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Effect of the incorporation of a fermented rooibos (*Aspalathus linearis*) extract in the manufacturing of rabbit meat patties on their physical, chemical, and sensory quality during refrigerated storage

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ABSTRACT

In the present study, rabbit meat patties were formulated with increasing incorporation levels of a fermented rooibos extract (*Aspalathus linearis*; R): Control (C), R1, R2 and R3 (0, 0.5, 1, 2% w/w R inclusion, respectively). Meat patties were subjected to a 7-day refrigerated storage trial, simulating retail display conditions. At day 0, 1, 3 and 6 of storage, patties drip loss, pH, L*a*b* colour traits (raw patties) and peroxide value (cooked patties) were analysed; at days 0 and 6, total volatile basic nitrogen (raw patties), fatty acid profile and sensory traits (cooked patties) were analysed. Increasing R incorporation levels lowered pH values of rabbit meat patties and increased their redness and yellowness indexes compared to the C group ($P < 0.0001$). R-treated cooked patties were protected from lipid oxidation compared to C ones ($P < 0.0001$), and this status was maintained up to the end of the trial. R1 rabbit meat patties showed sensory traits comparable to the C group, whereas R2 and R3 treatments provided negative outcomes. Based on the above-mentioned findings, rooibos can be considered a promising natural additive in the manufacturing of rabbit meat patties, up to the 0.5% incorporation level.

1. Introduction

To ensure optimal product quality, the meat industry has widely used synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole. However, safety concerns about synthetic antioxidants and changing consumer preference regarding food additives, have led to increasing scientific and commercial attention on the use of natural antioxidant alternatives (Shah, Bosco, & Mir, 2014). In processed products such as patties, grinding, mincing and mixing disrupt muscle structure, thereby accelerating lipid oxidation rate (Cullere, Hoffman, & Dalle Zotte, 2013) which can negatively influence meat colour, texture and flavour, thus leading to loss of nutritional value, ultimately reducing its shelf-life. The cooking procedure also promotes meat lipid oxidation as heating is known to inactivate catalase, glutathione peroxidase and superoxide dismutase, endogenous enzymes of the cell antioxidant defence system (Mei, Crum, & Decker, 1994).

Among natural antioxidants, rooibos (*Aspalathus linearis*) has gained interest as a source of unique phenolic compounds: it is rich in aspalathin, which is the major flavonoid in this shrub with potent antioxidant activity (Joubert & Schulz, 2006). The dihydrochalcones aspalathin, quercetin and nothofagin showed to be the most potent radical scavengers. Quercetin and aspalathin were found to be the most effective inhibitors of lipid peroxidation (Snijman et al., 2009). The antioxidant activity of phenolic acids and flavonoids found in rooibos tea showed to be comparable to that of α-tocopherol and to the widely used synthetic antioxidants (Von Gadow, Joubert, & Hansmann, 1997). During rooibos processing, a dusty by-product is formed which is not suitable for drying and to produce tea (Pokornỳ, 1991). Consequently, since the early 2000, extracts derived from this by-product have been used in beverages, food preparations, as well as in the pharmaceutical and cosmetic markets (Joubert, Viljoen, De Beer, & Manley, 2009; Joubert & De Beer, 2011). Considering food matrixes, the formulation of healthy meat products could be another possible application of this fermented rooibos extract, but research studies on this topic are still scarce. The fermented rooibos extract was assessed in the production of processed game meat products: ostrich salami manufactured without

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using nitrites and nitrates (Cullere et al., 2013) and in the manufacturing of ostrich (Hoffman, Jones, Muller, Joubert, & Sadie, 2014), blesbok, springbok and fallow deer droëwors (Jones, Hoffman, & Muller, 2015a, b). In the only other study on fresh meat, unfermented (green) rooibos effectively retarded the lipid oxidation of ostrich meat patties under refrigerated retail display (Cullere et al., 2013). In this context, it would be important to study the potential role of the fermented rooibos extract as additive also in other meat species with economical relevance, such as the rabbit, focusing on the oxidative evolution of treated meat products during retail display. Among meat derived from terrestrial animal, rabbit meat is rich in unsaturated fatty acids (Dalle Zotte, 2002) and for this reason, a certain degree of lipid oxidation mainly during processing, storage and cooking is expected, which can have a detrimental effect on meat safety, physical characteristics (colour and water holding capacity) and sensory acceptability. The latter is an extremely relevant aspect to be considered since a negative effect of the tested additive in this sense might require formulation adjustments or, in the worst scenario, hamper its utilization in this meat species.

Based on the above-mentioned premises, the present research studied the effect of the incorporation of increasing fermented rooibos extract inclusion levels in the manufacturing of rabbit meat patties in ensuring physical and chemical quality. Furthermore, a panel test was conducted to evaluate whether and to what extent rooibos could impact the sensory profile of the product.

2. Material and methods

2.1. Rooibos extract, meat patty preparation and sampling

The rooibos extract used in the present experiment was provided by the Post-Harvest and Wine Technology Division of the Agricultural Research Council of South Africa (Infruitec- Nietvoorbij, Stellenbosch, South Africa) which was prepared according to the procedure described in the work by Joubert et al. (2009): after high-temperature extraction, coarse filtration, cooling, microfiltration, reverse osmosis to obtain a semi-concentrated extract, further concentration under vacuum and spray drying, the extract was freeze-dried and pulverised. The phenolic composition of the fermented rooibos extract is reported by Cullere et al. (2013).

The trial took place at the Department of Animal Medicine, Production and Health (MAPS) of the University of Padova (Italy). Overall, 16 kg of rabbit meat (hind legs and loins) were purchased at local butcher shop and ground by means of a professional meat grinder, equipped with a plate with 5 mm diameter holes. Meat was divided in four equal batches, and each batch was further divided into 8 sub-portions which were manually mixed with different rooibos inclusion levels (n = 2 sub-portions/treatment/batch): Control (0% w/w rooibos extract), R1 (0.5% w/w rooibos extract), R2 (1% w/w rooibos extract) and R3 (2% w/w rooibos extract). Each sub-portion was ground again using the same meat grinder to maximize the homogeneity of the included natural additive. Ten patties were made per sub-portion.

A total of 320 meat patties (50 g each) were formed (n = 80/treatment), individually wrapped with food-grade polyvinyl chloride (PVC) film (thickness: 8.5 my ± 8%; breaking load: > 17 N/mm; extension: > 135%; temperature range: -30 °C/+40 °C) to prevent direct air contact and stored in a refrigerator for a 7-day retail display trial. The refrigerator was set at 4 °C ± 1 and continuous cold fluorescent light illumination (L58 W/ 20 Osram, Germany, at 870 lux - MT 940, Major) was provided for the whole period to simulate commercial retail conditions. Analyses were performed at days 0 (preparation of the patties), 1, 3 and 6 of refrigerated storage. For each treatment group, patties were dedicated to the following analysis:

- Forty raw patties were used to determine drip loss, pH and colour at days 0, 1, 3 and 6 of storage;
- Ten patties were dedicated to the analysis of total volatile basic nitrogen (TVBN, on raw patties) and to the sensory analysis of cooked patties at days 0 and 6 of storage. In detail, an aliquot weighing 10 g was collected, individually packed in polyethylene bags and frozen at −80 °C until analysis of the TVBN. The remaining part of the meat patties was frozen and subsequently used for the sensory analysis.
- Thirty patties were cooked: twenty were used to assess meat oxidative status (peroxide value) and ten were used to analyse the fatty acid profile. The analyses of peroxide were carried out on days 0, 1, 3 and 6 of storage, whereas that of the fatty acid profile at days 0 and 6;

2.2. Drip loss, pH, colour measurements and TVBN of raw patties

For drip loss calculation, every day of analysis rabbit meat patties were removed from the refrigerator, unwrapped and weighed. Afterwards, they were wrapped with new food-grade PVC and put back in the refrigerator. The pH was measured using a portable pH-meter (FG2-Five Go™, Mettler Toledo, Greifensee, Switzerland; calibration at pH 4.0 and 7.0), and lightness (L*), redness (a*) and yellowness (b*) colour values (Commission Internationale de l'Éclairage, 1976) were recorded using a RM200QC colorimeter (X-Rite Co, Neu-Isenburg, Germany. Measuring Area: 8 mm; Measuring Geometrics: 45/0 Image Capture; Illuminant/Observer: D65/10). For each sample, pH and L*a*b* colour measurements were performed in duplicate.

The progression of rabbit meat protein degradation was evaluated through the Total Volatile Basic Nitrogen (TVBN) determination. The analysis was performed on meat samples which were thawed overnight at room temperature, according to the reference method reported in the E.C. Regulation 2074/2005 (Chapter III). Results were expressed as mg of TVBN/100 g of meat.

2.3. Peroxide value and fatty acid profile of cooked patties

At days 0, 1, 3 and 6 of storage, patties were collected from the refrigerator and freed from the plastic film. Subsequently, they were *vacuum* sealed using a CSV-41n ORVED (Orved S.p.A, Musile di Piave, Venice, Italy) machine (99% *vacuum* level) and cooked in a water bath for 25 min at 80 °C. Then, they were cooled in an iced bath. After cooling, patties were dried with a paper towel, placed into labelled plastic bags and frozen at −80 °C. After 1 week of frozen storage, samples were thawed at +4 °C for 24 h and analysed. The peroxide value determination is intended to quantify primary products of lipid oxidation through an iodometric determination of the peroxides. It is based on a lipid extraction of the sample and subsequent iodometric titration with sodium thiosulphate. The analysis was performed according to the ISO 3960 (2017) method. Values were expressed as meq O_2/kg of lipids.

The fatty acid profile of rabbit meat patties was assessed at days 0 and 6 of retail display, on cooked sub-samples collected from the same patties subjected to peroxides analysis. After homogenisation, 2 g from each patty were sampled and ground: lipids extraction, fatty acids (FA) esterification, composition and chromatographic conditions were performed as detailed by Cullere et al. (2018). Briefly, a solution of chloroform/methanol 1:2 was used for lipid extraction, then the total lipid content was determined gravimetrically. Samples were trans-methylated and fatty acid methyl esters were analysed by gas chromatography (Shimadzu GC17A, equipped with an Omegawax (Sigma-AldrichCo. LLC., Saint Louis, USA) 250 column (30 m × 0.25μm × 0.25 μm)) and a flame ionization detector. Peaks were identified based on 37-Component FAME Mix (Supelco Inc., Bellefonte, PA, USA) and the obtained data were expressed as % of total detected FAME.

2.4. Sensory evaluation

After 1 month of frozen storage, rabbit meat patties were subjected to a sensory ranking test conducted by a panel of five staff members of the MAPS Department. Assessors were selected by a pre-test screening interview; the prerequisites to take part to the sensory test were to be in good health status and to be rabbit meat consumers. Panel members underwent four pre-test training sessions, in which purchased fresh rabbit meat was used as reference sample. Panellists were trained to familiarise with the food matrix and with the rooibos powder used in the experiment. Furthermore, appropriate descriptors and possible off-flavours were developed, discussed, selected, and intensity scores were assimilated according to ISO 13299 (2003). The sensory attributes established during the preliminary sessions were colour intensity, flavour (overall intensity, rabbit, rooibos and off-flavours intensity) and texture (toughness, juiciness). The selected off-flavours were rancid, rabbit, rooibos, liver, urine, acid and straw. Rooibos and rabbit were included both among flavours and off-flavours as, after a certain sensory threshold level, their sensory perception could be perceived as either positive or negative.

Sensory analysis was performed at days 0 and 6 of storage; the same five people were employed during the two tasting sessions. Analysis was carried out in a testing room provided with individual testing booths, neutral-coloured walls and furniture, and standard lighting conditions. Each assessor was equipped with plastic cutlery, a plastic dish and glass, expectorant cup, water, unsalted crackers, and two paper towels per session. Each day of analysis, n = 10 meat patties/experimental group were thawed for 24 h at +4 °C, *vacuum*-sealed, identified by a random three-digit code and cooked in a water bath set at 85 °C for 25 min. Each patty was cut into two equal pieces and assigned to two different panellists while still warm; therefore, each assessor evaluated eight samples per session (n = 2 samples/treatment group). On each plastic dish, four meat samples (n = 1 sample/treatment group) were simultaneously provided to each panellist. For all the set sensory attributes, samples were ranked from the least (rank 1) to the most intense (rank 4).

2.5. Statistical analysis

Data were analysed using the GLM (General Linear Model) procedures of SAS 9.3 statistical software package for Windows (SAS, 2004). Results concerning physical traits, oxidative status and fatty acid profile were analysed by day through a one-way ANOVA that considered rooibos inclusion level as fixed effect. Differences were considered statistically significant when $P < 0.05$. Ranked sensory scores were analysed by day with a one-way non-parametric ANOVA (Kruskal-Wallis test), while the specific off-flavours frequencies were evaluated by day through a χ^2-test. The single tasting was considered as experimental unit. Principal component analysis (PCA; Jackson, 1991) was performed on sensory data and resulted in two graphic arts. The first consists of a plot representing the sensory attributes that was obtained after Varimax rotation based on the two variables that mainly explained the PCA model variability, while the second consists of a scatter plot representing the sensory attributes based on the rooibos inclusion level.

3. Results and discussion

3.1. Physical traits

At days 0–1, groups R2 and R3 exhibited higher drip loss than C and R1 patties ($P < 0.0001$; Table 1). However, this trend was not maintained throughout the following days, as the situation reversed at days 1–3: C group had the most intense dripping, whereas group R3 recorded the lowest weight losses ($P = 0.0004$). During the last phase of the storage (days 3–6), the differences among the four groups opposed C and R3 groups once again. Interestingly, the situation observed at days 3–6 reflected that of days 0–1, with C and R3 meat patties exhibiting the lowest and the highest drip losses ($P = 0.0350$), respectively. Considering the whole experimental period, no statistical differences among groups were observed, probably because drip loss exhibited a fluctuating trend over the seven-days retail display, which could be explained by different contrasting phenomena. At day 0, C group displayed the highest pH values and R3 the lowest, while groups R1 and R2 were both intermediate ($P < 0.0001$) (Table 2). Such linear decreasing pH values were maintained throughout the whole storage period as similar results were observed also at days 1, 3 up to day 6, when the pH of C and R1 meat patties were similar and higher than that of groups R2 and R3 ($P < 0.0001$). This pH-lowering effect of the rooibos extract was mainly attributable to the intrinsic pH value of the extract which was 5.37. A similar result was also observed in a previous experiment by Cullere et al. (2013) on ostrich meat patties treated with unfermented (green) rooibos.

On the one hand, a negative correlation exists between ultimate pH and meat drip loss (Ryu & Kim, 2005) and, in the present trial, the tested rooibos extract demonstrated a linear pH-lowering effect with increasing inclusion levels at days 0–1. It is also known that the

Table 1
Effect of increasing inclusion levels of a rooibos extract (0% - C; 0.5% - R1; 1% - R2; 2% - R3) in the rabbit meat patties formulation on their drip loss (%), evaluated over a 7-day retail display.

	Groups				*P-value*	RSD[1]
	C	R1	R2	R3		
N. samples	40	40	40	40		
Days 0–1	1.18[b]	1.06[b]	2.20[a]	2.19[a]	< 0.0001	0.46
Days 1–3	2.37[a]	2.04[ab]	0.70[bc]	0.52[c]	0.0004	1.16
Days 3–6	0.05[b]	1.35[ab]	0.58[ab]	1.75[a]	0.0350	1.44
Days 0–6	3.60	4.45	3.48	4.47	0.1348	1.19

[1] Residual Standard Deviation.
[a, b] Means within the same row with different superscript significantly differ ($P < 0.05$).

Table 2
Effect of increasing inclusion levels of a rooibos extract (0% - C; 0.5% - R1; 1% - R2; 2% - R3) in the rabbit meat patties formulation on their pH and L*, a*, b* colour values, evaluated over a 7-day retail display.

	Groups				*P-value*	RSD [1]
	C	R1	R2	R3		
N. samples	40	40	40	40		
pH:						
Day 0	6.14[a]	6.10[b]	6.09[b]	6.00[c]	< 0.0001	0.02
Day 1	6.26[a]	6.16[b]	6.14[b]	6.07[c]	< 0.0001	0.03
Day 3	6.20[a]	6.15[b]	6.13[b]	6.09[c]	< 0.0001	0.03
Day 6	6.25[a]	6.23[a]	6.19[b]	6.04[c]	< 0.0001	0.02
Lightness (L*):						
Day 0	49.3[a]	43.5[b]	38.1[c]	32.0[d]	< 0.0001	2.41
Day 1	49.0[a]	39.3[b]	37.4[b]	32.5[c]	< 0.0001	2.10
Day 3	50.4[a]	41.7[b]	38.3[c]	34.3[d]	< 0.0001	1.72
Day 6	49.6[a]	41.0[b]	35.8[c]	31.8[d]	< 0.0001	1.43
Redness (a*):						
Day 0	3.01[c]	13.5[b]	15.5[a]	16.2[a]	< 0.0001	1.21
Day 1	2.17[c]	12.7[b]	14.7[a]	13.6[ab]	< 0.0001	1.08
Day 3	2.27[c]	11.5[b]	12.8[a]	12.6[a]	< 0.0001	0.71
Day 6	1.77[c]	11.0[b]	12.4[a]	12.9[a]	< 0.0001	0.81
Yellowness (b*):						
Day 0	10.5[b]	19.2[a]	19.7[a]	18.2[a]	< 0.0001	2.20
Day 1	9.81[c]	19.6[a]	19.3[a]	14.7[b]	< 0.0001	1.73
Day 3	10.1[c]	17.1[a]	16.3[ab]	14.6[b]	< 0.0001	1.42
Day 6	9.92[c]	18.2[a]	16.7[ab]	15.3[b]	< 0.0001	1.27

[1] Residual Standard Deviation.
[a, b, c] Means within the same row with different superscript significantly differ ($P < 0.05$).

interaction between proteins and polyphenols, with the formation of bonds between the amine and phenolic groups, could alter the surface charges of the proteins themselves, thus modifying their ability to retain water (Prigent, Voragen, Visser, Van Koningsveld, & Gruppen, 2007). On the other hand, considering the negative correlation between lipid oxidation and meat water holding capacity (Huff-Lonergan & Lonergan, 2005), the protective effect of rooibos against lipid oxidation might have reduced drip loss at days 1–3. Furthermore, rooibos was added as a freeze-dried powder, which might have rehydrated once in contact with the minced meat, thereby limiting water dripping.

Holistically, C and all rooibos groups were counterposed in terms of L*a*b* colour values (Table 2). Indeed, as expected, patties L* linearly decreased as rooibos inclusion levels increased ($P < 0.0001$), while patties of group R2 and R3 were the reddest ($P < 0.0001$) due to the dark, brick red colour of the rooibos extract. The inclusion of the rooibos powder also caused a sudden increase in meat yellowness compared to the C group, which was independent to the inclusion level and it was already noticed at the lowest rooibos concentration ($P < 0.0001$). An initial hypothesis was that this situation might reflect a pro-oxidant effect from rooibos extract inclusion in excess amounts. However, results depicting the oxidative status of rabbit meat patties along the retail display did not support such hypothesis. Diversely, this effect was, once again, mostly probably due to the combination of the brick red colour of the extract with the water present in the meat patties: in fact, after this type of extract is dissolved in water, it results a dark yellow-orange colour.

Results concerning the total volatile basic nitrogen (TVBN) of patties (Table 3) showed that, at day 0, the C group had the greatest TVBN content, while R2 and R3 groups exerted a protective action against protein degradation ($P = 0.0062$). At day 6 of retail display, meat patties did not benefit from any of the tested rooibos inclusion levels as the protein degradation process exhibited similar extent in groups C, R1 and R3 and resulted even worse in the R2 meat patties ($P = 0.0016$). TVBN is a parameter related to protein degradation, mediated, particularly, by microbial activity (Huang, Zhao, Chen, & Zhang, 2014). Considering the whole retail display, it was interesting to notice that the lowest TVBN values were recorded in patties manufactured with the lowest rooibos extract inclusion level (0.5%). However, as the microbiological aspect was not evaluated in this trial, it was not possible to verify whether the high TBVN values reported at day 6 were ascribable to a greater bacterial activity developed in groups R2, R3 and C compared to R1.

3.2. Lipid oxidation and fatty acid profile of cooked patties

The major problem of meat possessing a highly unsaturated FA profile, is a greater proneness to lipid oxidation which negatively influences its nutritional value (Jiménez-Colmenero, Carballo, & Cofrades, 2001). In addition, the grinding process increases the exposed surface area of the meat, thus making it even more susceptible to

Table 3
Effect of increasing inclusion levels of a rooibos extract (0% - C; 0.5% - R1; 1% - R2; 2% - R3) in the rabbit meat patties formulation on their total volatile nitrogen (TVBN) content (mg TVBN/100 g raw meat), evaluated over a 7-day retail display.

	Groups				*P-value*	RSD [1]
	C	R1	R2	R3		
N. samples	10	10	10	10		
Day 0	23.4[a]	21.0[ab]	19.6[b]	20.2[b]	0.0062	1.52
Day 6	23.6[b]	22.0[b]	26.4[a]	24.0[ab]	0.0016	1.42

[1] Residual Standard Deviation.
[a, b] Means within the same row with different superscript significantly differ ($P < 0.05$).

oxidation (Cullere et al., 2013). Lipid oxidation is also further exacerbated by the cooking process (Mei et al., 1994). Even if all the flavonoids in the rooibos extract have a certain antioxidant effect, the most potent radical scavengers were reported to be aspalathin, which was effective in inhibiting oxidative damage at low concentrations, quercetin and nothofagin; quercetin and aspalathin were also the most effective inhibitors of lipid peroxidation (Joubert & De Beer, 2011; Snijman et al., 2009). In fact, the tested additive effectively inhibited lipid oxidation in cooked rabbit meat already at the 0.5% extract inclusion level (Fig. 1). At day 0, groups R1, R2 and R3 exhibited the same ability in this sense ($P < 0.0001$), whereas the peroxide content of the C patties was the highest at day 0 and throughout the retail display ($P < 0.0001$). Meat patties of groups R2 and R3 displayed the lowest peroxide content both at day 1 ($P < 0.0001$) and day 3 ($P < 0.0001$). At the end of the trial however, the peroxide content of the three rooibos groups were equal and lower than C ($P < 0.0001$). In the only other study on fresh meat, unfermented rooibos (inclusion level: 2% w/w) retarded the lipid oxidation of ostrich meat patties up to eight days of retail display (Cullere et al., 2013). However, the present is the first study testing the effect of a fermented rooibos extract on the oxidative stability of cooked meat and the first dealing with white meat. The positive findings of the present research confirmed that the fermentation process which is typically performed on rooibos to develop its typical sensory features, doesn't hamper its antioxidant potential. The present findings confirm the outcomes of a previous research testing the antioxidant effect of the same fermented rooibos extract in the manufacturing of processed game meat products (Jones et al., 2015a).

Lipid profiles of cooked rabbit meat patties (Tables 4 and 5) observed in this trial globally reflected the typical FA composition normally observed in the meat of this species, with some differences among the experimental groups. At day 0, the control group exhibited the highest SFA content, while treated groups (R1, R2, R3) settled uniformly on lower ($P = 0.0003$) values. After 6 days of storage, SFAs remained higher in the meat patties of the C group compared to the R2 group, with the others being intermediate ($P = 0.0171$). In contrast, group C had the lowest MUFA percentage at day 0 of retail display, and at day 6 the MUFA of the C group remained lower than the R2 and R3 groups, with R1 being intermediate.

The overall PUFA proportion did not reveal significant differences among the four experimental groups, neither at day 0, nor at day 6 of retail display. However, at day 0, C and R groups were counterposed in terms of PUFA composition: C meat patties had the highest levels of α-linolenic acid, EPA, DHA and thereby total *n*-3 fatty acids, while treated groups (R1, R2, R3) were richer in C18:2 *n*-6 ($P = 0.0003$) and thereby in total *n*-6 ($P = 0.0197$). As a result, meat belonging to the C group possessed more *n*-3 FAs at time 0, providing a more desirable FA profile in terms of human health as denoted by the *n*-6/*n*-3 ratios ($P < 0.0001$). Even at day 6, rooibos-treated patties were richer in C18:2 *n*-6 ($P < 0.0001$) and thus in the *n*-6 series ($P = 0.0003$). As for C18:3 *n*-3, EPA and *n*-3 fatty acids, their amounts were similar in all groups thereby mitigating the discrepancies previously noticed in the *n*-6/*n*-3 ratios between the C and all R groups ($P = 0.0248$). The lack of differences between C and R patties in the overall PUFA contents at both times of analysis, indicated on the one hand a limited oxidative status of the meat and on the other hand, it demonstrated that the rooibos extract did not affect the overall PUFA class. The same hypothesis was drawn by Jones et al. (2015a), who did not find any difference between the PUFA proportion of control and rooibos treated blesbok droëwors.

Due to the absence of lipids in the tested rooibos extract, differences in the FA profile among treatments were not attributable to an additional source of fat but to different factors. Lipid oxidation affects FA integrity and cooking boosts oxidative reactions thus further altering fatty acids composition (Frankel, 1984). As rooibos exerted an antioxidant effect on rabbit meat patties already at day 0 of retail display,

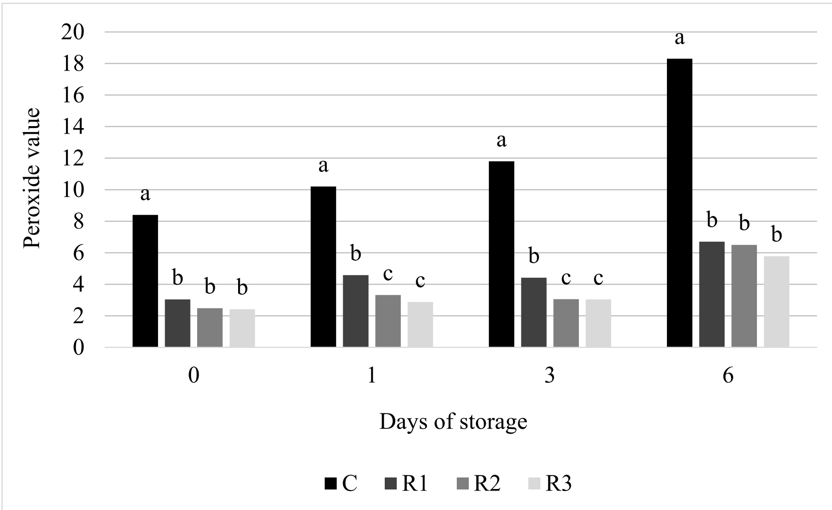

Fig. 1. Effect of increasing inclusion levels of a rooibos extract (0% - C; 0.5% - R1; 1% - R2; 2% - R3) in the rabbit meat patties formulation on their peroxide value (meq O2/kg lipids, cooked meat), evaluated over a 7-day retail display.
[a, b, c] Means within the same day of storage with different superscript significantly differ ($P < 0.05$).

Table 4
Effect of increasing inclusion levels of a rooibos extract (0% - C; 0.5% - R1; 1% - R2; 2% - R3) in the rabbit meat patties formulation on their fatty acid profile (% of total FAME, cooked meat) at day 0 of retail display.

	Groups				*P-value*	RSD [1]
	C	R1	R2	R3		
N. samples	5	5	5	5		
C14:0	2.36	2.32	2.46	2.26	0.2258	0.15
C15:0	0.62^{a}	0.57^{ab}	0.59^{ab}	0.56^{b}	0.0177	0.03
C16:0	24.5	24.0	24.0	23.9	0.3567	0.49
C17:0	0.68^{a}	0.61^{b}	0.60^{b}	0.61^{b}	< 0.0001	0.01
C18:0	6.70^{a}	6.34^{b}	6.18^{b}	6.44^{b}	< 0.0001	0.11
C20:0	0.094^{a}	0.100^{ab}	0.102^{ab}	0.105^{b}	0.0077	0.004
C22:0	0.025^{a}	0.023^{ab}	0.022^{b}	0.024^{ab}	0.0428	0.002
C23:0	6.79	6.27	6.95	6.43	0.2526	0.57
SFA	42.6^{a}	41.0^{b}	41.9^{b}	41.2^{ab}	0.0003	0.47
C14:1 *n*-5	0.22^{a}	0.20^{ab}	0.17^{b}	0.17^{b}	0.0021	0.02
C16:1 *n*-9	0.33	0.33	0.35	0.33	0.0458	0.01
C16:1 *n*-7	3.43^{Aa}	3.11^{ABb}	2.79^{Bc}	2.86^{Babc}	< 0.0001	0.17
C17:1	0.37	0.31	0.29	0.32	0.0655	0.04
C18:1 *n*-7	1.21^{A}	1.13^{B}	1.07^{C}	1.10^{BC}	< 0.0001	0.02
C18:1 *n*-9 *cis*	21.5^{B}	23.6^{A}	23.6^{A}	23.6^{A}	< 0.0001	0.26
C20:1 *n*-9	0.21	0.21	0.21	0.21	0.9231	0.01
C22:1 *n*-9	0.016	0.014	0.012	0.013	0.1551	0.001
MUFA	27.3^{b}	29.0^{a}	28.4^{ab}	28.6^{a}	< 0.0001	0.28
C18:2 *n*-6 *cis* (LA)	23.2^{b}	24.0^{a}	24.3^{a}	24.2^{a}	0.0003	0.32
C18:3 *n*-6 (GLA)	0.40^{a}	0.33^{b}	0.32^{b}	0.32^{b}	0.0015	0.03
C18:3 *n*-4	0.054^{a}	0.045^{b}	0.042^{b}	0.045^{b}	0.0001	0.003
C18:3 *n*-3 (α-LNA)	2.86^{a}	2.57^{b}	2.53^{b}	2.58^{b}	< 0.0001	0.08
C20:2 *n*-6	0.27^{a}	0.24^{b}	0.22^{b}	0.23^{b}	< 0.0001	0.011
C20:3 *n*-6 (DGLA)	0.339^{a}	0.273^{b}	0.234^{c}	0.267^{d}	< 0.0001	0.015
C20:4 *n*-6	1.86^{a}	1.56^{c}	1.33^{d}	1.58^{b}	< 0.0001	0.12
C20:3 *n*-3	0.08^{a}	0.06^{b}	0.06^{b}	0.06^{b}	< 0.0001	0.01
C20:5 *n*-3 (EPA)	0.048^{a}	0.037^{c}	0.031^{d}	0.040^{b}	< 0.0001	0.003
C22:4 *n*-6	0.45^{a}	0.38^{b}	0.31^{b}	0.39^{b}	< 0.0001	0.03
C22:5 *n*-6	0.175^{a}	0.145^{b}	0.125^{b}	0.147^{b}	0.0002	0.013
C22:5 *n*-3	0.23^{a}	0.22^{a}	0.19^{b}	0.22^{a}	0.0004	0.02
C:22 6 *n*-3 (DHA)	0.051^{a}	0.037^{c}	0.031^{c}	0.041^{b}	< 0.0001	0.004
PUFA	30.1	30.0	29.7	30.2	0.3032	0.42
n-6	24.9^{b}	25.3^{ab}	25.5^{ab}	25.5^{a}	0.0197	0.33
n-3	3.31^{a}	2.94^{b}	2.84^{b}	2.96^{b}	< 0.0001	0.08
n-6/*n*-3	7.52^{b}	8.62^{b}	8.96^{a}	8.63^{b}	< 0.0001	0.15

[1] Residual Standard Deviation.
[a, b, c, d] Means within the same row with different superscript significantly differ ($P < 0.05$).

Table 5
Effect of increasing inclusion levels of a rooibos extract (0% - C; 0.5% - R1; 1% - R2; 2% - R3) in the rabbit meat patties formulation on their fatty acid profile (% of total FAME, cooked meat) at day 6 of retail display.

	Groups				*P-value*	RSD [1]
	C	R1	R2	R3		
N. samples	5	5	5	5		
C14:0	2.34	2.29	2.29	2.26	0.8738	0.16
C15:0	0.59	0.56	0.56	0.57	0.5264	0.03
C16:0	24.5	23.9	23.7	23.9	0.1290	0.50
C17:0	0.64^{a}	0.61^{ab}	0.60^{b}	0.62^{ab}	0.0336	0.02
C18:0	6.65^{a}	6.40^{ab}	6.20^{b}	6.40^{ab}	0.0052	0.16
C20:0	0.097^{a}	0.107^{b}	0.105^{ab}	0.104^{ab}	0.0077	0.004
C22:0	0.023	0.022	0.023	0.024	0.8580	0.002
C23:0	6.21	6.57	6.22	6.41	0.3352	0.35
SFA	41.9^{a}	41.3^{ab}	40.6^{b}	41.1^{ab}	0.0171	0.59
C14:1 *n*-5	0.22^{a}	0.18^{b}	0.18^{b}	0.17^{b}	0.0045	0.02
C16:1 *n*-9	0.33	0.33	0.34	0.33	0.3789	0.01
C16:1 *n*-7	3.42^{A}	2.96^{B}	2.98^{B}	2.89^{B}	0.0011	0.18
C17:1	0.33^{a}	0.31^{ab}	0.30^{ab}	0.29^{b}	0.0387	0.02
C18:1 *n*-7	1.19^{a}	1.11^{b}	1.11^{b}	1.11^{b}	0.0002	0.02
C18:1 *n*-9 *cis-trans*	22.8^{b}	23.8^{ab}	24.2^{a}	23.6^{ab}	0.0080	0.44
C20:1 *n*-9	0.20	0.22	0.21	0.21	0.0597	0.01
C22:1 *n*-9	0.020^{a}	0.014^{b}	0.014^{b}	0.014^{b}	0.0023	0.002
MUFA	28.5^{b}	28.9^{ab}	29.3^{a}	28.6^{ab}	0.0234	0.41
C18:2 *n*-6 *cis* (LA)	23.1^{b}	24.0^{b}	24.5^{a}	24.3^{a}	< 0.0001	0.29
C18:3 *n*-6 (GLA)	0.37	0.32	0.32	0.33	0.0625	0.03
C18:3 *n*-4	0.050	0.044	0.045	0.044	0.1145	0.003
C18:3 *n*-3 (α-LNA)	2.59	2.60	2.60	2.59	0.9980	0.10
C20:2 *n*-6	0.25	0.24	0.23	0.24	0.4358	0.01
C20:3 *n*-6 (DGLA)	0.31^{a}	0.26^{b}	0.24^{b}	0.27^{b}	0.0006	0.02
C20:4 *n*-6	1.81^{a}	1.43^{b}	1.33^{b}	1.55^{ab}	0.0004	0.14
C20:3 *n*-3	0.07	0.06	0.06	0.06	0.2284	0.01
C20:5 *n*-3 (EPA)	0.04	0.03	0.03	0.04	0.3287	0.04
C22:4 *n*-6	0.43^{a}	0.36^{ab}	0.32^{b}	0.38^{ab}	0.0072	0.04
C22:5 *n*-6	0.17^{a}	0.13^{b}	0.12^{b}	0.14^{b}	0.0004	0.01
C22:5 *n*-3	0.25^{a}	0.20^{b}	0.18^{b}	0.22^{ab}	0.0019	0.02
C:22 6 *n*-3 (DHA)	0.044^{a}	0.036^{ab}	0.033^{b}	0.035^{ab}	0.0167	0.005
PUFA	29.6	29.8	30.1	30.2	0.1764	0.47
n-6	24.6^{b}	25.3^{a}	25.8^{a}	25.6^{a}	0.0003	0.33
n-3	3.00	2.94	2.90	2.94	0.5334	0.11
n-6/*n*-3	8.23^{b}	8.61^{ab}	8.89^{a}	8.73^{ab}	0.0248	0.31

[1] Residual Standard Deviation.
[a, b] Means within the same row with different superscript significantly differ ($P < 0.05$).

differences in the FA profile analysed at day 0 could be attributed to a protective effect of rooibos on the FA (mainly preventing breakages of double bonds). In fact, as it was observed in Table 6, C group exhibited the highest SFA content, but overall MUFA and n-6 PUFA percentages were the lowest. However, this was probably not the only mechanism as C group showed also the highest proportion of n-3 PUFA, thus suggesting that further research to elucidate the direct effect of the tested rooibos extract on meat fatty acids is required.

Table 6
Effect of increasing inclusion levels of a rooibos extract (0% - C; 0.5% - R1; 1% - R2; 2% - R3) in the rabbit meat patties formulation on their sensory scores, analysed at days 0 and 6 of retail display.

	Groups				*P-value*
	C	R1	R2	R3	
N. samples	20	20	20	20	
Day 0					
Colour intensity	1.15 ± 0.67[d]	2.20 ± 0.52[b]	2.90 ± 0.31[c]	3.75 ± 0.79[a]	< 0.0001
Flavour:					
Overall intensity	2.05 ± 1.10[b]	2.15 ± 0.87[b]	2.45 ± 0.89[b]	3.50 ± 1.05[a]	< 0.0001
Rabbit	3.55 ± 1.00[a]	2.90 ± 0.79[b]	2.15 ± 0.49[c]	1.40 ± 0.82[d]	< 0.0001
Rooibos	1.05 ± 0.22[d]	2.05 ± 0.22[c]	3.10 ± 0.31[b]	3.80 ± 0.70[a]	< 0.0001
Off-flavours intensity	1.60 ± 0.99[c]	2.15 ± 0.74[c]	2.75 ± 0.79[b]	3.65 ± 0.81[a]	< 0.0001
Texture:					
Toughness	1.60 ± 1.09[b]	2.30 ± 0.80[a]	2.80 ± 0.70[a]	3.15 ± 1.23[a]	< 0.0001
Juiciness	2.95 ± 1.31[a]	2.84 ± 0.76[a]	2.42 ± 0.84[a]	1.63 ± 1.06[b]	< 0.0001
Day 6					
Colour intensity	1.14 ± 0.66[d]	2.05 ± 0.22[c]	2.95 ± 0.22[b]	3.71 ± 0.90[a]	< 0.0001
Flavour:					
Overall intensity	2.00 ± 1.09[c]	1.57 ± 0.60[c]	2.76 ± 0.44[b]	3.67 ± 0.91[a]	< 0.0001
Rabbit	3.52 ± 0.98[a]	2.38 ± 0.86[b]	2.43 ± 0.68[b]	1.76 ± 1.22[c]	< 0.0001
Rooibos	1.43 ± 1.08[d]	2.09 ± 0.30[c]	2.90 ± 0.30[b]	3.57 ± 1.08[a]	< 0.0001
Off-flavours intensity	1.76 ± 0.99[c]	1.67 ± 0.66[c]	2.76 ± 0.44[b]	3.81 ± 0.68[a]	< 0.0001
Texture:					
Toughness	1.33 ± 0.80[d]	1.86 ± 0.36[c]	3.00 ± 0.63[b]	3.81 ± 0.40[a]	< 0.0001
Juiciness	2.57 ± 1.25[ab]	2.95 ± 0.92[a]	2.57 ± 0.60[ab]	1.90 ± 1.37[b]	0.0210

[a, b, c, d] Means within the same row with different superscript significantly differ ($P < 0.05$).

Table 7
Effect of increasing inclusion levels of a rooibos extract (0% - C; 0.5% - R1; 1% - R2; 2% - R3) in the rabbit meat patties formulation on the frequencies of the specific off-flavours (%), analysed at days 0 and 6 of retail display.

	Groups				χ^2	*P-value*
	C	R1	R2	R3		
N. samples	20	20	20	20		
Day 0						
Rancid	0.00[b]	5.00[ab]	20.0[ab]	30.0[a]	9.59	0.0224
Rabbit	5.00	5.00	5.00	1.00	0.64	0.8872
Rooibos	0.00[b]	5.00[ab]	25.0[ab]	35.0[a]	12.0	0.0073
Liver	5.00	5.00	10.0	15.0	1.72	0.6320
Urine	0.00	0.00	5.00	5.00	2.05	0.5618
Acid	0.00	0.00	10.0	15.0	5.76	0.1239
Straw	0.00	0.00	5.00	5.00	2.05	0.5618
Day 6						
Rancid	14.3[ab]	0.00[b]	14.3[ab]	33.3[a]	9.01	0.0292
Rabbit	0.00	0.00	0.00	0.00	–	–
Rooibos	0.00[b]	0.00[b]	33.3[a]	47.6[a]	22.6	< 0.0001
Liver	4.80	0.00	14.3	23.8	7.34	0.0618
Urine	0.00	0.00	9.50	9.50	4.20	0.2407
Acid	0.00	4.80	14.3	14.3	4.21	0.2399
Straw	0.00	0.00	0.00	0.00	–	–

[a, b] Means within the same row with different superscript significantly differ ($P < 0.05$).

3.3. Sensory analysis

Sensory traits are of outmost importance for the consumers' choice at purchase; as for rabbit meat, consumers expect a tender, lean, delicately coloured and flavoured meat (Dalle Zotte, 2002). At day 0, meat colour was gradually perceived as more intense as the level of rooibos incorporation increased ($P < 0.0001$; Table 6). This could determine an alteration of the normal visual perception of rabbit meat, which is generally associated with a light pinkish coloured meat that tends towards greyer tones after cooking. R3 patties reached the highest flavour overall intensity score ($P < 0.0001$) and recorded the highest rooibos flavour perception ($P < 0.0001$), which was responsible for the lowest rabbit flavour in R3 patties ($P < 0.0001$). The 1% and 2% rooibos inclusions led to an increasing off-flavours perception too ($P < 0.0001$). In contrast, 0.5% rooibos incorporation level showed similar sensory features of the C group. Rooibos inclusion raised the perceived toughness of patties as the level of incorporation increased ($P < 0.0001$) and it progressively reduced patties juiciness which reached the lowest value in the group R3 ($P < 0.0001$). At day 6 of storage, the previously described discrepancies among groups remained substantially unchanged. Overall, sensory scores at days 0 and 6 showed that 1% and 2% rooibos incorporation conferred undesired sensory characteristics to rabbit meat patties. In contrast, the rabbit meat sensory perception was not penalised when rooibos was added at 0.5% inclusion level.

Previously, the sensory impact of different rooibos tea extract levels (0.25%, 0.5% and 1.0%) were evaluated in traditional South African dried sausages manufactured with ostrich, blesbok and springbok meats. Hoffman et al. (2014) found that the addition of the extract to ostrich droëwors positively enhanced aroma and flavour intensities at all tested levels, thus suggesting it as a feasible natural and exotic flavouring additive for traditional South African meat products. Jones et al. (2015a) evaluated the sensory properties of enriched seasoned traditional South African blesbok and springbok droëwors and found that a significant effect on droëwors aroma and flavour was exerted in a dose-dependent manner: the increasing rooibos aroma and flavour progressively reduced the typical game aroma and flavour of ungulates droëwors, with a possible positive effect on product acceptability. In fact, many consumers do not appreciate the distinct aroma and flavour of game meat (Hoffman, Muller, Schutte, Calitz, & Crafford, 2005).

In the present experiment, the most frequently perceived off-flavours were rancid and rooibos, which were identified by assessors after the 0.5% threshold level (Table 7). At day 0, R2 and R3 groups exhibited the greatest percentages in terms of rooibos off-flavour ($P = 0.0073$). Interestingly, also the perception of rancidity followed the same trend ($P = 0.0224$).

This unexpected finding could be explained by two main hypotheses: as it emerged that rooibos can act as flavour enhancer, even though the oxidation level of treated groups was lower than the group C, the perception of the off-flavours related to lipid oxidation, such as rancid and acid flavours, could have been intensified as the rooibos inclusion level increased over the 0.5%. The second hypothesis was that rooibos flavour was perceived by panellists similar, to a certain extent, to rancid off-flavour as a result of some common sensory traits, therefore leading to the presented results. The latter aspect would however require further investigation.

At the end of the retail display, the specific off-flavour frequencies reflected the initial discrepancies among groups holistically. In addition, it is worth noting that, even though no statistical differences were detected, panellists seemed to perceive a certain increasing trend going from C to R3 meat samples for urine off-flavour. This descriptor is related to protein degradation, and its higher sensory perception in R2 and R3 samples reflected the higher TVBN contents in those meat patties.

Comparing our observations with previous studies, it appears that a positive evaluation of meat products containing rooibos is linked not only to the rooibos extract inclusion level, but also to the meat species and the type of product. Inclusion levels ≥1% are probably more suitable for game meat or ripened meat products that possess a stronger flavour, while lower levels of inclusion are preferable for white, fresh meat or meat products which are characterised by a more delicate flavour. In addition, since rooibos is a shrub that only grows in South Africa, higher levels of inclusion are probably more accepted by South African consumers/panellists, as they are more accustomed to consuming rooibos.

PCA was performed to evaluate the relationships among the studied sensory variables (Fig. 2). The first two principal components accounted for 64.09% of the total variance. The first PC, D1 (43.6% of the total variance), was positively loaded by overall off-flavours intensity (0.62), flavour intensity (0.61), toughness (0.68), colour intensity (0.81) and rooibos flavour perception (0.90). The second PC, D2 (20.50% of the total variance), was negatively loaded by juiciness (−0.40), but it was positively loaded by rancid flavour (0.88). The graph depicted an expected phenomenon: rabbit and rooibos flavours opposed, as well as juiciness was in contrast with rancid flavour, off-flavours and flavour intensities, toughness, colour intensity and rooibos flavour intensity. The scatter plots of Fig. 3 clearly showed that assessors well discriminated C from R2 and R3 groups, whereas C and R1 resulted in overlying, thus confirming the sensory scores previously described.

4. Conclusions

The present study is the first one providing an overview of the effect of a fermented rooibos extract in maintaining the oxidative stability of fresh rabbit meat stored in refrigerated conditions, as well as assessing its sensory features. Findings confirmed that rooibos is an effective antioxidant for rabbit meat with promising potential. Among the tested inclusion levels, the 0.5% was the preferable one as it lowered lipid oxidation and protein degradation of rabbit meat patties along the retail display and it guaranteed an optimal sensory profile of the product which did not differ from that of untreated rabbit meat patties. Further studies should test the efficacy of such extract in active packaging systems and compare them with the direct incorporation in the manufacturing of fresh meat products, considering also the microbial count within the studied variables.

Conflicts of interest

The authors of this manuscript have no conflict of interest.

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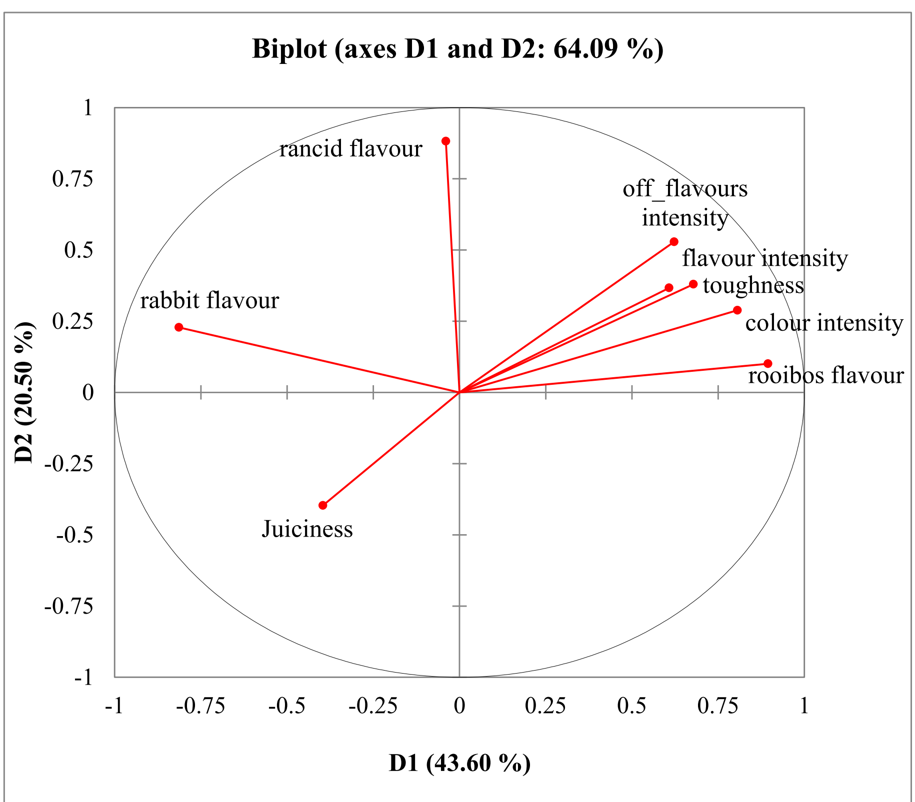

Fig. 2. PCA (Principal Component Analysis) to determine the effect of different rooibos inclusion levels (0% - C; 0.5% - R1; 1% - R2; 2% - R3) on the sensory traits of rabbit meat patties.

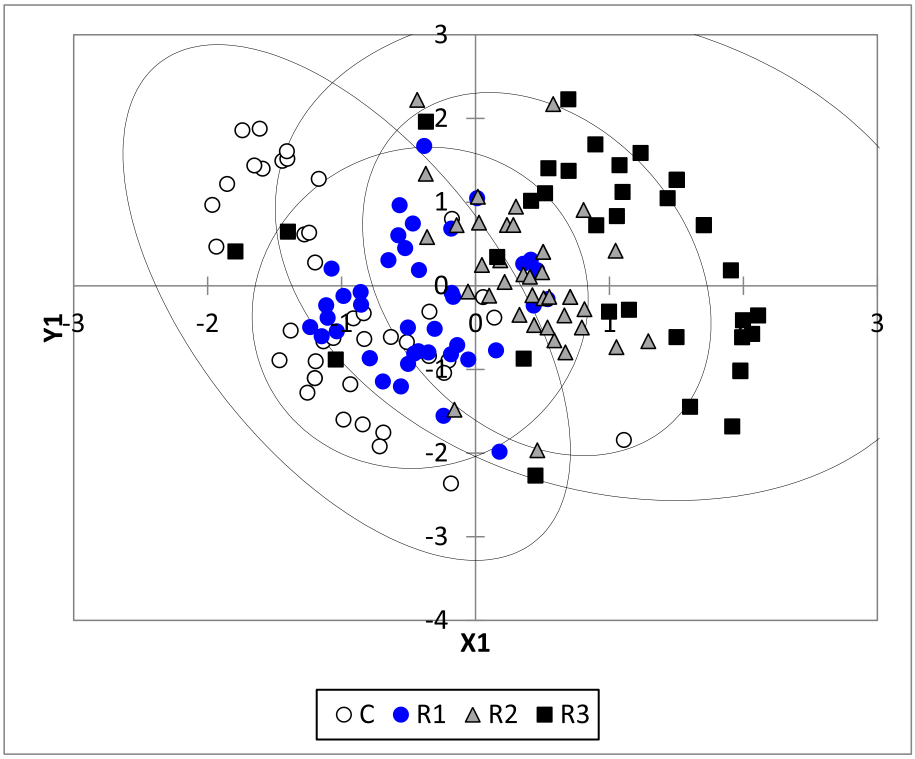

Fig. 3. Effect of different rooibos inclusion levels (C = 0%, R1 = 0.5%. R2 = 1%, R3 = 2%) on the sensory traits of rabbit meat patties displayed by scatter plots derived from PCA (Principal Component Analysis).

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