PRELIMINARY STUDY TO TEST ROOIBOS (*Aspalathus linearis*) AS A NATURAL ANTIOXIDANT APPLIED TO OSTRICH MEAT PATTIES AND ITALIAN TYPE SALAMI

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Abstract - Rooibos (*Aspalathus linearis*) is a South African fermented herbal tea which has been gaining attention in the market for its interesting functional properties among which is the protection from oxidative stress caused by superoxide, hydroxyl and peroxyl radical formation. It mainly acts as a chain-breaker, metal-chelator and also as a radical scavenger. Despite this antioxidant property, it has never been tested on meat products. Green rooibos is the unfermented form which possesses higher amounts of total polyphenols and flavonoids than the fermented form. An experiment was designed in order to test green rooibos antioxidant activity on refrigerated ostrich meat patties: one Control (0% green rooibos) and three Treatments (T1=2% dried leaves; T2=2% water extract; T3=2% freeze-dried extract) were studied. A second trial evaluated the antioxidant activity of increasing inclusion levels of fermented rooibos extract in the production of low-fat ostrich salami (Control=0%; T1=0.25%; T2=0.5%; T3=1% extract). Data on TBARS and weight losses were recorded and the rooibos antioxidant effect was tested by a one-way ANOVA. Green rooibos was effective in lowering the lipid oxidation rate in ostrich meat patties and fermented rooibos extract limited the TBARS content of ostrich salami at 15 days of ripening. Further studies are needed to test the lowest effective inclusion level, sensory qualities and economical sustainability of using this natural antioxidant.

Key Words – Ostrich meat, Fermented salami, Rooibos, Antioxidant activity.

I. INTRODUCTION

Rooibos (*Aspalathus linearis*) is a South African fermented herbal tea which has been gaining attention in the market for its interesting functional properties. Rooibos tea has more recently been used as a natural additive in many human food products such as yogurt, in the cosmetic industry and lately also in pet food [1].
Another type of rooibos, green rooibos, is now being produced. It possesses a higher level of total polyphenols, flavonoids and non-flavonoids than the fermented rooibos as the fermentation process oxidizes polyphenols and flavonoids such as aspalathin and nothofagin [2; 3]. Specifically, rooibos protects against oxidative stress caused by superoxide, hydroxyl and peroxyl radical formation and it mainly acts as a chain breaker, metal-chelator and, to a lesser extent, also as a radical scavenger [4; 5]. Despite all these positive characteristics, green and fermented rooibos have not been studied in meat products. Therefore, green rooibos' antioxidant activity was tested in a shelf-life trial on ostrich meat patties. In a second experiment, the antioxidant activity of a fermented rooibos extract, which is the most common and available form, as a natural additive for the production of low-fat ostrich salami was evaluated.

II. MATERIALS AND METHODS

Both experimental trials took place at the University of Stellenbosch in December 2011. In the first trial 2% (w/w) of green rooibos was added to 3 batches of ostrich meat patties. The rooibos was prepared in three different ways: dried leaves (Treatment 1), water extract (Treatment 2) and freeze-dried extract (Treatment 3). A meat batch without rooibos inclusion was considered as the Control (C). Frozen ostrich fan fillets were defrosted overnight at ±4 °C and the following day the meat was ground through a 5 mm diameter grinder (Butcherequip). Thereafter, the meat was divided in four equal parts of 200 g each and manually mixed with green rooibos extracts according to the experimental design.
Thereafter that the meat of each treatment was split in five patties of ≈ 40 g each patty was individually wrapped with plastic film to reduce direct air contact. The patties were stored at 4±1 °C under fluorescent light illumination (L58 W/ 20, Osram, Germany) at 870 lux (MT 940, Major) for a 9 days shelf-life trial. The TBARS (Thiobarbituric acid reactive substances) content of meat patties was measured at Day 0, 4, 6 and 8.

For the second trial 4 batches of 24 salami each (n=96) were prepared. One batch represented the control (C) without any fermented rooibos extract inclusion (0%). In the other 3 batches 0.25% w/w (T1), 0.5% (T2) and 1% (T3) fermented rooibos extracts were incorporated. The other ingredients were: a ratio of 75:25 defrosted ostrich meat (made up of a random selection of individually vacuum packed and frozen muscles) and pork fat, 2.4% salt, 0.5% ground black pepper and a starter culture mix of *L. plantarum* 423 and *Micrococcus sp*. in order to ensure $1x10^5$ cfu/g meat. Ingredients were minced (5 mm diameter), mixed and stuffed into cellulose casings in order to obtain raw salami of ≈ 500 g/each. The salami were ripened in a dedicated chamber for 30 days. After a day of fermentation (18 °C and 90% RH), the temperature was gradually decreased at one degree/day until 12 °C (RH 70-80%) was attained and maintained; at that point the RH was kept in the range of 75-85% for the rest of the ripening period. Salami weights were measured at DAY 0, DAY 7, DAY 15 and DAY 30 and weight loss percentages were subsequently calculated. Samplings for TBARS analysis were collected at Day 0, Day 15 and DAY 30. The green rooibos extracts (T2 and T3) for the first trial were prepared as follows: 2 grams of dried and crushed green rooibos leaves were steeped into 50 mL boiling distilled water for 10 minutes. After 20 minutes of cooling at room temperature and subsequent filtration through a Whatman n° 1 filter paper there were 25 mL left (T2). The water extract was then placed in a plastic cup, frozen at -80 °C for 30 minutes and then dried in a Christ Loc-1m drier overnight (T3). The fermented rooibos extract incorporated in the salami of the second trial was prepared according to the procedure described by Joubert *et al.* [6]. The TBARS analysis was performed following the method reported by Lynch *et al.* [7]. A one-way ANOVA tested the effects of the green rooibos type of preparation (experiment 1) and those of the fermented rooibos extract inclusion levels (experiment 2).

III. RESULTS AND DISCUSSION

As shown in Table 1 green rooibos presence, independently of the form in which it was added to the meat, significantly affected Thiobarbituric acid reactive substances (TBARS) formation during the storage period of ostrich meat patties (P<0.001). Malondyaldeyde (MDA) content of the Control group (mg MDA/kg meat) exhibited a rapid rise measured at 4 days of storage whereas it remained stable when 2% of green rooibos was included. By DAY 6 the TBARS of the control had already peaked at >7 mg MDA/kg meat. At DAY 8, the ostrich patties that were treated with green rooibos had TBARS values similar to that of the control on Day 4 (Table 1). The form in which the green rooibos was added had no effect on the TBARS values per day of measurement.

Table 1 TBARS values (mg MDA/kg meat) of ostrich meat patties prepared without (C) or with 2% dried leaves (T1), water extract (T2) and freeze-dried extracts (T3) from green rooibos.

	TREATMENTS					
	C	T1	T2	T3	*P*-value	RSD(1)
N. obs	24	24	24	24		
DAY 0	2.37	2.41	2.27	2.40	0.1450	0.11
DAY 4	4.33^A	2.44^B	2.20^B	2.42^B	<0.0001	0.16
DAY 6	7.25^A	2.83^B	2.70^B	2.67^B	<0.0001	0.61
DAY 8	7.83^A	4.17^B	3.78^B	3.98^B	<0.0001	0.27

$^{A, B}$ means with different superscripts differ within day (P<0.001); (1) Residual Standard Deviation

The initial MDA content of ostrich patties measured at DAY 0 was higher than that recorded by Leygonie *et al.* [8] on fresh ostrich meat (2.27 ≤ MDA ≤ 2.41 *vs* 1.2 < MDA ≤ 2.21), however, from DAY 2 until DAY 6 rooibos extract guaranteed a stability in the lipid oxidation rate. In fact the TBARS values were similar to those described from DAY 2 to DAY 6 by the previous mentioned work and, when compared to oxygen modified atmosphere packaging (MAP: 70% oxygen, 30% carbon dioxide), they were even lower (3.78 ≤ MDA ≤ 4.17 *vs* MDA > 4.5). Fernández-López *et al.* [9] packaged ostrich steaks under different conditions and showed lower MDA content (MDA ≤ 1.2 mg/kg meat) than measured in this investigation. However, the ostrich meat used in this trial was previously frozen at -20°C for 30 days and this, together with the fact that ostrich meat is rich in PUFA and heme-iron, certainly played an important role in the development of lipid oxidation [10]. Despite this, the TBARS content of ostrich meat patties studied exhibited lower values compared to those reported on the shelf-life of MAP ostrich meat (Day 4: MDA > 4 mg/kg and MDA > 3 mg/kg for 1:1 and 3:1

headspace ratios, respectively) [11] and those reported on freshly ground ostrich meat stored in AIR (80% nitrogen, 20% oxygen) or in O2 (80% oxygen, 20% carbon dioxide) MAP conditions (Day 6: MDA (AIR MAP) ≈ 5 mg/kg; MDA (O2 MAP) ≥ 20mg/kg) [12].

Similarly, the meat used for the investigation on ostrich salami was defrosted and this also affected lipid oxidation values measured at DAY 0 (Table 2). The statistical difference in the TBARS values at DAY 0 (Treatments 2 and 3 *vs* Control and Treatment 1) could be due to the fact that some of the vacuum-sealed plastic bags in which the frozen meat pieces were stored may have been damaged during handling. This allowed contact between meat and air, increasing the oxidation rate and influencing the baseline results obtained at DAY 0. Data recorded and analysed at DAY 15 showed that fermented rooibos extract was able to limit the formation of TBARS. In fact, increasing the rooibos inclusion level, resulted in a tendency towards a progressive lowering of lipid oxidation, which became statistically significant for 0.5% and 1% levels compared to 0.25% and 0%.

Table 2 TBARS values (mg MDA/kg meat) for ostrich salami prepared without (C) or with increasing levels of fermented rooibos extract: 0.25% (T1), 0.5% (T2) and 1% (T3).

	TREATMENTS					
	C	T1	T2	T3	*P*-value	RSD$^{(1)}$
N. obs	12	12	12	12		
DAY 0	4.81^{B}	4.70^{B}	5.63^{A}	5.04^{AB}	0.0056	0.31
DAY 15	5.81^{A}	5.52^{A}	3.96^{B}	3.93^{B}	0.0001	0.49
DAY 30	9.55	12.43	6.63	10.80	0.0618	2.73

$^{A, B}$ means with different superscripts differ within day (P<0.01); $^{(1)}$ Residual Standard Deviation

Salami formulated with natural antioxidants were also studied by Marangoni *et al.* [13] and Chichoski *et al.* [14]: in the first study the antioxidant activity of *Coriandrum Sativum* L. essential oil was evaluated whereas in the second the basil essential oil (*Ocimum basilicum* L.) was tested. In both cases natural extracts effectively controlled lipid oxidation. At DAY 30 the variability of the TBARS values was high and this condition, associated to the limited number of salami used, resulted in no differences (P=0.0618; Table 2).

As mentioned, among the properties of flavonoids present in rooibos extracts is the ability to chelate metals such as iron, a well known pro-oxidant, whose content in ostrich meat can increase lipid oxidation [15]. Rooibos could thus have acted as an iron-chelator in both trials and, as a final result, it limited the TBARS substances formation.

The ability of rooibos extract to bind the water was evidenced by the results presented in Table 3 where, during the whole ripening process, T2 and T3 salami presented lower weight losses (WL) compared to C and T1. Despite this, the water activity values that were measured at DAY 30 for T2 and T3 were below the safety maximum of 0.92 (0.84 and 0.83 for T2 and T3, respectively) thereby ensuring a product with no risks from a microbial point of view. Additionally, salami are generally sold when they reach a weight loss higher than 35% and this is perfectly in line to that obtained from DAY 15 (44.9 and 46.4% of WL for T2 and T3, respectively).

Table 3 Weight losses (WL) of ostrich salami prepared without (C) or with increasing levels of fermented rooibos extract: 0.25% (T1), 0.5% (T2) and 1% (T3).

	TREATMENTS					
	C	T1	T2	T3	*P*-value	RSD$^{(1)}$
N. obs	62	63	63	63		
WL D7	44.2^{A}	41.7^{A}	30.1^{B}	30.7^{B}	<0.0001	4.6
WL D15	49.2^{A}	49.2^{A}	44.9^{B}	46.4^{B}	<0.0001	2.0
WL D30	55.3^{A}	56.3^{A}	53.4^{B}	54.3^{B}	<0.0001	1.1

$^{A, B}$ means with different superscripts differ within day (P<0.001); $^{(1)}$ Residual Standard Deviation
D= Day

IV. CONCLUSIONS

These two trials demonstrated that the antioxidant potential attributed to both green and fermented extracts was effective in prolonging the shelf-life of ostrich meat patties and in lowering the lipid oxidation rate of ostrich salami. Despite these encouraging results, further studies are needed to understand if and to which extent the rooibos presence affects the sensory quality of the product and at which extract concentration can both the antioxidant effect and sensory quality of the final product be ensured. It is also necessary to evaluate whether the rooibos incorporation is economically feasible for the industry and acceptable to consumers.

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