ANTIOXIDANT COMPOSITION OF *MORINGA OLEIFERA* LEAF POWDER AND ITS EFFECT ON THE LIPID OXIDATION OF SOUTH AFRICAN PORK DROËWORS

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The study assessed the effect of using *Moringa oleifera* leaf powder (MLP) to minimise lipid oxidation in the processing and storage of droëwors, a traditional South African dried meat product. The *β*-carotene, *α*-tocopherol and total phenolic compounds (TPC) of MLP were determined. Four batches of pork droëwors, containing 0, 0.5, 1 and 2% MLP were produced and sampled during drying and after 7 days of storage for analysis of thiobarbuturic acid reactive substances (TBARS). The MLP was found to contain substantial levels of *β*-carotene (23.2 ± 2.8 mg/100 g), *α*-tocopherol (76.7 ± 1.9 mg/100 g) and TPC (7.5 ± 0.2 mg gallic acid eq/g). Lipid oxidation occurred more rapidly when MLP was not added and TBARS were significantly (P < 0.05) higher in the control from 72 hrs. No effect of MLP level was observed, indicating that MLP inclusion at 0.5% is sufficient to inhibit lipid oxidation.

Key Words – Lipid oxidation, *Moringa Oleifera*, pork, droëwors.

I. INTRODUCTION

Droëwors is a shelf-stable, ready to eat dried sausage; produced and consumed widely in South Africa. It is traditionally made from beef and animal fat, although meat from other species such as ostrich and game are increasingly being used (1,17). Pork, however, is not typically used in droëwors production. Traditional droëwors recipes advise against using pork because it is reportedly prone to rancidity when dried. Pork has a higher fat content and polyunsaturated:saturated fat ratio than beef (0.58 in pork vs 0.11 in beef) (2); making it more susceptible to oxidation (3). Lipid oxidation is the main non-microbial cause of quality deterioration in muscle foods (4). It results in the development of off odours, off flavours and deterioration of colour, texture and nutritive value of meat and meat products (5). Formulations containing synthetic antioxidants are commonly used in meat processing to reduce the detrimental effects of lipid oxidation on product quality (6). However, synthetic additives have been implicated with human health risks. The recent International Agency for Research on Cancer (IARC) report on the carcinogenicity of processed meat (7) has increased consumer concern about the safety of synthetic additives (15); validating research for suitable natural alternatives (6). Feeding *Moringa oleifera* leaves to livestock as a functional feed additive has been effective in reducing lipid oxidation in meat (8,9,10). However, the dietary inclusion of Moringa has been reported to negatively affect feed conversion efficiency in pigs due to the presence of anti-nutritional factors (11). *Moringa* leaves are safe for human consumption and reportedly contain high levels of antioxidant compounds (12), raising the question of whether direct incorporation of *Moringa*

oleifera leaf powder (MLP) into droëwors during processing would increase the oxidative stability of the product. This study aimed to quantify antioxidant compounds in MLP and determine its effect on lipid oxidation during the processing and storage of pork droëwors.

II. MATERIALS AND METHODS

Moringa oleifera leaf powder (MLP) of Senegalese origin, processed by air drying fresh Moringa leaves under shade before being ground thrsough a 2 mm sieve screen, was purchased for the trial (Racines, SA, France).

α-tocopherol analysis

To 100 mg of MLP (in triplicate), 1 ml NaCl 0.9 % was added and vortexed. Ethanol (with 1% pyrogallol) and saturated KOH was added, samples were placed at 70 °C for 30 min, and then extracted twice with n-hexane. The organic phases were collected, evaporated under nitrogen and diluted in ethanol for HPLC analysis. Separation was performed with a C-18 reverse phase column and mobile phase ethanol:methanol (60:40). Fluorescent detection was done at 296-330 nm excitation and emission respectively. Standard curves were performed using commercial standards.

β-carotene analysis

To 100 mg of MLP (in triplicate), 3 mL of distilled water was added and stirred with a magnetic stirrer for 2 min. Twenty five mL of a mixture of ethanol and hexane (40:30) with 0.1% BHT was added and agitated for 4 min before filtering through a sintered filter. The residue was re-extracted twice using the same procedure. The totality of the extract was transferred to a separatory funnel and 50 mL of NaCL 10% was added. The mix was agitated and the organic phase washed with 40 mL of water. The aqueous phase was removed and the yellow organic phase was evaporated to dryness and dissolved in dichloromethane:methanol: MTBE and injected in an HPLC system with a C-30 reverse column. Detection was performed at 450 nm.

Total phenolic compounds

Extraction and analysis of total phenolic compounds (TPC) was conducted according to procedures detailed in ISO 14502-1:2005(E), with modifications. The standard curve was prepared using galic acid and results were reported as mg gallic acid equivalent per gram of dry matter. Two hundred mg of MLP (in triplicate) was vortexed with 5 mL of pre-heated methanol (70%), incubated in a water bath at 70°C for 10 min, allowed to cool to room temperature and centrifuged at 4000 rpm for 5 min. The supernatant from each extraction tube was carefully decanted and the extraction procedure was repeated on the residues. The 2 extracts were combined and extraction solution was added to bring the volume up to 10 mL. Extracts were diluted (1 mL extract in 20 mL distilled H_2O) and 1 mL of diluted extract was added to 1 mL distilled H_2O, 5 mL Folin-Ciocalteu phenol reagent and 4 mL sodium carbonate solution, vortexed and allowed to stand for 60 min at room temperature before the absorbance was read at 760 nm.

Droëwors production

Two kg of lean pork meat and pork fat in the ratio of 80:20 were cut into cubes (3 x 3 cm) and minced together through a 5 mm screen. The mixture was re-weighed and salt (2%) and pepper (0.5%) were thouroughly incorporated to mixture. The mince was separated in 4 batches of 500 g, to which no (C), 0.5 (M0.5), 1 (M1) and 2% (M2) MLP was added. The treatments were minced separately through a 2 mm screen into natural sheep casings (22 mm diameter) and hung vertically in a drying chamber at 35°C and 40% relative humidity for 72 hrs.

Lipid oxidation measurement

Triplicate samples (5 g each) were taken at 0, 1.5, 5.75, 27.25, and 72 hrs during drying. The droëwors were stored under ambient conditions and sampled after 7 days (168 hrs). The content of thiobarbituric acid reactive substances (TBARS) was determined by acid precipitation using the technique described by Descalzo et al (4). Briefly, samples were homogenized with 12.5 mL of TCA [20% trichloroacetic acid in 1.6% hypo-phosporic acid (HPO_3)] and 12.5 mL distilled water for 180 sec using a Stomacher 400 Laboratory Blender (Seward Medical, London, UK). Slurries were filtered (0.45 µm) and duplicate samples of filtrate (3 mL) were added to an equal volume of 0.02M

2-thiobarbituric acid. An equal volume of distilled water was added to the third replicate to act as a turbidity blank for each sample. Samples were vortexted for 10 sec, incubated in a water bath at 70°C for 1 hrs until pink colour development, allowed to cool for 30 min and the absorbance was read at 532 nm. TBARS were calculated using 1,1,3,3-tetraethoxypropane (TEP) as a standard. Results were expressed as mg of malonaldehyde (MDA) equivalents/kg of fresh meat.

Statistical analysis

Data on α-tocopherol, β-carotene, TPC quantities in MLP and TBARS in pork were analysed using PROC GLM procedures of SAS (13) and pair wise comparisons of least square means were done. Differences were significant at $P < 0.05$.

III. RESULTS AND DISCUSSION

α-tocopherol, β-carotene and TPC of MLP

The quantities of *β*-carotene, *α*-tocopherol and total phenolic compounds are presented in Table 1. The TPC of the methanol/water extracts of MLP found in this trial (7.5 mg gallic acid eq/g) is comparable to that reported by Wangcharoen and Gomolmanee (2011), who found average TPC values ranging from 8.6 – 9.7 mg gallic acid eq/g in aqueous extracts of MLP and 4.6 – 5.6 mg gallic acid eq/g in ethanolic extracts of MLP. Differences in the TPC are expected with the use of different extraction solutions. The mean *β*-carotene content found in MLP this trial (23.2 mg/100 g) was slightly higher than the *β*-carotene content reported by Moyo *et al.* (12), who found 18.5 mg/100 g). Moringa leaves have been reported to contain high levels of vitamin E and *β*-carotene and this is consistent with the results found in this trial. Variations in the quantities of antioxidant compounds in Moringa have also been attributed to differences in the agrological zones in which it is grown (14). For this reason, it was important to quantify the antioxidant compounds in the MLP being used in this study, as it was of Senegalese origin and there is presently no literature available on the antioxidant compounds in Moringa of Senegalese origin.

Table 1 *β*-carotene, *α*-tocopherol and TPC of MLP

Antioxidant component	means ± standard error
β-carotene (mg/100 g)	23.2 ± 2.8
α-tocopherol (mg/100 g)	76.7 ± 1.9
TPC (mg gallic acid eq/g)	7.5 ± 0.2

Lipid oxidation

The evolution of TBARS for each treatment are presented in Table 2. The control shows the onset of lipid oxidation between 27.25 and 72 hrs of drying and oxidation continued during storage to reach 1.13 mg MDA/Kg at 168 hrs. TBARS of dry cured pork ham have been reported to be 1.38 – 1.57 MDA/Kg meat after an 18 month curing and drying process (16). Some reported TBARS in droëwors after 2 weeks of storage include 2.8 mg MDA/Kg in springbok droëwors made with beef fat (17). TBARS of M0.5, M1 and M2, which started to increase during storage, were significantly lower (0.34 - 0.35 mg MDA/Kg in each treatment) from 72 hrs than in the control and no significant differences were shown between the MLP treatments at any time during drying and storage. Jayawardana et al. (15) similarly found significantly lower TBARS values in chicken sausages with 0.5, 0.75 and 1% MLP added compared to the control treatment, with no significant differences between the MLP treatments after 1 week of storage.

Table 2 Evolution of TBARS during 72 hrs of drying and 7 days (168 hrs) of storage

Time (hrs)	Treatment			
	C	M0.5	M1	M2
0	0.19^{aA} ± 0.035	0.15^{aA} ± 0.035	0.15^{aA} ± 0.035	0.19^{aA} ± 0.035
1.5	0.18^{aA} ± 0.035	0.16^{aA} ± 0.035	0.22^{aA} ± 0.035	0.19^{aA} ± 0.035
5.75	0.23^{aA} ± 0.035	0.17^{aA} ± 0.035	0.18^{aA} ± 0.035	0.20^{aA} ± 0.035
27.25	0.26^{aA} ± 0.035	0.17^{aA}± 0.035	0.17^{aA} ± 0.035	0.20^{aA} ± 0.035
72	0.53^{bB} ± 0.035	0.16^{aA} ± 0.035	0.19^{aA} ± 0.035	0.22^{aA} ± 0.035
168	1.13^{bC} ± 0.035	0.35^{aB} ± 0.035	0.34^{aB} ± 0.035	0.34^{aB} ± 0.035

^{ab}means in the same row with different superscripts are significantly different ($P < 0.05$); ^{ABC}means in the same column with different superscripts are significantly different ($P < 0.05$)

IV. CONCLUSION

Moringa oleifera leaf powder was found to contain significant levels of antioxidant compounds, making it a potentially suitable plant source of antioxidants for use in food processing. MLP significantly inhibited lipid oxidation in pork droëwors. Since there was no significant difference in the TBARS values between the MLP treatments, it would be advisable to use the lowest levels of MLP (0.5%) in order to reduce cost and minimize potential changes in the product appearance and flavor; which will be assessed in further studies.

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