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ORIGINAL RESEARCH ARTICLE

Effects of *Cymbopogon citratus* extract on quality of chevon patties during storage

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ABSTRACT
Lipid oxidation and growth of undesirable microorganisms in meat and meat products render them unacceptable for human consumption. A need therefore arises to explore the use of natural antioxidants and antimicrobials to prevent deterioration as synthetic ones have been found to pose high health risks to consumers. The effect of Lemon grass (Cymbopogon citratus) extract (LGE) on shelf stability of cooked Chevon patties during refrigerated storage was investigated. The experiment was a factorial experiment in a completely randomized design with five antioxidant treatments (Treatment A (0.2% Vit E), B (control), C (0.1% LGE), D (0.2% LGE), E (0.3% LGE) and four storage days (0, 3, 6, 9). Proximate composition of patties were determined using standard method, sensory qualities were evaluated using a 9-point hedonic scale, lipid oxidation was monitored by malondialdehyde formation with 2-thiobarbituric acid (TBARS) assay. Nutrient composition of patties at all treatment levels, except ash were affected ($p<0.05$) by the antioxidant treatments. Lipid oxidation rates of patties and pH were reduced ($p<0.05$) by antioxidant treatments during storage. Total Plate Count (TPC) was also ($p<0.05$) reduced and all counts were below 7logCFU/g, the Maximum Permissible Limit (MPL) for TPC. Sensory qualities were not ($p<0.05$) influenced by the antioxidant treatments however, a high Overall Acceptability score (6.70±1.16) was observed for treatment D, closely followed by treatment A (6.60±0.97).
Lemon grass extract at 0.2% level of inclusion had comparable effects with alpha-tocopherol in most parameters measured and can successfully replace its use at this level to improve the shelf-life of Chevon patties and also provide a meat product with natural additives.

Keywords: Lemongrass extract; alpha-tocopherol; antioxidant; antimicrobial; chevon patties

INTRODUCTION
Processed meat products suffer chemical spoilage during their preparation, storage, and distribution. Meat and meat products are prone to spoilage even when stored under refrigerated conditions. This therefore makes their shelf-life highly dependent on the microbiological status and conditions of storage of the meat and meat product (Hayam *et al.*, 2013). Lipids which play important role in technological, nutritional and sensory function of food are liable to undergo oxidation that leads to the formation of a number of undesirable compounds. Numerous factors affect lipid oxidation such as temperature, light, concentration of oxygen in the surrounding atmosphere, amount and composition of phospholipids, presence of anti-oxidants, pro-oxidants, metal ions, haem pigments, enzymes, mechanical processes etc (Biswas *et al.*, 2012). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have gained vast use in the meat processing industry. However, their application has recently been restricted because of their carcinogenic properties (Naveena *et al.*, 2008). As a result of this, there is an increasing interest in researches for production and isolation of biologically active compounds from natural sources. The use of natural antioxidant as well as antibacterial compounds, such as extracts of spices and herbs, essential oils, organic acids, salts, and bacteriocins have been reported to improve the shelf life of meat (Jamilah *et al.*, 2008; Jałosńska and Wilczak, 2009) and to prevent bacterial and fungal growth. These protective abilities of bioactive materials are mostly attributed to plant polyphenols and their antioxidant, antimicrobial, antiviral or anticarcinogenic effects (Shadab *et al.*, 2001). There is however a challenge of quantifying the amount of plant extracts to be included in meat products that will perform the same role as that of the synthetic ones.

Lemon grass (*Cymbopogon citratus)* is a perennial, aromatic tall tropical grass that is commonly used as herbs for flu, headache, malaria, coughs elephantiasis, pneumonia digestive problems, diarrhea, stomach upsets and vascular disorders (Ozer *et al.*, 1995). It is also used as a stimulant, diuretic, antispasmodic and a mild irritant (Simon *et al.*, 1984). The fresh stalks and leaves have a clean lemon-like odour. The oil of Lemon grass is sherry coloured with pungent taste, it has citral as the principal constituent and it is used in perfumery, pharmaceutical industries, and as a preservative (Shadab *et al.,* 1992). Other constituents of the oil are terpineol, S-myrcene, citronellol, limonene, geranoil, dipentene, methyl heptenon and nerol (Simon *et al.*, 1984). Hayam *et al.* (2013) reported that lemon grass and lime peel extracts can play important role as antioxidant and antibacterial agents in refrigerated chicken patties. Paracetamol-induced lipids peroxidation was also inhibited in rats by lemon grass and green tea (Ojo *et al.*, 2006). This study was therefore carried out to determine the antioxidant and antimicrobial capabilities of LGE in comparison with alpha-tocopherol on the quality of cooked Chevon patties during storage.

MATERIALS AND METHODS

Collection and extraction of Lemon grass

Matured Lemon grass leaves were harvested during the early rainy season and identified at the Department of Crop Protection and Environmental Biology of the University of Ibadan. The leaves were removed from stalk and thoroughly washed with distilled water and air-dried until crispy (for eight days). They were thereafter milled into fine powder using dry blender (VTCL Grinder, Model: Smart Leaf, India). Extraction was carried out using conventional Soxhlet extraction method (hot continuous extraction) for 6 hours using 98% methanol as the solvent medium at ratio 1:50 (Mandana *et al.,* 2011). Water-bath, set at the boiling point temperature for the solvent was thereafter used for concentration of the extract and evaporation of solvent residue. Extract was stored until further use.

Preparation of Chevon Patties

Chevon was obtained from a reputable meat market within Ibadan metropolis. The skin, bones and fat trimmings were manually removed while the meat was cut into small pieces and minced using a meat grinder (Breville grinder, model: VTP141, UK) through a 5mm sieve plate. Ten (10) kg minced meat was divided equally into five (5) batches to make up the treatments. Treatment A was formulated with 0.2% alpha-tocopherol inclusion (positive control), treatment B (no antioxidant inclusion-negative control), Treatments C, D and E were prepared by adding LGE at the rates of 0.1%, 0.2% and 0.3% respectively. The extracts and alpha-tocopherol were thoroughly mixed into the minced meat together with other ingredients and thereafter formed into patties (100g each) using a meat former. Patties were cooked using an electric oven set at 125°C for 30min to an internal temperature of 72°C. They were placed on plastic meat trays to cool, wrapped with foil paper and kept in a refrigerator at 4°C for 9 days. Patties samples were evaluated in triplicates at 3 days interval during the storage period.

Analysis of Chevon patties

Physico-chemical properties of Chevon patties

Proximate analysis was carried out using procedures described by AOAC (1990) for moisture, protein, fat and ash determinations. 1g patty samples were weighed and thoroughly homogenised with distilled water (1:10 w/v) and pH was measured using a pH meter (PHS-3C, Philips) fitted with glass electrode. Water Holding Capacity (WHC) was determined as described by Suzuki *et al.* (1991). 1g patty samples from each treatment were weighed individually onto two filter papers and pressed between two plexi glasses for a minute using a vice. The samples were then oven dried at 65°C for 48 h to determine the moisture content. The amount of water released from the samples was measured indirectly by measuring the area of the filter paper wetted relative to the area of pressed sample. WHC was calculated as follows:

Equation:

$$WHC = \frac{100 - [(Ar - Am) \times 9.47)]}{Wm \times Mo} \times 100$$

Where, Ar = Area of water released form meat (cm^2); Am = Area of meat sample (cm^2); Wm = Weight of meat in mg; Mo = Moisture content of meat (%); 9.47 is a constant factor.

Product yield was calculated using the following formula:

$$\text{Yield} = \frac{\text{Weight of product}}{\text{Initial weight of sample}} \times 100$$

Lipid Oxidation Analysis

Thiobarbituric Acid-Reactive Substances (TBARS) assay was performed to determine rate of lipid oxidation according to the method described by Witte *et al.* (1970). 10g of sample was thoroughly ground using a mortar and pestle and thereafter 25ml 20% Trichloroacetic acid (TCA) and 20 ml distilled water were added. The mixture was thoroughly homogenized for 2minutes and then filtered using Whatman filter paper (No 1). The filtrate was mixed with an equal volume of 0.02M Thiobarturitic acid (TBA) and inoculated at 100°C for 35 minutes. It was then cooled in tap water for 10 minutes. Absorbance of the solution was measured at 532nm using a UV-VIS Spectrophotometer (Jenway 6305 Spectrophotometer, UK). TBA values were expressed as mg malonaldehyde per kg of patty.

Microbial Analysis of Chevon patties

Total plate count, Mould and yeasts counts and Coliform bacteria counts were determined on nutrient agar, potato dextrose agar and MacKonky agar media as recommended by the American Public Health Associations for Foodstuff Examination (APHA, 1992). Briefly, 28g, 39.9g and 50g of nutrient agar, potato dextrose agar and MacKonky agar respectively were weighed into 1000ml conical flasks and made up by addition of distilled water to dissolve the agar and the mixture was placed in a water bath and stirred continuously until a homogenized solution was obtained. It was thereafter tightly corked and autoclaved at 121°C for 15 minutes. It was allowed to cool at room temperature. The medium was then poured and swirled gently into the Petri dishes containing drops of samples obtained via serial dilution (10g of patty samples homogenized with 90ml sterile peptone water). Medium was allowed to gel. Incubation was at 37°C, for 24-72h depending on the agar. After incubation, colonies were counted and results were expressed as $\log_{10}$ CFU/g of patty sample.

Table 1: Proximate composition (%) of chevon patties with varied levels of antioxidants

Parameters	Treatments				
	A	B	C	D	E
Moisture	57.60±0.07 ^{d}	59.10±0.04 ^{b}	57.95±0.02 ^{c}	57.35±0.04 ^{e}	59.28±0.02 ^{a}
Protein	17.31±0.06 ^{b}	16.89±0.12 ^{d}	17.06±0.02 ^{c}	17.54±0.03 ^{a}	17.45±0.03 ^{a}
Fat	9.92±0.00 ^{d}	9.97±0.00 ^{c}	9.99±0.00 ^{a}	9.98±0.00 ^{b}	9.91±0.00 ^{e}
Ash	7.33±0.58	7.00±0.00	7.67±0.58	7.67±0.58	7.33±0.58

A: alpha-tocopherol,; B: control; C: 0.1% lemon grass extract; D: 0.2% lemon grass extract; E: 0.3% lemon grass extract

a,b,c,d,e - means along the same row with different superscripts are significantly ($p<0.05$) different.

Table 2: Physical characteristics of chevon patties with varied levels of antioxidants (%)

Parameters	Treatments				
	A	B	C	D	E
WHC	55.35±3.38	48.61±2.84	68.86±5.94 ^{a}	57.14±9.55	56.58±3.00
Product yield	74.50±0.91 ^{a}	66.02±0.34 ^{d}	73.10±0.18 ^{b}	75.50±0.79 ^{a}	68.66±0.44 ^{c}

A: alpha-tocopherol,; B: control; C: 0.1% lemon grass extract; D: 0.2% lemon grass extract; E: 0.3% lemon grass extract;

WHC: water holding capacity

a,b,c,d - means along the same row with different superscripts are significantly ($p<0.05$) different.

Sensory Evaluation

A total of ten (10) trained individuals aged between 20 and 40 years were used to assess three replicate samples of each treatment of the

cooked Chevon patty. Samples were evaluated for aroma, flavour, colour, juiciness, tenderness, and overall acceptability according to Cross *et al.* (1978). The samples were rated on a nine point hedonic scale with maximum score of 9 (nine) for extremely high condition while the lowest score of 1 (one) was assigned to the poorest condition (Mahendraker *et al.*, 1988). Equal bite size from the five treatments was coded and served to each panelist. Each sample was evaluated independently of the other. Water was offered to rinse the mouth in-between tasting to reduce the carry-over effect of one sample to another, the room was well illuminated and all distractions were completely abated.

RESULTS AND DISCUSSION

The nutritional composition of Chevon patties with varied antioxidant levels (table 1) showed that inclusion of antioxidants had significant ($p<0.05$) effects on the moisture, protein and fat contents of the cooked Chevon samples. The ash content was however not significantly ($p>0.05$) affected. Significant ($p<0.05$) differences were observed across treatments for moisture with treatment E (59.28±0.02) being significantly higher than others. Proximate values for fat ranged significantly between 9.91±0.00 in treatment E and 9.99±0.00 in treatment C. Protein contents of treatments A (17.31±0.06), B (16.89±0.12), and C (17.06±0.02), also varied significantly ($p<0.05$). The significant changes caused by the addition of the extract on the nutritional composition of the patties could be as a result

Table 3: pH values of Chevon patties with varied antioxidant levels

Storage days	Treatments				
	A	B	C	D	E
0	6.09 ± 0.01^{ef}	6.07 ± 0.02^{b}	6.11 ± 0.03^{a}	6.11 ± 0.01^{c}	6.19 ± 0.02^{a}
3	6.11 ± 0.03^{fg}	6.06 ± 0.04^{c}	6.19 ± 0.08^{a}	6.11 ± 0.03^{i}	6.19 ± 0.04^{d}
6	6.52 ± 0.07^{e}	6.20 ± 0.03^{i}	6.25 ± 0.01^{h}	6.17 ± 0.06^{g}	6.20 ± 0.05^{e}
9	6.35 ± 0.07^{j}	6.29 ± 0.04^{k}	6.36 ± 0.09^{k}	6.45 ± 0.02^{ij}	6.35 ± 0.05^{h}

A: alpha-tocopherol,; B: control; C: 0.1% lemon grass extract; D: 0.2% lemon grass extract; E: 0.3% lemon grass extract;
a,b,c,d,e - means along the same row with different superscripts are significantly ($p<0.05$) different.
f,g,h,i,j,k: means along the same column with different superscripts are significantly ($p<0.05$) different.

Table 4: Lipid oxidation of Chevon patties with varied antioxidant levels

Storage days	Treatments				
	A	B	C	D	E
0	1.14 ± 0.01^{b}	0.65 ± 0.02^{bc}	0.87 ± 0.01^{b}	1.32 ± 0.01^{b}	2.10 ± 0.01^{a}
3	3.12 ± 0.00^{a}	1.45 ± 0.01^{de}	2.31 ± 0.58^{cd}	2.64 ± 0.41^{efg}	3.01 ± 0.01^{de}
6	2.72 ± 0.04^{fgh}	3.77 ± 0.01^{h}	6.38 ± 0.04^{de}	1.45 ± 0.00^{gh}	3.41 ± 0.02^{def}
9	2.94 ± 0.03^{gh}	4.18 ± 0.00^{h}	6.42 ± 0.02^{fgh}	3.84 ± 0.02^{fgh}	6.59 ± 0.01^{de}

A: alpha-tocopherol,; B: control; C: 0.1% lemon grass extract; D: 0.2% lemon grass extract; E: 0.3% lemon grass extract;
a,b,c,d - means along the same row with different superscripts are significantly ($p<0.05$) different.
e,f,g,h - means along the same column with different superscripts are significantly ($p<0.05$) different.

of the differences in inclusion rates of the antioxidants in the patties. This report contradicts the findings of Wills *et al.* (2006) who reported that, addition of natural antioxidants had no effect on proximate composition of beef patties. It is also not in agreement with the findings of Sallam *et al.* (2004) who recorded that the addition of different forms of garlic had no significant effect on the protein, ash and fat contents of chicken sausage.

Water holding capacity of a product is its ability to retain water after the application of external force and this has direct effect on the consequent yield of the product. The positive correlation of water holding capacity and

product yield was observed in this study (Table 2). The product yield however varied significantly across treatments with treatment D having highest value (75.50±0.79). This however did not agree with the findings of Omojola and Adediran, (2015) who observed that product yield of Chicken patties did not increase with higher water holding capacity when ginger, garlic and Roselle were used as antioxidant treatments in the product.

Gradual significant ($p<0.05$) increase in pH values (Table 3) for all the treatments was observed over the storage days. Same trend of increase occurred in all the treatment samples except in treatments A and B. In all, it was observed that pH values for treatment B (control) were slightly lower than those of the other treatments. The increase observed is in agreement with other studies carried out on chicken sausage (Sallam *et al.,* 2004), lamb patties (Hayam *et al.,* 2013), and frozen pork patties (Sasse *et al.,* 2009). Hayam *et al.* (2013) also reported increase in pH of Chicken patties treated with lemongrass and lime peel extracts over the storage days. This increase in pH observed in all the treatments could be as a result of tissue protein breakdown of the meat in the Chevon patties over the storage days (Gill, 1983) and the storage of meat at refrigeration temperature (4°C) causes a number of chemical reactions including enzymatic reactions (Hayam *et al.,* 2013) resulting in increased pH of meat.

Table 5: Microbial load of Chevon patties treated with varied levels of antioxidants (cfu/g)

Storage days	Treatments				
	A	B	C	D	E
0	$1.02\pm0.00^{k\ l}$	1.56 ± 0.00^{h}	1.04 ± 0.07^{k}	1.18 ± 0.00^{j}	1.80 ± 0.00^{ef}
3	1.77 ± 0.01^{f}	1.72 ± 0.01^{g}	1.76 ± 0.01^{fg}	1.41 ± 0.00^{i}	1.52 ± 0.00^{h}
6	2.13 ± 0.00^{b}	2.00 ± 0.00^{d}	2.06 ± 0.00^{c}	1.85 ± 0.00^{e}	2.16 ± 0.00^{b}
9	2.05 ± 0.00^{c}	2.27 ± 0.00^{a}	2.28 ± 0.00^{a}	2.17 ± 0.00^{b}	2.12 ± 0.00^{b}

A: alpha-tocopherol,; B: control; C: 0.1% lemon grass extract; D: 0.2% lemon grass extract; E: 0.3% lemon grass extract; abcde - means along the same row with different superscripts are significantly ($p<0.05$) different. fghijk: means along the same column with different superscripts are significantly ($p<0.05$) different.

Table 6: Sensory characteristics of Chevon patties with varied levels of antioxidants

Parameters	Treatments				
	A	B	C	D	E
Aroma	6.50±2.12	5.30±2.21	5.20±2.25	5.70±1.64	4.80±2.10
Colour	6.30±0.82	6.20±1.87	5.60±1.43	5.60±1.58	5.90±0.88
Flavour	5.90±2.33	4.80±2.10	4.90±2.02	5.70±2.00	6.00±1.83
Tenderness	6.40±1.51	5.80±1.62	5.00±1.49	5.30±1.89	6.30±1.42
Juiciness	5.30±1.42	5.80±0.79	5.30±1.42	5.70±1.34	5.50±1.96
Overall acceptability	6.60±0.97	6.30±1.16	6.30±0.82	6.70±1.16	6.00±0.67

A: alpha-tocopherol,; B: control; C: 0.1% lemon grass extract; D: 0.2% lemon grass extract; E: 0.3% lemon grass extract;

The rate of lipid oxidation given by the TBARS (thiobarbituric acid reactive substance) values of patties in all treatments increased gradually during the storage days with the lowest and highest values observed on day 0 and 9 respectively. The antioxidant inclusion was observed to have significant effect on the oxidative stability of cooked Chevon patties during the storage period at 4^{0}C (Table 4).The comparable ($p<0.05$) values observed between treatment A (alpha-tocopherol) and treatment D (0.2% Lemon grass extract) does not agree with the reports by Higgins *et al.* (1998) that direct addition of alpha-Tocopherol did not

significantly ($p>0.05$) affect lipid oxidation in cooked turkey meat, as exogenous α-Tocopherol added postmortem can only be incorporated into the neutral fraction thus having a low inhibition on oxidation. The higher TBARS values of the patties compared to that of the control could be as a result of cooking which exposed the membrane lipids to lipid oxidant catalysts, thus disrupting the muscle membrane systems leading to loss of structural integrity. It could also be as a result of increase in ionic iron concentration from heat-induced release of protein-bound iron after cooking, inactivation of antioxidant enzymes in meat or the formation of the hypervalent ferrylmyoglobin (or activated metmyoglobin) (Harel and Kanner, 1985; Asghar *et al.*, 1988; Rhee, 1988). TBARS values for treatment A were lower than that of the treatment B (control) and this aligned with the findings of Olorunsanya *et al.* (2010) who reported that addition of exogenous α-Tocopherol significantly ($p<0.05$) reduced lipid oxidation in cooked pork patties than in raw. This is also related to the findings of Nunez de Gonzalez *et al.* (2008) who reported an increase in TBARS of precooked sausage pork patties treated with dried plum refrigerated or frozen over the raw. Ahn *et al.* (2002) reported that rosemary and α-Tocopherol retarded oxidation during and immediately after cooking, but lemongrass did not show the same effect in the cooked chevon patties.

The microbial analysis of Chevon patties treated with varied levels of antioxidants stored at 4°C for 9 days presented in table 5 showed that total plate count (TPC) increased significantly ($p<0.05$) over the storage period. Both the addition of the antioxidants and the storage time had significant ($p<0.05$) effects on the Chevon patties. In general, Chevon patties in treatment B was observed to have significantly ($p<0.05$) higher values when compared with the others. All counts in the treatment were below 7 log10 CFU/g which is the MPL (Maximal Permissible Limit) for TPC recommended by ICMSF (1986). This trend was also observed by Biswas *et al.* (2012) in precooked chicken patties supplemented with curry leave powder. In studies carried out by Farice *et al.* (2015), it was also observed that the bacteria load in beef patties was significantly reduced at the incorporation of lemon grass extract. Fungi and Coliform bacteria growth was inhibited by the addition of the extract during the storage period. The result of this study supported the indications of the potential of lemon grass extract as an antimicrobial in meat and meat product.

Since the use of naturally occurring preservatives can alter the taste of food due to the associated flavours, the sensory characteristics of the Chevon patties treated with antioxidants were analysed (Table 6). The non-significant effects observed among the treatments showed that inclusion of lemon grass extract did not affect the sensory characteristics of the treated Chevon patties when compared with the control (treatment B). This therefore indicated that consumers accepted patties formulated with the Lemon grass extract although Chevon patties with 0.2% LGE was the most accepted by the panelists. The result of this study did not agree with the findings of Sayed *et al.* (2014) who observed that inclusion of rosemary extract into beef patties significantly affected its aroma, colour, flavour and overall acceptability.

Conclusion

The findings of this study concluded that Lemongrass extract is a promising source of bioactive compound which can be used as a natural meat preservative. Lemon grass extract provided greater benefit in controlling microbial growth and spoilage in Chevon patties during cold storage but was observed to have little effect on the lipid oxidation profile of the cooked Chevon patties as high values were observed during the study. The sensory profile of the cooked Chevon patties was satisfactorily high as the inclusion rates of the extract did not affect the overall acceptability of the patties. It is therefore suggested that lemon grass extract at 0.2% level of inclusion which was observed to have comparable effects with alpha-tocopherol on the parameters measured, can be used to improve the shelf-life of Chevon patties and also provide a meat product with natural additives.

Conflict of interests

The authors have declared that no conflict of interests exists.

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