ROOIBOS (*Aspalathus linearis*) IS AN EFFECTIVE FUNCTIONAL INGREDIENT IN RETARDING LIPID OXIDATION OF COOKED RABBIT MEAT PATTIES

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Abstract – In the present trial, the effect of a fermented rooibos tea extract as a natural antioxidant on the shelf-life of rabbit meat patties was investigated. With this purpose, three treatments with the inclusion of 0.5% (R1), 1% (R2) and 2% (R3) fermented rooibos tea extract were compared to a control group without any extract (C: 0%). The inclusion of 0.5%, 1% and 2% fermented rooibos tea extract lowered the peroxides content of rabbit meat patties compared to untreated ones (P<0.0001). Moreover, the rooibos presence affected also the pH values of rabbit meat patties, with R2 and R3 groups providing lower values compared to R1 and C groups (P<0.0001). Differently, although a lower drip loss in patties treated with the rooibos tea extract compared to the control group was expected, no effect was observed in this sense. Consequently, further studies are needed to deeply understand the mechanisms through which the rooibos presence would affect quality aspects of meat and meat products.

I. INTRODUCTION

Lipid oxidation represents an important issue for the meat industry because it negatively affects meat colour, texture, flavour, and determine loss of nutritional value, ultimately reducing its shelf-life [1]. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used to ensure optimal product quality. However, due to safety concerns and a changing consumer's preference regarding food additives, since the last decade natural antioxidants have been gaining more and more scientific and commercial attention [2]. One emerging natural source of unique phenolic compounds such as aspalathin, is a South African leguminous shrub named rooibos (*Aspalathus linearis*) which has been appreciated from centuries by locals as a beverage. Traditional rooibos preparation includes an oxidation (fermentation) step, essential to develop the characteristic sweetish flavour and red-brown colour which determines lower antioxidant levels compared to the unfermented (green) form [3]. 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 BHT and BHA [4]. Considering food matrixes, the antioxidant potential of rooibos was only tested in two recent studies [5; 6]. Unfermented rooibos was applied to ostrich meat patties in a shelf-life trial, whereas the classic fermented rooibos was evaluated in processed meat products: nitrates and nitrites-free ostrich salami and ostrich droëwors. The preliminary study by [5] provided promising results in terms of lipid stability of fresh ostrich meat and salami. Differently, even if the presence of rooibos generated positive sensory scores, the oxidative stability of the droëwors remained unaffected [6]. Consequently, further confirmations of the positive effects of rooibos on the quality of meat and meat products are required. In the specific case of rabbit meat, which is very rich in unsaturated fatty acids [7], a certain degree of lipid oxidation, mainly during processing, storage and cooking is expected [8; 9]. Thus, in the present trial, we studied the effect of a fermented rooibos extract in prolonging the shelf-life of rabbit patties during display conditions.

II. MATERIALS AND METHODS

The trial took place at the Department of Animal Medicine, Production and Health of the University of Padova (Italy). A total of 16 kg of rabbit meat from hindleg and loin cuts were ground. The rooibos extract was prepared according to the procedure described by Joubert *et al*. [10]. Meat was then divided in four batches, and manually mixed with different rooibos inclusion levels: Control (0% rooibos), R1 (0.5% rooibos), R2 (1% rooibos) and R3 (2% rooibos). Then the mixture was ground again in order to allow a better distribution of the rooibos extract. Afterwards, 60 patties per treatment of 50 g each were prepared, individually wrapped with plastic film in order to prevent direct air contact, and then placed in a fridge at 4 °C for a 7 days shelf-life trial. Continuous cold fluorescent light illumination was ensured for the whole period, in order to simulate commercial display conditions. Just before wrapping, n=40 patties per treatment were weighed for drip loss determination and pH was measured twice. The same measurements were repeated at days 1, 3 and 6 of storage. At days 0, 1, 3 and 6 a total of 80 patties (5/treatment/day of storage) were removed from the fridge, freed from the plastic film, wrapped with aluminium sheets and individually identified, vacuum sealed in four plastic bags (one/treatment) and cooked in a water bath for 25 minutes at 80 °C. After cooling, patties were frozen until peroxide value analysis (internal method of the Istituto Zooprofilattico Sperimentale delle Venezie) and expressed as meq O_2/kg meat. A One-way Anova [11] tested the rooibos inclusion level as fixed effect and the significance level was calculated at the 5% confidence level. For drip loss determination the initial weight of the patties was considered as covariate.

III. RESULTS AND DISCUSSION

The presence of rooibos extract tea significantly affected the peroxides content of rabbit meat patties throughout the trial (Table 1), improving their shelf-life. Already at day 0, independently to the inclusion level, patties treated with rooibos tea extract showed a higher oxidative stability compared to the control group (P<0.0001). This result could depend on the three week frozen storage prior to analysis. As a result, rooibos extract had time to effectively counteract the oxidative process. At days 1 and 3, groups R2 and R3 provided the best protection against lipid oxidation even if also R1 showed a lower peroxides value compared to C group. At day 6, the oxidative degree of the meat worsened in all groups. However, the presence of the rooibos tea extract (R1, R2 and R3) still generated lower peroxides values compared to the untreated samples (C). This result could be interesting for the meat industry in terms of costs, as the inclusion of a 1% rooibos tea extract was enough to provide satisfactory protection against lipid oxidation. Cullere *et al.* [5] showed a protective effect against oxidation of the same fermented rooibos tea extract on ostrich salami. Specifically, after 15 days of ripening, salami incorporated with 0.5% and 1% rooibos tea extract had a lower TBARS content compared to the control group. Differently, Hoffman *et al.* [6] did not find any significant effect of fermented rooibos (0.25%, 0.5% and 1% of inclusion level) on ostrich droëwors in terms of protection against lipid oxidation. The only other work that studied the effect of rooibos on fresh meat was that of Cullere *et al.* [5] where 2% unfermented rooibos was applied to ostrich patties and significantly retarded the oxidative process compared to a control group (0% unfermented rooibos).

Table 1. Peroxide (meq O_2/kg meat) values (measured at day 0, 1, 3, 6) of cooked rabbit patties without (C) or with 0.5% (R1), 1% (R2) and 2% (R3) fermented rooibos levels

	Treatments				RSD[(1)]
	C	R1	R2	R3	
No.	20	20	20	20	
Day 0	8.4^{A}	3.0^{B}	2.5^{B}	2.4^{B}	0.60
Day 1	10.2^{A}	4.6^{B}	3.3^{C}	2.9^{C}	0.41
Day 3	11.8^{A}	4.4^{B}	3.1^{C}	3.0^{C}	0.45
Day 6	18.3^{A}	6.7^{B}	6.5^{B}	5.8^{B}	1.12

[A,B,C] Means in the same row with unlike superscripts differ (P<0.0001); [(1)] Residual Standard Deviation

The results presented in Table 2 on the pH values of rabbit meat patties showed that the presence of the rooibos tea extract lowered the pH values compared to the control group (P<0.0001).

Specifically, increasing the rooibos extract inclusion level, the pH progressively decreased. As a consequence, R3 group provided the best results throughout the experiment followed by groups R2 and R1, with C patties exhibiting always the worst values. These results were probably a consequence of the better oxidative status of the treated patties compared to those belonging to the C group. In the only other research studying rooibos inclusion on fresh meat quality [5] it was observed that the inclusion of a 2% dried unfermented rooibos leaves to ostrich meat patties provided lower pH values compared to a control group until the sixth day of an eight-days shelf-life trial.

Table 2. pH values (measured at day 0, 1, 3, 6) for rabbit patties without (C) or with 0.5% (R1), 1% (R2) and 2% (R3) fermented rooibos levels

	Treatments				RSD[(1)]
	C	R1	R2	R3	
No.	40	40	40	40	
Day 0	6.14^{A}	6.10^{B}	6.09^{B}	6.00^{C}	0.02
Day 1	6.26^{A}	6.16^{B}	6.14^{B}	6.07^{C}	0.03
Day 3	6.20^{A}	6.15^{B}	6.13^{B}	6.09^{C}	0.03
Day 6	6.25^{A}	6.23^{A}	6.19^{B}	6.04^{C}	0.02

[A,B,C] Means in the same row with unlike superscripts differ (P<0.0001); [(1)] Residual Standard Deviation

Results presented in Table 3 dealt with drip loss of rabbit meat patties. The presence of rooibos tea extract had a significant effect on the drip loss between Days 0-1, 1-3 and 3-6. Results at Day 0-1 showed that R2 and R3 groups had higher drip loss values than those exhibited by C and R1 groups (P<0.001). But at Day 1-3 the situation was completely reversed, with a progressive drip loss lowering with increasing rooibos extract inclusion levels. In the third window of time (Day 3-6), the trend of drip loss changed again with C patties showing a lower value compared to R3 group, with R1 and R2 being intermediate (P<0.05). As a result of this inconstant pattern, the total drip loss (Day 0-6) was not significantly affected by treatments (P>0.05). This result was partly unexpected and it was probably the result of the interaction of two different phenomena. On one hand, the protection of the rooibos tea extract against lipid oxidation (Table 1) should have had a positive effect also on drip loss, as lipid oxidation is known to affect the ability of myofibrillar proteins to entrap water [12]. On the other hand, however, in food products the interaction protein-polyphenol through hydrogen binding is known to occur. The strength of this complex, which depends on the specific structures of the phenolic group of polyphenols and the amide group of protein, would alter charge distribution on the protein surface and consequently also the water holding capacity [13; 14]. Even if there are few studies on the relationship between polyphenols-protein complex and functional properties of proteins, this effect seemed to partly explain our unexpected results and also those of other studies evaluating the ability of natural preservatives to enhance the shelf-life of meat products. In fact, in the above mentioned works, a protection against lipid oxidation didn't always provide also an improvement in terms of WHC as well as a drip loss lowering [15].

Table 3. Drip losses (%) of rabbit meat patties without (C) or with 0.5% (R1), 1% (R2) and 2% (R3) fermented rooibos levels

	Treatments				RSD[(1)]
	C	R1	R2	R3	
No.	40	40	40	40	
Day 0-1	1.18^{B}	1.06^{B}	2.20^{A}	2.19^{A}	0.46
Day 1-3	2.37^{A}	2.04^{AB}	0.70^{BC}	0.52^{C}	1.16
Day 3-6	0.05^{b}	1.35^{ab}	0.58^{ab}	1.75^{a}	1.44
Day 0-6	3.60	4.45	3.48	4.47	1.19

[A,B,C,D] Means in the same row with unlike superscripts differ (P<0.001); [a,b] means in the same row with unlike superscripts differ (P<0.05); [(1)] Residual Standard Deviation

IV. CONCLUSION

The practical application of the rooibos tea extract to meat and meat products could generate interesting outcomes for the meat industry. In fact, fermented rooibos tea extract confirmed to be an optimal antioxidant source also for rabbit meat patties enhancing their shelf-life through the reduction of the peroxides content and pH values. However, as literature data on the application of this natural source of antioxidants on fresh meat and meat products is still scarce and results are sometimes contrasting, further studies are needed

to deeply understand the mechanisms through which the rooibos presence would affect quality aspects of meat and meat products. Moreover, the sensory acceptability of the product should also be further investigated.

ACKNOWLEDGEMENTS

Authors thank E. Joubert, of Post-Harvest and Wine Technology Division (Agricultural Research Council of South Africa; Infruitec- Nietvoorbij, Stellenbosch, South Africa), for providing the fermented rooibos extract. Research funded by the University of Padova (Ricerca Scientifica fondi quota EX 60% code: 60A08-2910/12).

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