J. Agric. Res. & Dev. 16(1)68-77. Copy@2017. Faculty of Agriculture, University of Ilorin. Nigeria

INFLUENCE OF POSTMORTEM APPLICATION OF SHADDOCK (*Citrus maxima)* PEEL POWDER ON LIPID OXIDATION OF *SEMIMEMBRANOSUS* MUSCLE IN GOAT.

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https://dx.doi.org/10.4314/jard.v16i1.8

ABSTRACT

The presence of phenolic compounds in Shaddock peel powder (SPP) and its effect on lipid oxidation in cooked and raw semimembranosus (SM) muscle in goats were examined. The SPP was applied to cooked and raw minced SM at the rate of 0, 0.5, 1.0 and 1.5% of the weight of the meat and compared with 0.01% Butylatedhydroxyl anisole (BHA), a synthetic antioxidant. Oxidative stability of the samples were assessed at 0, 3, 6 and 9 days of refrigerated storage (4^{o}C). The BHA samples had lower (P< 0.05) Thiobarbituric acid reactive substance (TBARS) value compared to other treatments. The TBARS value in the 0.5%, 1% and 1.5% SPP did not differ (P> 0.05) but was lower than that of the control samples (0% SPP). Regardless of the additive, the TBARS values in cooked samples were greater (P< 0.05) than in the raw samples. The TBARS value increased (P< 0.05) over refrigerated storage (P< 0.05). There were significant (P< 0.05) interactions between the antioxidant treatments, storage days and state of meat. The current results emphasized that the addition of 1.5% SPP was as potent as BHA in reducing lipid oxidation in raw and cooked chevon for 9 and 6 days respectively.

***Keywords**: Shaddock peel powder, BHA, Lipid Oxidation, Semimembranosus.*

INTRODUCTION

Goat meat is a popular red meat and enjoys great acceptability in many developing countries (Devendra, 2015). Meat can be kept edible for a much longer time though not indefinitely if proper hygiene is observed during production and processing, and if appropriate food safety, food preservation and food storage procedures are applied (Lawrie and Ledward, 2006). Shelf life is the length of time in which the defined quality of food and other perishable products remain acceptable under expected (or specified) conditions of distribution, storage, and display (Gray *et al*., 1996). A significant portion of meat and meat products spoiled every year and this could have a substantial economic and environmental impact (Iulietto *et al*., 2015).One of the major causes of meat spoilage is lipid oxidation, which leads to discoloration, drip loss, off-odor, and off-taste, and may even produce potentially toxic compounds(Gray *et al*., 1996; Adeyemi *et al*., 2016). Synthetic antioxidants are capable of attenuating lipid oxidation in foods (Adeyemi *et al*., 2013). However, the high cost, scarcity and the potential health hazards of synthetic antioxidants have stimulate interest in the use of natural antioxidants in foods (Olorunsanya *et al*., 2010, Adeyemi *et al*., 2013; Nakyinsige *et al*., 2016). The phytochemical compounds in citrus and their antioxidant properties have been documented (Anwar *et al.*, 2008; Devatkal *et al.*, 2010; Adeyemi *et al.*, 2013). Thus, the objective of this study was to determine the phytochemical compounds and the effects of Shaddock peel powder on lipid oxidation in refrigerated, minced, raw and cooked *semimembranosus* muscles in goats.

MATERIALS AND METHODS

The processing of shaddock peel powder and goat meat was carried out at the Agricultural Laboratory, Kwara State University, Malete, while the extraction and phytochemical screening of the SPP and lipid oxidation analysis was carried out at the Laboratories of Animal Production and Chemistry Departments, University of Ilorin, Ilorin, Nigeria. Ripe and fresh shaddock fruit (*Citrus maxima*) was obtained at Flower Garden Area Ilorin, Kwara State. The fruit were peeled and their edible part was separated. The shaddock peel was washed with distilled water and then air dried for seven days. The peel obtained was ground with an electric blender to obtain a fine powder and kept in an air-tight container.

Extraction of shaddock peel using methanol

About 500 ml of methanol was distilled against impurities before it was added to the SPP. The shaddock peel powder sample (101.44g) was put into a 1 liter amber bottle containing 500 ml of distilled methanol, shook vigorously, covered tightly with foil paper, well taped and placed on the shelf for 5 days to get the extract. On the fifth day, a greenish colouration was observed in the sample after which the sample was decanted using funnel and cotton wool into another amber bottle and dried up after 7 days.

Phytochemical screenings of the shaddock peel powder (qualitative analysis) for both methanol and ethyl acetate extract.

Test for Flavonoids

Approximately 0.5g of the extract was heated with 10ml ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of diluted ammonia solution. A light yellow coloration indicates the presence of flavonoids.

Test for Phenolic compounds

About 50g of the extract was dissolved in 5ml of distilled water and few drops of neutral 5% ferric chloride solution were added. A dark green coloration indicates the presence of phenolic compound (Awe and Shodipo, 2001).

Test for Tannins

About 0.5g of the extract was boiled in 10ml of water in a test tube, filtered and 0.1% ferric chloride was added and a brownish green coloration was observed (Awe and Shodipo, 2001).

Test for Terpenoid

About 5ml of the extract was mixed with 2ml of chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration indicates the presence of terpenoids (Prashant *et al.,* 2011).

Meat Preparation

A matured Red Sokoto buck was obtained from Kahm Integrated Farm Oke-Oyi, Ilorin, Nigeria. The goat was slaughtered by cutting through the jugular vein with a sharp knife. The goat was de-skinned and eviscerated. The *semimembranosus* muscle was sampled from the right and left hind limbs, and minced with a food processor (National MK-5080M).

Influence of Postmortem Application of Shaddock on *Semimembranosus* Muscle

Application of Treatments

The minced SM was divided into one hundred and sixty (160) parts of 20g each. Graded levels of 0% (control), 0.5, 1.0 and 1.5% SPP and 0.01% BHA were added to each 20g of meat sample and mixed thoroughly. Eighty (80) parts of the samples were cooked for 15 min in microwave oven while the other 80 parts were left raw. Both cooked and raw meat samples were put in a Petri-dish with labeling corresponding to the applied treatments and stored in a refrigerator (PVDD 240L) for 9 days at a temperature of 4 °C. The lipid oxidation was monitored on 0, 3, 6 and 9 d of refrigerated storage.

Analysis of Lipid Oxidation in Goat Meat

Lipid oxidation was assessed following the method described by Tarladgris *et al.* (1964). Approximately 10 g of the meat sample was homogenized with 47.5 ml of distilled water with 2.5 ml of diluted hydrochloric acid (0.2 M) in a specimen bottle using a glass rod. The homogenized mixture was rinsed with 50 ml of distilled water into a round bottom flask and the mixture was distilled through a distillate apparatus (BUCHI Distillation unit K-350) to collect about 10 ml of the distillate. About 5 ml of the distillate was mixed with 5 ml of Thiobarbituric acid (TBA) (0.02M) and boiled for 35 min in water bath. The mixture was cooled for 10 min under a running tap water for color formation. The duplicate absorbance readings were measured at a wavelength of 538 nm against a blank that contains 5ml of Thiobarbituric acid (TBA) reagent and 5ml of dilute hydrochloric acid solution using a spectrophotometer (Cecill- 2011). The absorbance values were multiplied by 7.8 (Tarladgris *et al*. 1964) to obtain the TBARS values in milligram per malonaladehyde per kilogram of sample (mg/MDA/kg).

Statistical Analysis

The experimental data were analyzed as a 5*2*4 factorial arrangement in a completely randomized design with antioxidant treatments (0, 0.5, 1.0, 1.5% SPP and 0.01% BHA), state of the meat (raw or cooked) and storage days (0, 3, 6, 9).The data obtained were analyzed using the MIXED procedure of (SAS, 2003). Means were separated with Tukey HSD test at $P < 0.05$ level of significance.

RESULTS

The phytochemical profile of shaddock peel powder is shown in Table 1. Shaddock peel powder contained flavonoids, phenols, tannins and terpenoids.

Table 1: Phytochemical Analysis of shaddock Peel Powder.

Phytochemical compounds	
Flavonoids	+
Phenols	+
Tannins	+
Terpenoids	+

+ indicates presence

The main effect of antioxidant treatments, state of meat, and storage days on the oxidative stability of chevon is shown in Table 2. The TBARS value in the control meat was greater ($P< 0.05$) than that of chevon preserved with SPP and BHA. The TBARS value in the chevon treated with 0.5%, 1% and 1.5% SPP did not differ ($P> 0.05$). The BHA treated samples had lower ($P<$

0.05) TBARS values compared with other treatments. Regardless of antioxidant treatment, cooked chevon samples had greater (P< 0.05) TBARS values compared with raw meat samples. The TBARS value in chevon samples on 0 and 3 day of refrigerated storage did not differ (P> 0.05) but was lower (P< 0.05) than that observed on day 6 and 9 of chill storage. The TBARS value observed on day 6 was lower than that observed on day 9.

Table 2: Effects of Antioxidant treatments, State of Meat and Storage Days on Lipid Oxidation of Goat Meat

	TBARS (mg MDA/kg)					
Factor						
Antioxidant treatments	0%	0.5% SPP	1.0% SPP	1.5% SPP	0.01% BHA	SEM
	0.85^{a}	0.49^{b}	0.44^{b}	0.39^{b}	0.13^{c}	0.01
State of meat	Cooked	Raw				
	0.71^{a}	0.21^{b}				0.02
Storage days	0	3	6	9		
	0.03^{c}	0.04^{c}	0.72^{b}	1.05^{a}		0.03

^{a,b,c} means having different superscript along the same row are significantly different (P< 0.05). SEM=standard error of means

There was a significant interaction (P< 0.05) between antioxidant treatments and state of meat on the oxidative stability of chevon. The TBARS value of cooked chevon samples treated with 0, 0.5, 1 and 1.5% SPP was greater (P< 0.05) than the raw chevon samples. However, the TBARS value of the raw BHA chevon was similar to that of the cooked BHA chevon.

Table 3: Interaction Effect of Antioxidant treatments and State of Meat on Lipid Oxidation of *Semimembranosus* Muscle of Goats.

State of meat	**Antioxidant treatments**	**TBARS (mg MDA/kg)**
Cooked	0%	1.39^{a}
	0.5% SPP	0.76^{b}
	1.0% SPP	0.73^{b}
	1.5% SPP	0.54^{cb}
	0.01% BHA	0.14^{e}
Raw	0%	0.39^{cd}
	0.5% SPP	0.22^{c}
	1.0% SPP	0.15^{e}
	1.5% SPP	0.23^{c}
	0.01% BHA	0.14^{e}
	SEM	0.05

$^{a, b, c}$ means having different superscripts along the same column are significantly different (P< 0.05)

The interaction effect of antioxidant treatments and storage days on the TBARS value in chevon is shown in Table 4. The TBARS value observed on d 0 did not differ from those observed on d

3 in all treatments. On day 6, the TBARS value in the 0% and 1.5% SPP was significantly lower than that observed on day 9. Contrarily, the TBARS value of the 0.5, 1 and 1.5% SPP and 0.01% BHA did not differ (P< 0.05) from that obtained on day 9.

Table 4: Interaction Effect of Antioxidant and Storage Days on Lipid Oxidation in *Semimembranosus* Muscle in Goats

Antioxidant treatment	Storage days	TBARS(mg MDA/kg
0%	0	0.03^{d}
0.5% SPP		0.03^{d}
1.0% SPP		0.03^{d}
1.5% SPP		0.03^{d}
0.01% BHA		0.03^{d}
0%	3	0.08^{d}
0.5% SPP		0.04^{d}
1.0% SPP		0.02^{d}
1.5% SPP		0.02^{d}
0.01% BHA		0.02^{d}
0%	6	1.31^{b}
0.5% SPP		0.82^{c}
1.0% SPP		0.95^{cb}
1.5% SPP		0.37^{d}
0.01% BHA		0.15^{d}
0%	9	1.98^{a}
0.5% SPP		1.07^{cb}
1.0% SPP		0.77^{c}
1.5% SPP		1.12^{cb}
0.01% BHA		0.32^{d}
SEM		0.08

$^{a, b, c}$ means having different superscript along the same column are significantly different (P< 0.05). SEM=standard error of means

The interaction effect of storage days and state of meat on the oxidative stability of *semimembranosus* muscle in goats is shown in Table 5. The TBARS value observed on d 0 and 3 in the cooked and raw meat samples did not differ. On day 6 and 9, the cooked samples had greater (P< 0.05) TBARS value than the raw samples.

The interaction of antioxidant treatment, storage days and state of meat on the TBARS value of *semimembranosus* muscle in goats was significant. On days 0 and 3, the TBARS value of the raw and cooked samples for all the antioxidant treatments did not differ (P> 0.05). On day 6 and 9, the raw and cooked control samples had significantly greater (P< 0.05) TBARS value compared with other antioxidant treatments. On days 6 and 9, the raw and cooked BHA samples had lower (P< 0.05) TBARS value compared with other antioxidant treatments.

Table 5: Interaction Effect of State of Meat and Storage Days on Lipid Oxidation of *semimembranosus* Muscle of Goats

Storage days	State of meat	TBARS
0	Cooked	0.03^{d}
3		0.05^{d}
6		1.13^{b}
9		1.63^{a}
0	Raw	0.03^{d}
3		0.03^{d}
6		0.31^{c}
9		0.48^{c}
SEM		0.05

$^{a, b, c}$ means having different superscript along the same column are significantly different ($P< 0.05$). SEM=standard error of means

DISCUSSION

Shaddock peel powder contained flavonoids, phenols, tannins and terpenoids. Phenolic compounds in citrus are known to be concentrated in the peels where they play vital roles in the chemical and physical defense systems of seeds and fruits during oxidative damage and microbial infections (Rafiq *et al*., 2016). The current finding is in tandem with those of Anwar *et al*. (2008) and Devatkal *et al*. (2010) who observed that tangerine peels contains myriad polyphenolic compounds that exhibited antioxidant properties. The application of SPP reduced lipid oxidation in *semimembranosus* muscle in goats. This observation could be attributed to the antioxidant properties of phenolic compounds in the SPP. This finding is consistent with that of Devatcal *et al*. (2010) who observed that tangerine peels reduced lipid oxidation in goat meat patties. In addition, lemon, grape and orange seed extracts reduced lipid oxidation in minced broiler meat (Adeyemi *et al*., 2013). However, the antioxidant potency of 0.5, 1.0 and 1.5% SPP did not differ. The BHA exhibited greater antioxidant capacity than the SPP. This observation concurs with that of Adeyemi and Olorunsanya (2012) who observed that BHA exhibited higher antioxidant potency than cowpea seed coat extracts. However, the current observation is contrary to that of Devatcal *et al*. (2010) who observed that the antioxidant potency of tangerine peel extract was similar to that of synthetic antioxidant. In addition, the antioxidant potency of grape, orange and lemon seed extracts was similar to that BHA in broiler meat samples (Adeyemi *et al*., 2013).

The cooked chevon exhibited a greater oxidative deterioration than the raw chevon. This observation could be attributed to the fact that the increase in temperature, associated with cooking could activate some lypolytic enzymes such as phospholipase and lipase that are capable of enhancing lipid oxidation (Krinsky, 1994). In addition, cooking disrupts lipid membrane systems, which causes loss of structural integrity and facilitates the reaction of oxygen and molecular weight metal with unsaturated fatty acids resulting in the generation of oxidative reactions (Asghar *et al.*,1988). The current finding is consistent with those of Adeyemi *et al*. (2013), who observed an increase in TBARS value during the refrigerated storage of cooked and raw broiler meat. Contrarily, cooked pork patties had lower TBARS value than the raw pork patties (Olorunsanya *et al*., 2010).

Influence of Postmortem Application of Shaddock on *Semimembranosus* Muscle

Table 6: Interaction Effect of Antioxidant, Storage Days and State of Meat on Lipid Oxidation in *Semimembranosus* Muscle of Goats

State of meat	Antioxidants	Storage days	TBARS (mg MDA/kg)
Cooked	Control		0.04^{f}
	0.5%		0.03^{f}
	1.0%		0.02^{f}
	1.5%		0.04^{f}
	BHA	0	0.03^{f}
Raw	Control		0.03^{f}
	0.5%		0.03^{f}
	1.0%		0.03^{f}
	1.5%		0.02^{f}
	BHA		0.03^{f}
Cooked	Control		0.10^{ef}
	0.5%		0.05^{f}
	1.0%		0.04^{f}
	1.5%		0.04^{f}
	BHA	3	0.03^{f}
Raw	Control		0.05^{f}
	0.5%		0.03^{f}
	1.0%		0.01^{f}
	1.5%		0.01^{f}
	BHA		0.02^{f}
Cooked	Control		2.17^{b}
	0.5%		1.34^{c}
	1.0%		1.48^{c}
	1.5%		0.50^{edf}
	BHA		0.17^{edf}
Raw	Control	6	0.46^{edf}
	0.5%		0.30^{edf}
	1.0%		0.43^{edf}
	1.5%		0.24^{edf}
	BHA		0.12^{edf}
Cooked	Control		3.25^{a}
	0.5%		1.62^{cb}
	1.0%		1.37^{c}
	1.5%		1.59^{edf}
	BHA		0.31^{d}
Raw	Control		0.70^{edf}
	0.5%	9	0.53^{edf}
	1.0%		0.18^{edf}
	1.5%		0.56^{ed}
	BHA		0.34^{edf}
	SEM		0.10

$^{a, b, c, d, e, f}$ means having different superscript along the same column are significantly different ($P< 0.05$). SEM=standard error of means

The TBARS value of goat meat increased as refrigerated storage progressed. This suggests a loss in the structural integrity of muscle cells and the formation of pro-oxidants like peroxides. Similar findings were reported during refrigerated storage of cooked and raw broiler meat (Adeyemi *et al*., 2013) and pork patties (Olorunsanya *et al*., 2010).The presence of the second and third order interactions revealed the interrelationships among antioxidant treatments, state of meat and storage days. Regardless of antioxidant treatments, the TBARS value observed on days 0 and 3 in the current study was less than 0.6 mg MDA/kg needed to bring about an objectionable flavour in meat associated with lipid oxidation (Greene and Cumuze, 1982). Regardless of antioxidant treatment, the TBARS value of raw meat samples did not exceed 0.6 mg MDA/kg. However, on day 6, the TBARS value of cooked chevon samples for the control, 0.5% and 1% SPP exceeded 0.6 mg MDA/kg sample. On day 9, only the cooked BHA treated chevon had a TBARS value lower than 0.6 mg MDA/kg. Contrarily, except the control, the TBARS value of other antioxidant treatments was lower than the 0.6 mg MDA/kg benchmark. The implication of this finding is that raw chevon samples treated with 0.5, 1 and 1.5% SPP could be stored for 9 days in the refrigerator while cooked chevon samples treated with the 0.5 and 1% SPP should not be stored beyond 3 days in the refrigerator. The cooked chevon treated with 1.5% SPP could be stored up to 6 days.

CONCLUSION

The addition of 0.5, 1.0 and 1.5% of SPP reduced lipid oxidation in both cooked and raw goat meat, though their antioxidant effect was more pronounced in raw meat samples. The BHA exhibited the greatest antioxidant potency among the antioxidant treatments. Irrespective of the additive, the TBARS values in cooked samples were greater than in the raw samples. The TBARS value increased over refrigerated storage. The addition of 1.5% SPP was as potent as BHA in reducing lipid oxidation in raw and cooked chevon for 9 and 6 days respectively. The addition of 1.5% SPP could be used as an alternative to BHA in attenuating lipid oxidation in *semimembranosus* muscle in goats.

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