitexin, isovitexin, luteolin, chrysoeriol, quercetin, isoquercetin [26, 27]. Previous studies have explored the antioxidant potential of rooibos in meat preservation [28] demonstrating, for the first time, that green and red rooibos, in the form of dried leaves, aqueous extracts or lyophilized powder, lowered lipid oxidation in ostrich patties and salami. Further studies have shown that the use of 0.25% red rooibos tea extract (RRTE) improved lipid stability and sensorial properties in ostrich droëwors 15 days after drying [29]. Moreover, RRTE was also able to delay oxidation in springbok and blesbok droëwors during conservation. Pork feet boiled in rooibos infused water had significantly lower TBARS levels [30] while 0.5% RRTE significantly lowered lipid peroxides in rabbit patties during 6 days of storage [31]. Despite these promising results, no studies have yet investigated how different marinating techniques (wet vs dry), with or without the addition of fermented or unfermented rooibos, modulate the oxidative response and the initial redox state of pork loin patties during thermal treatment, considering that cooking is a determinant factor for the chemical state of meat at the time of consumption. While rooibos-derived polyphenols have demonstrated antioxidant potential in meat systems, their effectiveness may depend on both the marination technique used for their

incorporation and the thermal treatment applied during cooking. In particular, liquid immersion marinating remains the most common method in home meat preparation, while injection, vacuum tumbling and dry marinating are the most common in the meat processing industry. The first method consists in injecting the required amount of brine into the food, while the second one involves rotating meat products in a sealed drum under vacuum conditions in the presence of a marinade solution. Finally, dry marination is based on evenly distributing dried seasonings (i.e. powdered functional ingredients) over the surface of the meat [32]. These different marination techniques are mainly used not only to improve flavour and tenderness but also to influence water retention, cooking yield and oxidative stability [33].

Therefore, in the present study we evaluated the combined effects of wet or dry rub marinades for pork loin and four cooking methods (oven, pan, sous-vide and microwave) on classic lipid peroxidation markers (peroxides and TBARS). These methods were selected because they represent markedly different heat treatment conditions in terms of heat transfer mechanism, temperature, cooking time, and oxygen exposure, all known to influence oxidative reactions and meat quality. Pan cooking involves direct contact with a hot surface, resulting in rapid heat transfer and high surface temperatures, while oven treatment relies on convective heat transfer, producing a slower and more gradual increase in meat temperature. Microwave cooking fundamentally differs from conventional heating in that it generates heat volumetrically through dielectric interactions with water molecules, allowing for rapid cooking but often creating heterogeneous temperature distributions. In contrast, sous-vide cooking uses prolonged low-temperature heating under vacuum conditions, minimizing oxygen exposure and thermal stress, while improving overall moisture retention and preserving heat-sensitive compounds. Moreover, since thermal treatment may affect the stability of endogenous antioxidants compounds such as CoQ₁₀, both meat content and oxidative status of this lipophilic antioxidant was also analyzed [34]. Finally, cooking-related moisture retention of meat and sensorial analysis were also investigated to provide a comprehensive evaluation of rooibos as a natural and functional ingredient to improve the nutritional and sensorial quality of cooked meat products.

Materials and methods

Chemicals and reagents

All chemicals were purchased from Merck KGaA (Darmstadt, Germany) unless otherwise stated: trichloroacetic acid (CAS 76-03-9), thiobarbituric acid (CAS 504-17-6),

butylhydroxytoluene (BHT) (CAS 128-37-0), chloroform (CAS 67-66-3), acetic acid (CAS 64-19-7), propanol (CAS 71-23-8), potassium iodine (CAS 7681-11-0), Folin-Ciocalteu reagent (CAS 521-24-4). Ultrapure water was obtained from a Milli-Q-system by Merck Millipore (Merck KGaA, Darmstadt, Germany) and used throughout the experiments. The water for rooibos extracts and marinades was bottled, mineral water, Acqua Sant'Anna S.p.A. (Vinadio, CN, Italy) purchased from local supermarkets.

Extract preparation

Fermented and non-fermented rooibos (*Aspalathus linearis*) long-cut samples were both purchased from a local retailer located in Jesi (AN, Italy) sourcing from Skimmelberg (South Africa), with both samples originating from the same commercial supplier to ensure consistency. Rooibos is endemic to the Cederberg region of the Western Cape, South Africa, and thus this region represents the inferred origin of the plant material. Product labels include batch numbers and “consumed by” dates only. Detailed information regarding harvest season, processing conditions, and packaging time was not provided and not available from the manufacturer. This could represent a limitation of the study, since these factors may affect the phytochemical composition and sensory characteristics of rooibos products and, consequently, sample comparability.

A liquid extract of both was prepared using a 1:5 ratio of water to rooibos leaves (w/w) [35]. Mineral water was used instead of deionized/MilliQ water in order to reproduce conditions that reflect those encountered in domestic and food service settings rather than those of a controlled laboratory environment. Boiling, mineral water was poured over the leaves in a beaker, covered with parafilm and infused in a water bath kept at 100 °C for 5 min. One part of the extract was then filtered through Whatman no. 4 filter paper and frozen at −80 °C until use (liquid extracts). The other part was first filtered through a sieve before passing through a Buchner filter under vacuum, and the resulting liquid was mixed with maltodextrin (CAS 9050-36-6) in a 1:10 ratio of maltodextrin to liquid (w/w). The extract obtained was injected into a spray-drier (Mini Spray-drying S-300, BUCHI, Meierseggsstrasse, Germany) with the following settings: drying gas 32 m³/h, injection temperature 220 °C, spray gas 1000 L/h, exit temperature 150 °C, injection pump 10 mL/min. The resulting dried extracts were stored under vacuum in plastic pouches (2–3 g in each) at room temperature in the dark until use. Only one batch of each dry and liquid extract was prepared and used throughout all the experiments, since the aim of the study was focused only on the variability induced by the dry or wet marinades for pork loin cooked using four different methods, although we

do acknowledge that this is a limitation regarding batch-to-batch variability.

Marinade preparation

The wet marinade was obtained by adding a 2% NaCl salt solution to pork meat at a ratio of 1:1 (w/w) [36]. In addition, for the rooibos-treated samples, the fermented and unfermented extracts, were separately added to pork meat at a ratio of 1% by weight of the meat (v/w). Instead, the dry marinade was prepared by adding 2% NaCl in crystalline form to the meat, whereas, for the rooibos-treated samples, an amount of 135.80 mg of fermented dried extract and 120.67 mg of unfermented dried extract per 100 g of meat were separately added as a rub. These quantities were chosen to retain the same total polyphenolic content as those previously determined in the corresponding solution used for the liquid marinade determined using the Folin–Ciocalteu reagent.

Sample preparation

The *Longissimus dorsi* muscle of pork, commonly known as pork loin, was obtained from a local butcher located in Matelica (MC, Italy) with 24–72 h postmortem age. The meat was transported under refrigerated conditions to the university and processed the same day of purchase. For each experimental trial, the meat was cut into 30 pieces, 50 g each, and divided into 6 groups (5 pieces for each treatment condition). Each group was placed in sufficiently large aluminum trays so that the meat slices did not overlap with each other. In the case of the wet marinade, this completely covered the meat portions in each tray. Each tray was then closed with the supplied lid and left to marinate at 4 °C for ≈ 24 h. This marination procedure was applied to the whole meat cuts before mincing, to ensure that the marinade components could diffuse throughout the muscle matrix and induce the desired structural and compositional modifications. After marinating, all samples were minced with a household mincer (Mini chopper SILVER CREST, SMZ 260 J1, 260W, Bochum, Germany) for 20 s. The resulting minced meat was used to form 5 hamburgers weighing 45 ± 1 g each. One burger was intended for raw material analysis, while the others were assigned to four specific cooking methods. This experimental design was replicated using three different pork loins over three different days.

Cooking methods

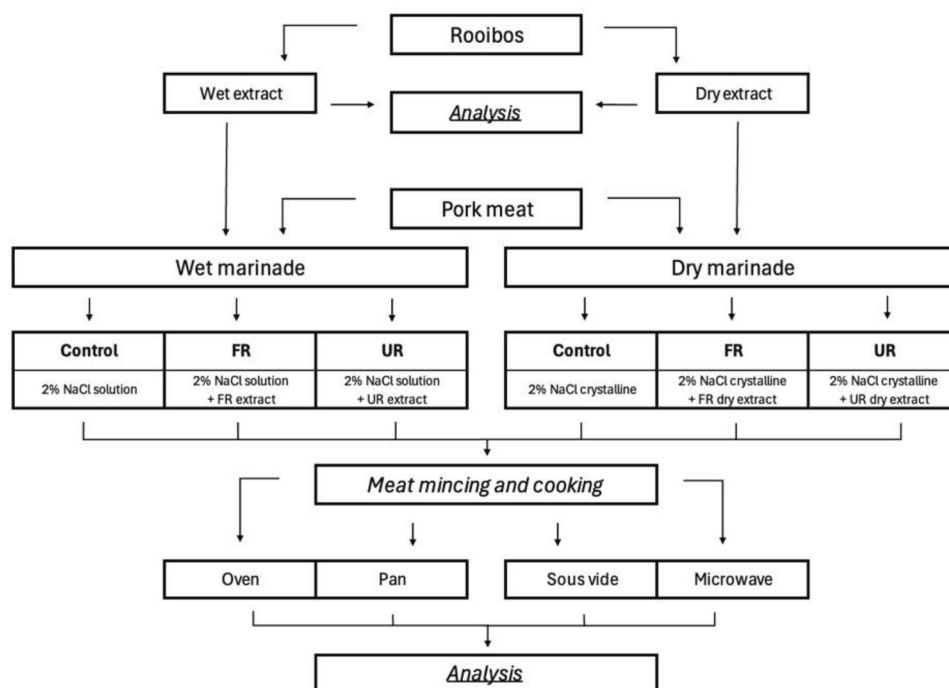
Four cooking methods were designated for this study: oven, microwave, sous vide and pan cooking. All cooking times and modes were tested and fine-tuned to achieve

a burger core temperature of 76 ± 1 °C, measured with a probe thermometer (Hendi, AAA0000199886, De Klomp, Netherlands). The specifications for each cooking method are as follows: Oven (AEG, Electrolux, Stockholm, Sweden), set to 180 °C in fan mode and 17 min cooking time [37, 38]; Microwave (Caso Design MIG 25 inverter microwave, Arnsberg, Germany), set to 540 W and 30 s cooking time for each side [39]; Sous vide (ANOVA precision cooker, 900W, A3-2-220 V-EU, San Francisco, USA), set at 80 °C and 16 min cooking time in a vacuumed pack [40]; Pan cooking using an induction pan (HEMLAGAD, IKEA, Delft, The Netherlands) on an induction hob (TILLREDA, IKEA, 2000W, Delft, The Netherlands) set at level 3 (600 W) and a 6 min cooking time for each side [41–43]. After cooking, each burger was allowed to cool to room temperature and weighed for cooking loss analysis. For all the other analyses, equal portions of the outer and inner burgers were taken, mixed and stored at -80 °C until analysis. A schematic overview of the workflow is shown in Fig. 1. The four cooking methods were replicated using hamburgers obtained from three different days of sample preparation.

Determination of total polyphenolic content

The total polyphenolic content (TPC) in the extracts was determined using the Folin–Ciocalteu reagent [44]. Although this reagent is widely applied for estimating TPC, it lacks specificity because the reagent also reacts with a variety of non-phenolic reducing substances, representing an inherent methodological limitation. Briefly, 50 μ L of each extract diluted 100-fold were transferred into wells of a transparent 96-well microplate to which 150 μ L of a 10-fold diluted aqueous solution of Folin–Ciocalteu reagent was added followed by the addition, after 10 min incubation in the dark, of 100 μ L 10% Na_2CO_3 (CAS 497-19-8) (w/w) aqueous solution. Samples were then incubated at room temperature in the dark for 120 min before measurement at 760 nm against water as blank on a microplate reader (Synergy HT, Biotek, Winooski, VT, USA). For the dry extract, 100 mg of powder was rehydrated in 1 mL of water and analyzed as reported above. The results are expressed as mM gallic acid equivalents (mM GAEq) using the linear regression value calculated from a gallic acid calibration curve. Gallic acid standards (CAS 149-91-7) (20 mM in Milli-Q water) were serially diluted in Milli-Q water to obtain a calibration curve covering the working range (0.6 to 0.05 mM, see Supplementary Materials). The standard curve was prepared using the same protocol described above, replacing the sample with gallic acid at different concentrations.

Fig. 1 Schematic overview of experimental plan. *FR* fermented rooibos, *UR* unfermented rooibos



Polyphenols characterization in the extracts

The characterization of the polyphenols of the liquid and dry rooibos extracts was carried out using an Agilent 1200 series HPLC with diode-array detection. Liquid extracts were freeze-dried and stored dry until analysis. Freeze-dried liquid extracts and spray-dried extracts were reconstituted in 10% dimethylsulphoxide (CAS 67-68-5) and ascorbic acid (CAS 50-81-7) (final concentration ≈ 9 mg/mL) was added to prevent oxidation during analysis. The solutions were filtered through 0.22 μm hydrophilic polyvinylidene fluoride syringe filters (Merck). Aspalathin (CAS 6027-43-6) and ferulic acid (CAS 537-98-4) were analyzed using the method described by Muller et al. [45]. Quantification was performed using the peak areas at specific wavelengths depending on the compound using external calibration curves. Values for the wet and dry marinades are expressed as $\mu\text{g}/100$ g pork meat.

Peroxide value

The peroxide value was determined using an iodometric titration of the peroxides with sodium thiosulphate (CAS 7772-98-7). The protocol followed was described by Ayari et al. [46] with minor modifications. Briefly, 3 g of pork loin patties was weighed and homogenized in 12 mL of chloroform using an ultra-turrax (IKA T18 ULTRA-TURRAX, Staufen, Germany). The solution was kept at 60 $^{\circ}\text{C}$ for 3 min, with hand shaking every 30 s before centrifugation for 5 min at 2400 g at room temperature. The supernatant

was then transferred to a flask containing 18 mL acetic acid and 0.5 mL potassium iodide for subsequent titration. Values are expressed as mEq O_2 per 100 g of meat sample using the following equation: $\text{mEq O}_2 = (V_{\text{eq}} \cdot N_{\text{thiosulfate}} / g_{\text{sample}}) \cdot 100$ (V_{eq} = equivalent volumes; $N_{\text{thiosulfate}}$ = normality of thiosulfate).

Thiobarbituric acid reactive substances (TBARS) value

TBARS in pork loin patties were determined using the spectrophotometric method described by Nour et al. [47] with minor changes. Briefly, 100 mg of sample, 750 μL of 15% trichloroacetic acid in 0.2 M HCl (CAS 7647-01-0) and 10 μL of 0.1 mM BHT were homogenized using a Tissue Lyser (Retsch, MM 400, Haan, Germany) at 30 Hz for 2 min. After centrifugation (5 min at 1800 $\times g$), 400 μL of supernatant was mixed with 400 μL of 0.375% 2-thiobarbituric acid and incubated at 95 $^{\circ}\text{C}$ in a water bath for 30 min. After cooling, the absorbance was recorded at 532 nm on a microplate reader. A calibration curve was constructed using 1,1,3,3-tetraethoxypropane (CAS 102-52-3) as standard. TBARS values are expressed as mg of malondialdehyde (MDA) per 100 g of meat.

CoQ₁₀ determination

1-Propanol (1 mL) was added to 50 mg of pork loin patties and subsequently homogenized using the tissue lyser described above. After centrifugation, 40 μL of extract was

injected into an HPLC system with electro-chemical detector (ECD) (Shiseido Co. Ltd., Tokyo, Japan) equipped with a post-separation reducing column (Shiseido CQR) to evaluate both oxidized and reduced CoQ₁₀ forms, as described previously [48]. Total CoQ₁₀ content in pork meat and its oxidative status are expressed as mg/100 g of meat and as percentage of ubiquinone/total CoQ₁₀, respectively.

Cooking loss assessment

For comparing the weight of the hamburgers before and after cooking which can differ due to water loss, each sample was weighed before (raw) and after each cooking method and expressed as g weight.

Sensory analysis

The objective of the sensory analysis was to evaluate consumer acceptability of pork hamburgers marinated using two techniques (wet and dry) and incorporating two types of rooibos extracts (fermented and unfermented). Based on prior results regarding meat oxidation and the protective effects of rooibos, sensory testing was limited to two cooking methods: oven cooking, representing a traditional approach, and microwave cooking, selected as a more modern and time-efficient alternative. Exclusion criteria for the sensory analysis were pregnancy and lactating women, low propensity to consume pork, subjects suffering from dysgeusia and olfactory disorders. Eleven volunteers aged between 25–59 years of age were recruited and were handed a questionnaire where they had to record their findings regarding the following sensory attributes: taste (spicy, salty, pork taste, persistence and overall taste), juiciness, tenderness, aspect and smell, giving a score from 1–10. Between each sample tasting, volunteers were provided with unsalted white bread and natural mineral water. All participants provided written informed consent prior to participation in the sensory evaluation. Since the sensory panel consisted of only 11 volunteers, the sensory analysis should be considered exploratory rather than conclusive, and the results should therefore be regarded as preliminary pilot data requiring validation through future consumer studies. This small sample size represents a key limitation as it does not allow the findings to be generalized to overall consumer acceptability, which typically requires a substantially larger number of participants to ensure representative and statistically reliable results.

Statistical analysis

The study was conducted on three different pork loins (*Longissimus dorsi*) from three different animals ($n = 3$)

purchased on different days, each analyzed in triplicate for TBARS analysis and in duplicate for CoQ₁₀ analysis and peroxide value. The results are expressed as mean value $\pm$ standard deviation (SD). Statistical analysis was performed using two separate two-way ANOVA models: the first one evaluated both the effects of cooking method and marinade type on control samples, while the second model was used to analyze the effect of rooibos treatment and marinade type (including their interactions). When significant main effects or interactions were detected, post hoc analyses were performed using Tukey's multiple comparisons test for all pairwise comparisons and Šidák's test for selected planned comparisons. A p value ≤ 0.05 was considered statistically significant and $p \leq 0.01$ highly significant.

Results

Total polyphenol content

Total polyphenol content (TPC) was quantified in both wet and dry rooibos extracts to standardize dosage for marination and to assess differences between fermented and unfermented preparations. The results are presented in Fig. 2. In wet extracts, the unfermented rooibos showed a significantly higher TPC than the fermented sample (267.9 ± 6.3 vs. 217.3 ± 7.5 mM gallic acid equivalents; $p = 0.0008$). This trend was also observed in the dry extracts, where the unfermented rooibos again displayed a higher TPC than the fermented counterpart.

Polyphenols characterization by UHPLC

The characterization of phenolic compounds was performed to determine whether there were any quantitative differences between the dry and liquid extracts. Table 1 shows the phenolic composition of the wet and dry marinades. Most phenolic compounds were present at higher levels in unfermented rooibos marinades compared to the fermented ones. Some exceptions include the eriodictyol glucosides and ferulic acid, which were detected only in fermented rooibos marinades, and vitexin and isovitexin, showing similar contents. This is expected based on the known effect of rooibos fermentation on the phenolic composition of extracts [49]. Among the phenolic compounds, aspalathin was present at much higher levels than all the other compounds in unfermented rooibos marinades, likely being the main contributor to the antioxidant outcomes.

Fig. 2 Total polyphenol content in fermented (FR) and unfermented (UR) rooibos extracts prepared for **a** wet and **b** dry marination, measured using the Folin–Ciocalteu method. Data are expressed as mM gallic acid equivalents (GAE). ** $p \leq 0.01$ indicates statistical significance between FR and UR extracts

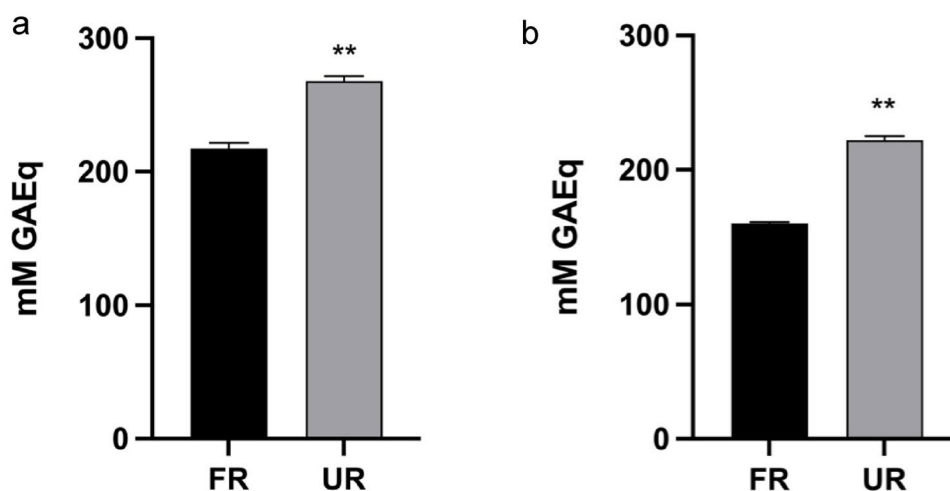


Table 1 Content of rooibos polyphenols in the wet and dry marinades prepared using fermented and unfermented rooibos expressed as μg compound per 100 g pork meat as determined using HPLC with diode-array detection

Compounds	Wet marinade		Dry marinade	
	Fermented	Unfermented	Fermented	Unfermented
(S)-6- β -D-glucopyranosylerythrodityol	151.6	nd	151.8	nd
(R)-6- β -D-glucopyranosylerythrodityol	144.3	nd	143.6	nd
(S)-8- β -D-glucopyranosylerythrodityol	30.2	nd	30.8	nd
(R)-8- β -D-glucopyranosylerythrodityol	43.9	nd	44.5	nd
Z-2-(β -D-glucopyranosyloxy)-3-phenyl-propenoic acid	122.4	159.3	121	141.6
Aspalathin	61.3	3056.2	96.7	2646.2
Nothofagin	14.6	217.1	14.5	189.6
Isoorientin	206.7	366.2	201.8	352.9
Orientin	291.7	467.2	281.9	446.7
Bioquercetin	70.6	237.7	71.0	212.5
Vitexin	63.0	62.7	60.9	58.2
Hyperoside	38.0	52.2	37.1	44.3
Rutin	5.6	138.5	6.7	124.0
Isovitexin	64.4	71.5	64.1	66.9
Isoquercetin	13.7	71.1	12.7	59.7
Luteoloside	10.1	16.1	9.0	14.5
Ferulic acid	20.4	nd	29.2	nd

Peroxide value in pork meat

Peroxide value (PV), expressed as $\text{mEq O}_2/100 \text{ g}$ meat, was measured to assess the accumulation of lipid hydroperoxides following cooking under different experimental conditions for both wet and dry salt-only marinades (without rooibos) (Fig. 3). Across all groups, cooked samples showed higher PVs than raw meat. Among the cooking methods, pan-cooked samples exhibited the lowest PVs for

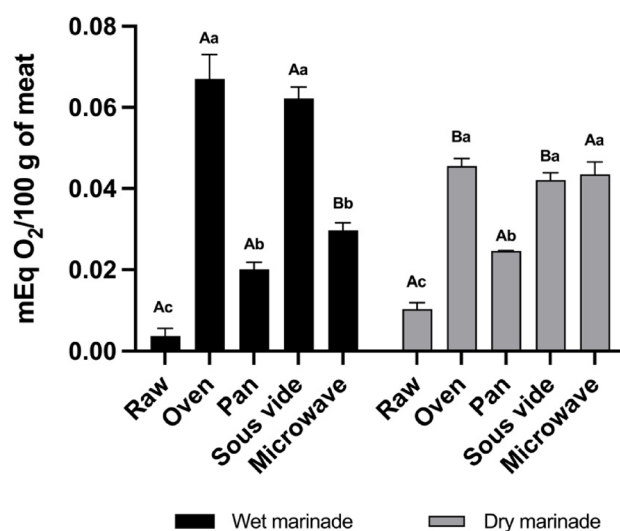


Fig. 3 Peroxide levels in raw pork loin and after four cooking methods (oven, pan, sous-vide, microwave), for both wet and dry salt-only marinades (no rooibos). Results are expressed as milliequivalents (mEq) of O_2 per 100 g of meat. Lowercase letters (a–c): significant differences within the same marination type ($p \leq 0.05$). Uppercase letters (A–B): significant differences between marination types for the same cooking method ($p \leq 0.05$)

both wet ($0.020 \pm 0.003 \text{ mEq O}_2/100 \text{ g}$) and dry ($0.025 \pm 0.000 \text{ mEq O}_2/100 \text{ g}$) salt marinades. In wet-marinated samples, oven and sous vide cooking led to the highest PVs, while microwave-cooked samples had intermediate values. For dry-marinated meat, PV levels were similar across cooking methods, except for pan-cooked samples which remained lowest. Statistically significant differences were observed between wet and dry marinades for specific cooking methods. In microwave-cooked samples, the dry marinade produced significantly higher PVs than the wet marinade ($p = 0.013$), while oven and sous vide cooking yielded lower PVs in dry-marinated meat compared to wet-marinated counterparts ($p = 0.0002$ and $p = 0.0031$, respectively).

When rooibos extracts were used, both liquid and dry formats were associated with reduced PVs compared to untreated controls (Fig. 4). This reduction was most evident with unfermented rooibos while fermented one was associated with reduced PVs primarily when used in dry marinade form. Across all conditions, no significant differences in PVs were observed between rooibos-treated and control samples when pan cooking was applied.

Thiobarbituric acid reactive substances (TBARS) levels

TBARS levels, expressed as mg malondialdehyde (MDA)/100 g meat, were determined to evaluate the extent of secondary lipid oxidation following cooking in both wet and dry salt-only marinated pork (Fig. 5). In the absence of rooibos, all cooking methods increased TBARS levels compared to raw meat, for both wet (0.033 ± 0.007 mg

MDA/100 g) and dry (0.078 ± 0.002 MDA/100 g) marinades. Among cooking techniques, pan cooking resulted in the lowest MDA levels across both marinade types. In wet-marinated meat, oven and sous-vide cooking produced the highest TBARS levels. In dry-marinated meat, the highest levels were observed with oven and microwave cooking, while pan and sous-vide cooking resulted in lower MDA concentrations. A significant difference between marination strategies was detected only for microwave cooking, where dry-marinated samples exhibited higher TBARS levels than wet-marinated ones ($p = 0.0001$).

Use of rooibos extracts was generally associated with decreased TBARS levels (Fig. 6). For both marinade types, unfermented rooibos reduced MDA concentrations in oven, sous-vide, and microwave cooking, but not in pan-cooked samples. Fermented rooibos showed reduced TBARS in oven-cooked meat under both marinade types, in sous-vide (wet marinade), and in microwave (dry marinade). No

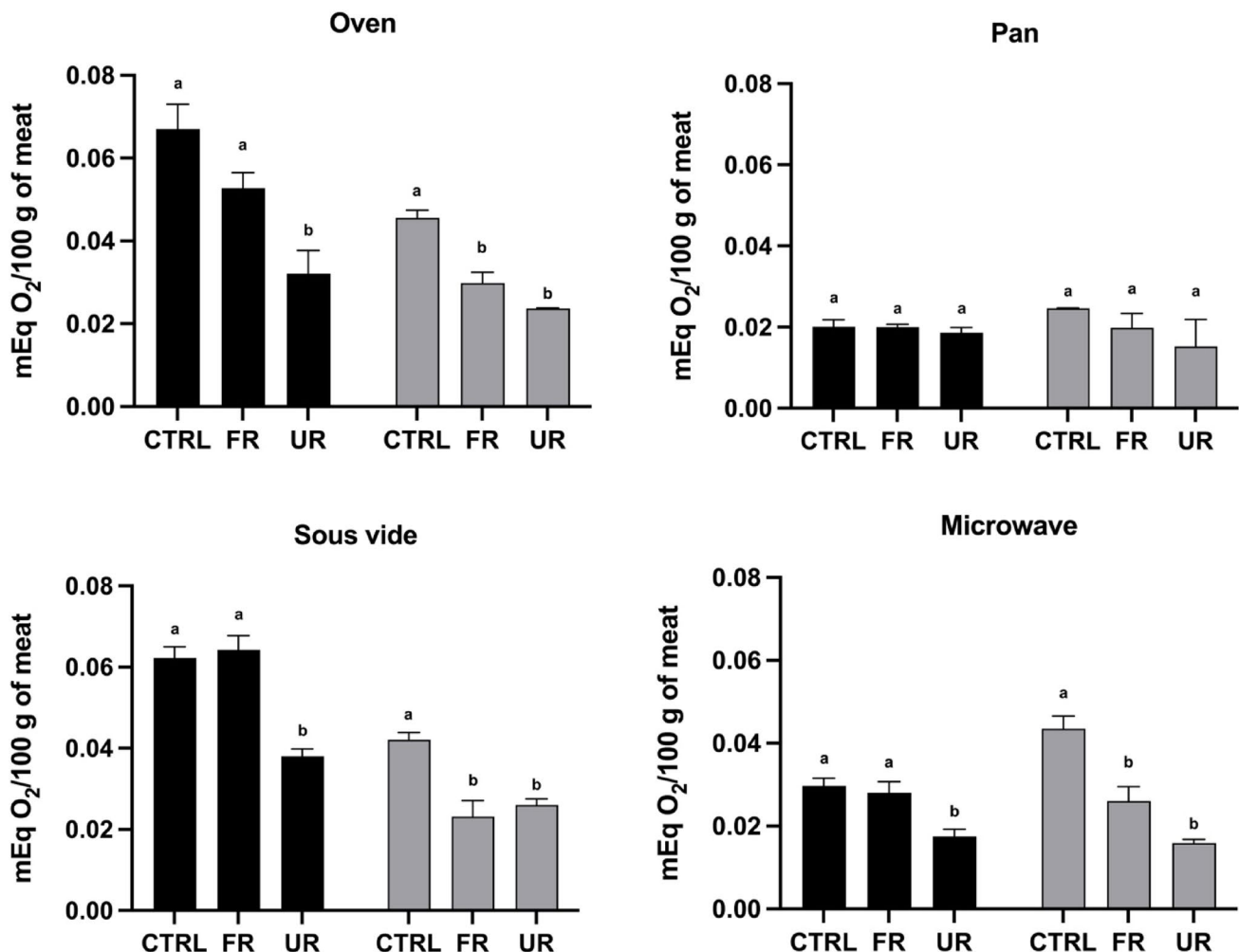


Fig. 4 Peroxide levels in pork meat marinated with rooibos extracts: wet (black bars) vs dry (grey bars) across four cooking methods. Results are expressed as mEq O₂ per 100 g of meat. Lowercase letters

(a–b): significant differences within the same marination method ($p \leq 0.05$). CTRL salt-only marinade, FR fermented rooibos, UR unfermented rooibos

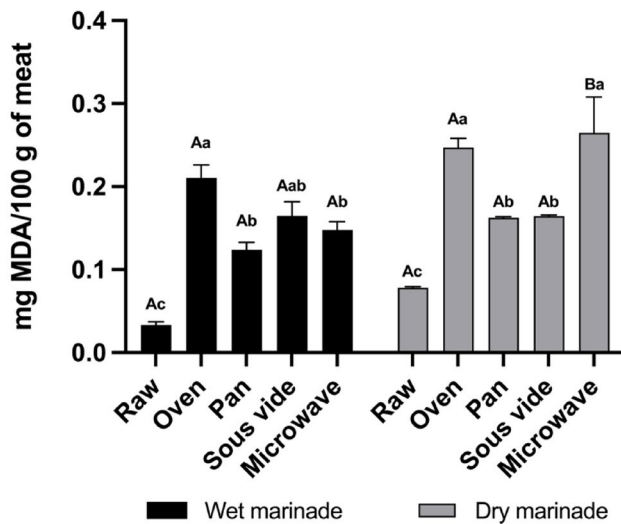


Fig. 5 TBARS levels expressed as mg of malondialdehyde (MDA) per 100 g of meat, in raw and cooked pork (oven, pan, sous-vide, microwave) for both wet and dry salt-only marinades (no rooibos). Lowercase letters (a–c): significant differences within the same marinade type ($p \leq 0.05$). Uppercase letters (A–B): significant differences between marinade types for a given cooking method ($p \leq 0.05$)

statistically significant differences were observed between rooibos-treated and control samples under pan cooking.

CoQ₁₀ content and redox status

Total CoQ₁₀ content in pork meat, expressed as mg CoQ₁₀/100 g meat, was quantified to evaluate the effects of both cooking and marinade types. As shown in Fig. 7, CoQ₁₀ levels increased significantly in all cooked samples compared to raw meat, regardless of the cooking method or salt-only marination type. No statistically significant differences in CoQ₁₀ concentrations were observed among the four cooking methods within each marination group. However, for wet-marinated samples, CoQ₁₀ levels were higher after oven and sous-vide cooking compared to dry-marinated samples (oven: $p = 0.0008$; sous-vide: $p = 0.0021$). For raw meat, CoQ₁₀ content was also higher in wet-marinated samples than in dry-marinated ones ($p = 0.0018$). Rooibos treatment, whether fermented or unfermented, did not significantly alter total CoQ₁₀ content under any experimental condition (Supplementary Tables 1–2).

The redox state of CoQ₁₀, expressed as the percentage of oxidized CoQ₁₀ over total CoQ₁₀, was also assessed (Fig. 8). In general, cooking was associated with a decrease in the proportion of oxidized CoQ₁₀ for most cooking methods. For salt-only wet-marinated meat, sous-vide cooking resulted in lower oxidized CoQ₁₀ percentages compared to oven and microwave cooking ($p < 0.000$). In salt-only dry-marinated meat, sous-vide also produced lower oxidation

values compared to all other methods ($p < 0.000$ for each comparison).

Figure 9 reports oxidized CoQ₁₀ percentages in rooibos-treated samples. In wet-marinated meat, a significant reduction in oxidized CoQ₁₀ was observed with unfermented rooibos during sous-vide cooking. For dry-marinated samples, both fermented and unfermented rooibos extracts were associated with increased oxidized CoQ₁₀ percentages under most cooking methods, except microwave cooking, where no significant differences were observed.

Cooking-induced weight loss and water retention

Cooking loss was evaluated by comparing meat weight before and after thermal processing across different salt-only marination strategies and cooking methods (Fig. 10). All cooking treatments resulted in weight reduction ranging between 20%–25% compared to raw meat. Wet-marinated samples showed on average greater weight loss than dry-marinated ones across all cooking methods. Among the techniques tested, microwave and sous-vide cooking resulted in the least weight loss, particularly for the dry-marinated groups (final weight: 38.26 ± 0.51 g for microwave, 37.72 ± 0.52 g for sous-vide). Conversely, oven and pan cooking induced greater losses for both marinade types. No statistically significant differences in cooking loss were observed between samples treated with or without rooibos extracts, regardless of the cooking technique or type of rooibos used (Supplementary Tables 3–4).

Correlation weight/CoQ₁₀ level

Figure 11 presents the relationship between weight of cooked meat and total CoQ₁₀ concentration, expressed as mg CoQ₁₀/100 g meat. A statistically significant inverse correlation was observed ($p < 0.0001$), indicating that variations in post-cooking weight are associated with changes in CoQ₁₀ content per unit of weight. The correlation pattern was consistent across cooking methods and marination strategies.

Sensory analysis

Sensory analysis was conducted on pork hamburgers marinated with fermented or unfermented rooibos, prepared using either wet or dry marination, and cooked using oven or microwave methods. Eleven volunteers evaluated each sample for attributes including spicy flavour, general taste, smell, juiciness, tenderness, saltiness, and visual appearance. Given the limited panel size, the results summarized in Fig. 12 are regarded merely exploratory rather than inferential. In microwave-cooked samples with wet marinade

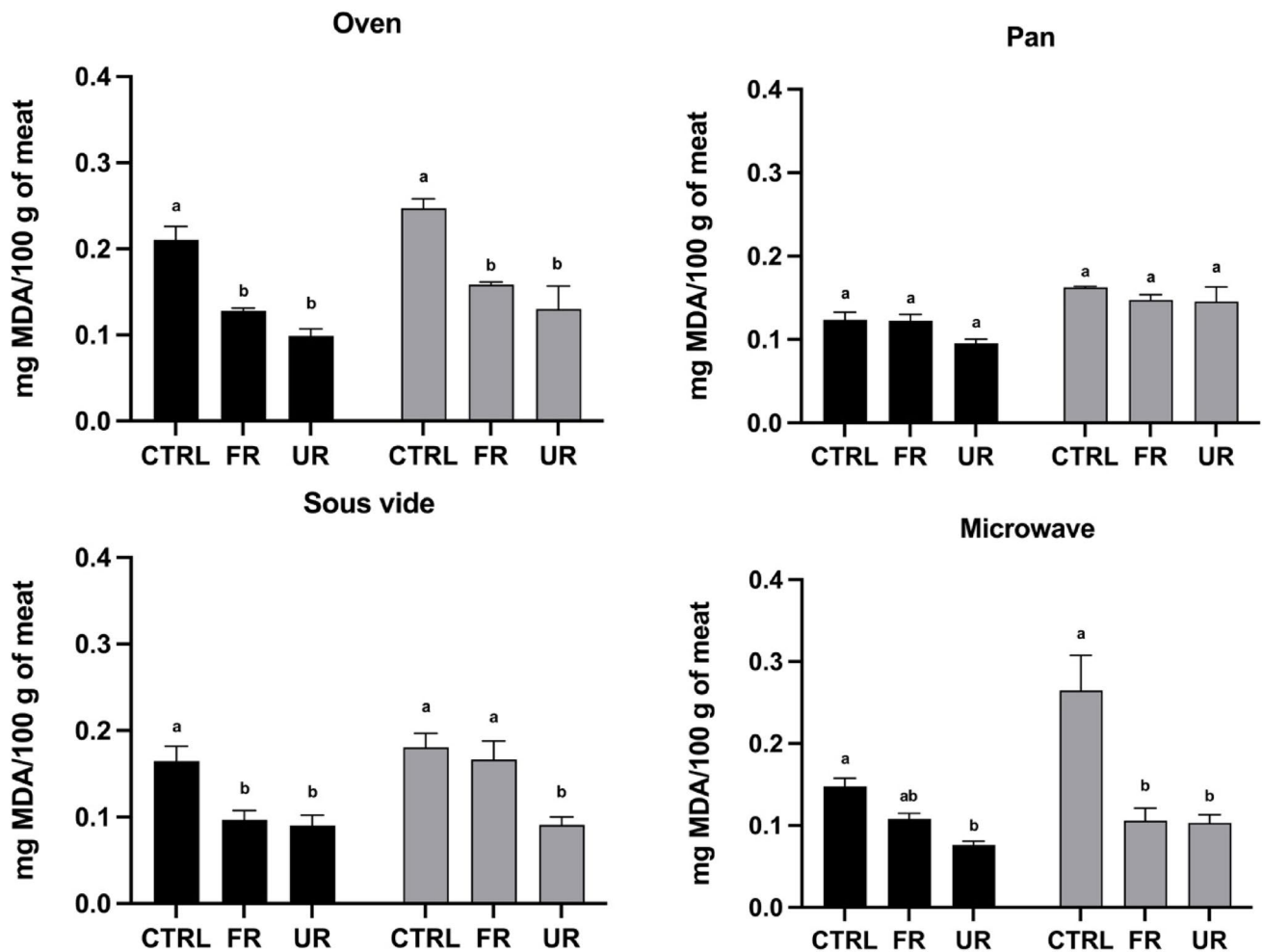


Fig. 6 TBARS levels in pork meat marinated with both wet (black bars) and dry (grey bars) rooibos extracts, after four different cooking methods (oven, pan, sous-vide, microwave). Values are expressed as mg of malondialdehyde (MDA) per 100 g of meat. Lowercase let-

ters (a–b): significant differences within the same marinade type ($p \leq 0.05$). Uppercase letters (A–B): significant differences between marinade types for a given cooking method ($p \leq 0.05$). CTRL salt-only marinade, FR fermented rooibos, UR unfermented rooibos

(Fig. 12a), average scores for spicy flavour, general taste, and smell were highest in the fermented rooibos group. For the unfermented rooibos group, mean scores were comparable to the control for most attributes, with lower average values recorded for tenderness, juiciness, and saltiness. For microwave-cooked samples with dry marinade (Fig. 12b), average scores across all sensory parameters were similar among the three groups. In oven-cooked samples with wet marinade (Fig. 12c), most attributes yielded comparable scores across all groups, with a slight increase in juiciness noted in the fermented rooibos group. In oven-cooked samples with dry marinade (Fig. 12d), higher average values were recorded for juiciness, tenderness, general taste, appearance, and saltiness in the fermented rooibos group compared to the other conditions.

Discussion

Growing consumer interest in healthier food choices has driven the food and culinary industries to explore natural ingredients rich in antioxidants and bioactive compounds, capable of improving both the nutritional and sensory quality of food products. In this study, we investigated the potential role of both fermented and unfermented rooibos (*Aspalathus linearis*), in mitigating cooking-induced lipid oxidation in pork loin. Its antioxidant efficacy was evaluated using four different cooking methods: traditional oven, pan, sous-vide, and microwave cooking. Furthermore, since most of the studies conducted to date have primarily focused on the role of natural antioxidant compounds added directly to food, either through encapsulation, formulation enrichment, or direct incorporation [12, 29, 31], we deemed it relevant to also investigate the contribution of culinary practices, commonly adopted both at the industrial and

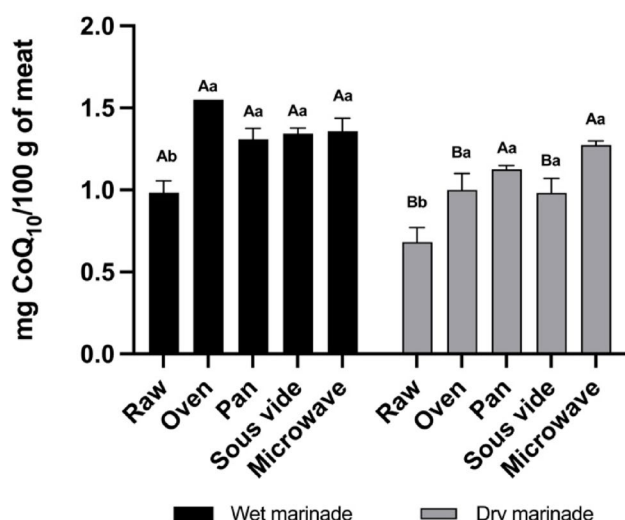


Fig. 7 Total CoQ₁₀ levels (mg per 100 g meat) in raw pork and after four cooking methods (oven, pan, sous-vide, microwave) with either wet or dry salt-only marinades. Lowercase letters (a–b): significant differences within each marinade type ($p \leq 0.05$). Uppercase letters (A–B): significant differences between marinade types for the same cooking method ($p \leq 0.05$)

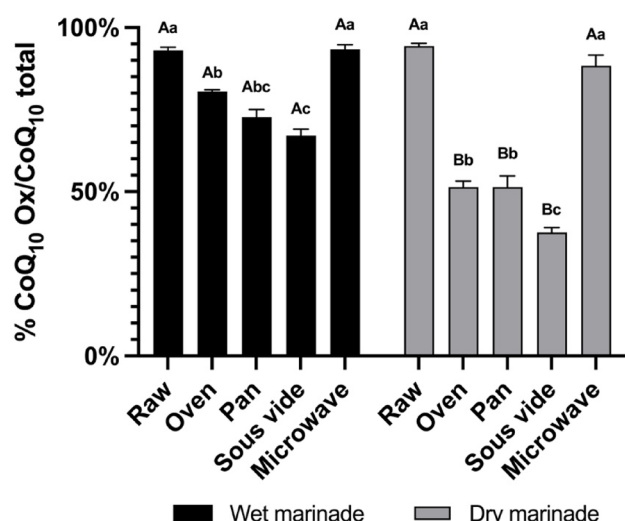


Fig. 8 Oxidized CoQ₁₀ (% of total CoQ₁₀) in raw pork and after four cooking methods (oven, pan, sous-vide, microwave) for both wet and dry salt-only marinades (no rooibos). Lowercase letters (a–c): significant differences within the same marinade ($p \leq 0.05$). Uppercase letters (A–B): significant differences between wet and dry marination under each cooking condition ($p \leq 0.05$). A decrease in oxidized CoQ₁₀ indicates a shift toward a more reduced and antioxidant-effective form

household level, for the delivery of rooibos-derived antioxidants into pork meat. Consistent with this approach, the choice to use commercial mineral water to prepare the rooibos extracts was also aimed at reproducing conditions more representative of common household and restaurant practices. Although the mineral composition of the water can influence the extraction and stability of phenolic

compounds, for example through complexation with metal ions or changes in pH, all extracts were prepared under the same experimental conditions, ensuring comparability between treatments. Specifically, we compared the effects of two marination methods: liquid marination, typically used in home cooking, and dry marination, more commonly applied during industrial meat processing. To ensure a consistent baseline for comparison, both rooibos extracts were normalized by total polyphenol content, to assess the impact of qualitative differences in polyphenolic profiles rather than concentration. As extensively reported in the literature, fermentation decreases the total polyphenol amount, particularly in terms of aspalathin, the highest component in green rooibos [50], while promoting the production of other flavones such as orientin and isoorientin [23, 51, 52]. These differences in composition appeared to influence antioxidant efficacy in the cooked pork samples. In agreement with previous literature findings, the present study confirmed that cooking promotes lipid peroxidation, resulting in the formation of primary (hydroperoxides) and secondary (malondialdehyde, MDA) oxidation products [6, 12]. Among the cooking methods tested, pan cooking induced the least oxidative damage, while oven, sous-vide, and microwave cooking caused higher levels of lipid oxidation. The protective effects of rooibos were particularly evident in these more oxidative settings, highlighting that its benefits are greater under high-stress cooking conditions. Of the two, unfermented rooibos demonstrated greater antioxidant performance than the fermented form, especially in reducing MDA levels in oven- and sous-vide-cooked meat. This allows us to hypothesize that aspalathin, the antioxidant that most characterizes green rooibos, plays a fundamental role in counteracting lipid peroxidation. Although pan cooking induces only mild oxidation, microwave cooking and sous-vide cooking trigger significant oxidative stress, especially when the meat is dry-marinated. In these cases, rooibos extracts effectively counteracted the formation of both hydroperoxides and MDA. Hence, these results highlight the strong protective role of rooibos against lipid peroxidation promoted during cooking of pork loin. The interpretation of cooking effects, however, is complicated by variability across studies. Some report lower oxidation with sous-vide than oven [53], while others show the opposite [6]. Our data indicate that pan cooking consistently generated the lowest oxidative markers, meanwhile microwave cooking showed contrasting results, depending on the marinating strategy: oxidation was minor compared to oven or sous-vide when meat was wet-marinated (Figs. 3 and 5). This suggests that oxidation kinetics are affected by cooking dynamics and heat distribution, together with the degree of hydration. In fact, dry-marinated samples undergo less water loss after cooking (Fig. 10), which may contribute to

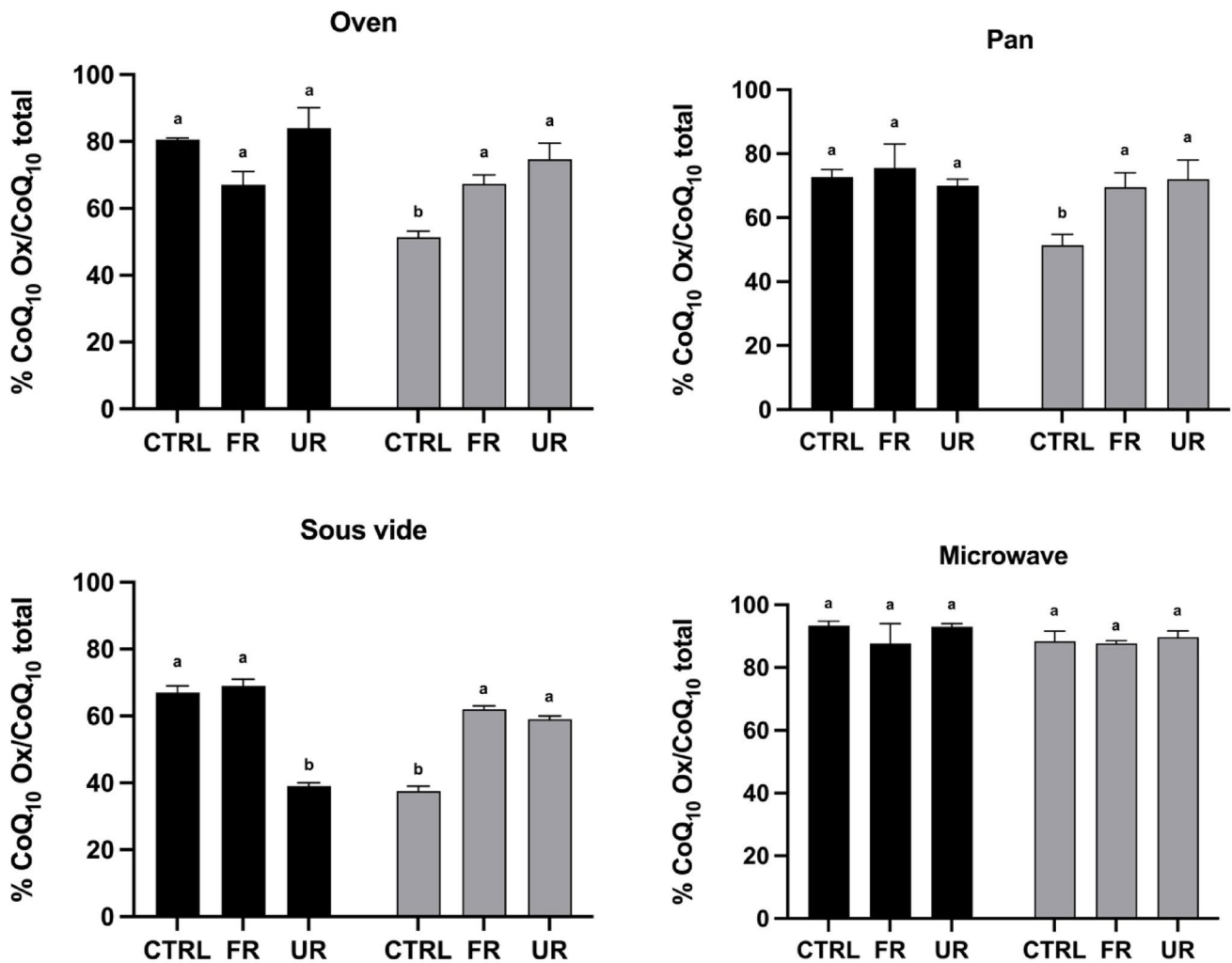


Fig. 9 Oxidized CoQ₁₀ (% of total CoQ₁₀) in pork meat marinated with rooibos extracts: wet (black bars) and dry (grey bars), after four different cooking methods. Lowercase letters (a–b): significant differences within the same marinade type ($p \leq 0.05$). Uppercase letters (A–B):

significant differences between marinade types for a given cooking method ($p \leq 0.05$). CTRL salt-only marinade, FR fermented rooibos, UR unfermented rooibos

the higher oxidation observed in microwave-cooked meat compared with wet-marinated samples. Given that microwaves induce heating through the rapid vibration of water molecules, reduced moisture content can impair heat dispersion and promote oxidative reactions. Pork loin, due to its leanness and high phospholipid content, is particularly susceptible to oxidation. Phospholipids, although a minor component of total lipids, are rich in PUFAs and localized in cell membranes, making them more susceptible to peroxidation [5, 54, 55]. Indeed, Pikul et al. [56] estimated that phospholipids account for up to 90% of MDA formation in meat. As expected, MDA levels in our study remained close to or below the 2.5 mg/kg rancidity threshold, with only dry-marinated microwave-cooked samples slightly exceeding this value (2.65 ± 0.61 mg/kg) [57, 58]. These data are consistent with the expected oxidative status of cooked and commercially relevant marinated meat products intended for

normal consumption conditions. Regarding marination strategy, our results suggest nuanced effects. While wet marination has been previously shown to reduce TBARS formation during extended meat aging [59, 60], our design focused on a 24-h marination followed by immediate cooking. Under these conditions, dry-marinated samples exhibited slightly higher pre-cooking oxidation than wet ones, but delayed hydroperoxide formation after oven and sous-vide cooking. However, final MDA levels were in general not significantly different between marination methods. Additionally, dry-marinated samples retained more post-cooking weight, likely due to salt-induced osmotic effects enhancing water retention. Beyond lipid oxidation, we evaluated CoQ₁₀ levels and redox state as an additional index of nutritional quality and oxidative environment. All cooking methods increased CoQ₁₀ content, likely due to water loss concentrating lipophilic compounds or enhanced tissue

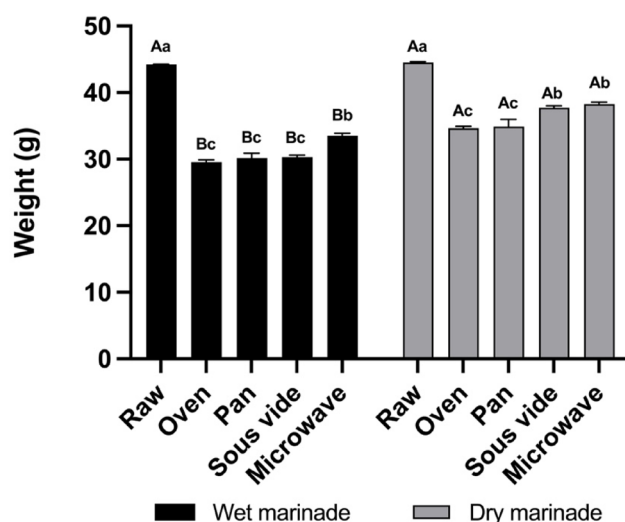


Fig. 10 Cooking-induced weight loss in raw pork and after four cooking methods (oven, pan, sous-vide, microwave) for both wet and dry salt-only marinades (no rooibos). Values are expressed as weight (g) of each sample. Lowercase letters (a–c): significant differences within the same marinade type ($p \leq 0.05$). Uppercase letters (A–B): significant differences between marinade types for a given cooking method ($p \leq 0.05$)

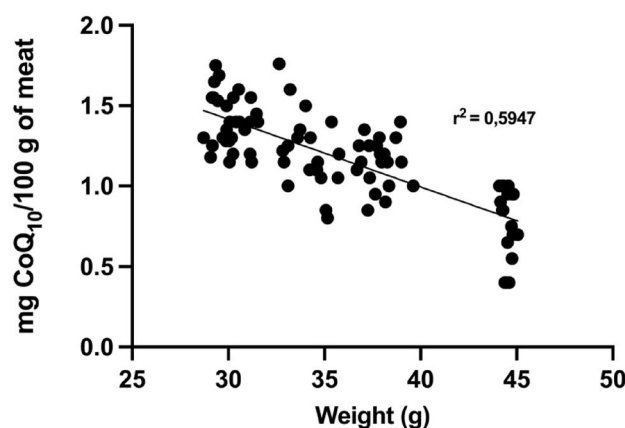


Fig. 11 Correlation analysis between total CoQ₁₀ content (mg per 100 g of cooked meat) and meat weight (g). A significant inverse relationship was observed (Pearson's $r = 0.5947$, $p \leq 0.05$)

extractability through protein denaturation [61]. Notably, this increase showed a strong inverse correlation with post-cooking meat weight, reinforcing the idea that dehydration may artificially inflate CoQ₁₀ concentration values. Crucially, cooking appeared to cause a shift in the CoQ₁₀ redox balance toward the reduced form, ubiquinol. This was particularly evident in dry-marinated samples, possibly suggesting that the cooking matrix under these conditions created a reducing environment [62]. These results are consistent with previous findings on salmon, where cooking conditions altered CoQ₁₀ concentration and redox state depending on matrix composition and water loss [48]. Conversely, microwave cooking seems to preserve the original

redox state of the molecule probably because it is a blander thermal condition. Interestingly, the use of wet marinade with unfermented rooibos during sous-vide further enhanced CoQ₁₀ reduction, suggesting a synergistic antioxidant effect. On the other hand, dry marinades with rooibos tended to increase oxidized CoQ₁₀, possibly due to limited diffusivity of aqueous antioxidants into the tissue. Rooibos has previously been used as a natural antioxidant in meat products such as droëwors [29], [28], and ostrich meat [31], confirming its suitability in complex food matrices. However, those studies primarily investigated lipid oxidation or color, whereas our study provides additional insights into redox-active micronutrients such as CoQ₁₀, expanding the functional profile of rooibos beyond traditional oxidation endpoints. However, it would be useful to delve deeper into the role of various cooking methods on the redox changes of CoQ₁₀ in order to better understand the underlying mechanisms.

Finally, we aimed to assess whether the presence of rooibos, introduced via two marination techniques, would be perceptible to the non-specialist assessors using an exploratory approach. To investigate this, we selected two distinct cooking methods: oven, representing a traditional approach, and microwave heating, considered a more modern and time-efficient technique. These methods were chosen based on previous results showing the most significant effects on lipid peroxidation, with both fermented and unfermented rooibos providing the highest oxidative protection under these conditions. Sensory evaluation results showed that using rooibos to marinate pork loin did not compromise, or in some cases even improved, the consumer-perceived attributes. In this context, red rooibos enhanced several sensory qualities, especially in the dry-marinated and oven-cooked samples and in the wet-marinated and microwaved meat, likely due to the formation of aromatic compounds during fermentation. Conversely, unfermented rooibos did not significantly alter sensory characteristics. Importantly, despite rooibos's powerful antioxidant activity, it did not introduce bitterness or off-flavors, attributes often associated with polyphenol-rich extracts. However, it is reiterated that the sensory findings should be interpreted with caution, given the small number of panelists. Therefore, further studies including larger consumer panels are warranted to confirm the observed trends. Overall, the objective of the study was not to induce severe rancidity in cooked meat products, but rather to evaluate the oxidative changes associated with different cooking methods and to investigate whether rooibos-based marinades could mitigate cooking-induced lipid oxidation. Although all burger samples remained within acceptable sensory quality limits, cooking still promoted measurable oxidative processes in control samples, as demonstrated by increased hydroperoxide and MDA formation.

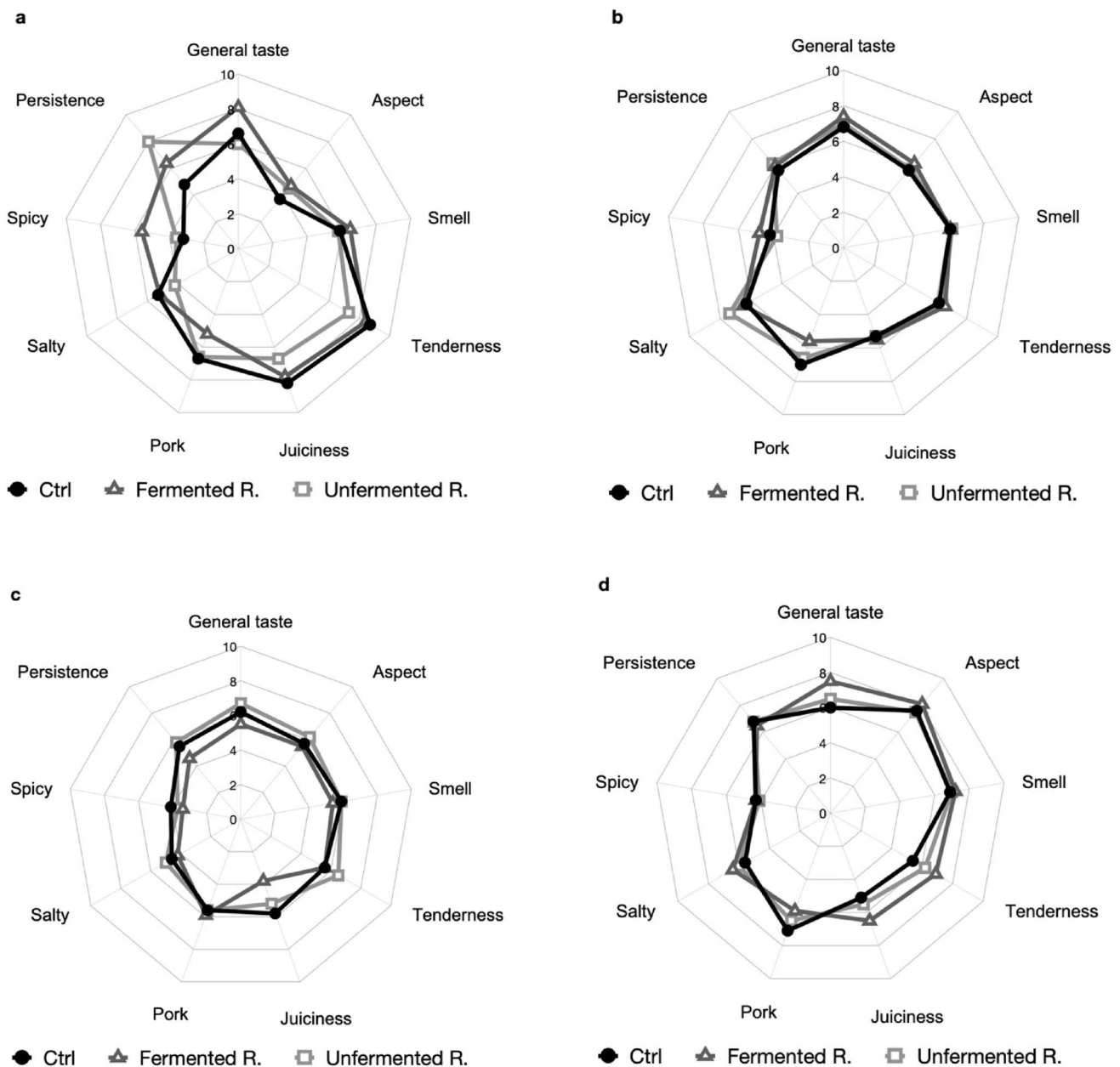


Fig. 12 Sensory evaluation of pork meat marinated with salt-only (circle), fermented rooibos (triangle), or unfermented rooibos (square). **a** Wet marinade and microwave-cooked, **b** dry marinade and microwave-cooked, **c** wet marinade and oven-cooked, **d** dry marinade and

oven-cooked. Attributes assessed include smell, aspect, tenderness, juiciness, persistence, general taste and specific taste like spicy, salty and pork; on a 0–10 point scale

Rooibos marinades, particularly green rooibos, significantly limited the formation of both primary and secondary lipid oxidation products compared to untreated controls. In addition, red rooibos marinades partially improved selected sensory characteristics of the burgers, supporting the potential technological and organoleptic relevance of rooibos-based formulations. Therefore, the practical significance of the study lies in demonstrating the ability of rooibos extracts to modulate early oxidative changes occurring during cooking, even before the development of overt sensory rancidity.

Conclusion

The present study provides new perspectives on the use of rooibos as a culinary strategy to improve the oxidative and sensorial quality of pork loin. Fermented and unfermented rooibos extracts have been effective in mitigating cooking-associated oxidation, particularly under conditions of high temperatures such as in oven and sous-vide cooking.

Although unfermented rooibos has shown a higher antioxidant capacity, fermented rooibos appears to contribute more to the improvement of sensorial properties of cooked pork loin. These results highlight the potential of rooibos as a multifunctional ingredient, able to increase both the stability of meat products as well as consumer preference, while using standard cooking procedures. Future studies could investigate the effects of rooibos extracts on long-term meat conservation and its microbiological stability, further supporting the use of rooibos in meat industrial transformation.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00217-026-05279-2>.

Author contributions MM: Conceptualization, Formal analysis, Data curation, Writing—review & editing. SB: Conceptualization, Formal analysis, Data curation, Writing—review & editing. DDB: Data curation, Writing—review & editing. BF: Formal analysis. LI: Formal analysis. PL: Data curation, Funding acquisition. LT: Writing—review & editing, Funding acquisition. AF: Writing—review & editing, Funding acquisition. ED: Conceptualization, Writing—original draft, Writing—review & editing. PO: Conceptualization, Writing—original draft, Writing—review & editing, Funding acquisition.

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Data availability The data that support the findings of this study can be made available upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Human ethics and consent to participate This study involving human sensory evaluation was conducted in accordance with the ethical principles of the Declaration of Helsinki (2013 revision). All procedures were reviewed and approved by the Research Ethics Committee of the Università Politecnica delle Marche (Protocol n. 0071372). All participants received written information describing the project and the procedures involved, and all participants provided written informed consent to participate before the sensory evaluation.

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