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COMPARATIVE ASSESSMENT OF BEEF, CHEVON AND MUTTON BILTONG CURED WITH *OCIMUM GRATISSIMUM* PASTE

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ABSTRACT

Meat not processed after slaughter deteriorates, hence processing is needed to hinder microbial growth for longer shelf life in biltong. The study was carried out to comparatively assess responses of biltong from different meat types (beef, chevon and mutton) to *Ocimum gratissimum* paste (Ogp) as curing agent. The paste was obtained by blending the leaves of basil into paste (1.360 kg). Meat chunk of beef, chevon and mutton were stripped into approximately 8 - 10 x 2 x 1.5 cm along the grains. There were six treatments and each replicated twenty times. In a Completely Randomized Design using factorial arrangement treatments were tagged. T^1, T^3, T^5 for controls (no Ogp) while T^2, T^4, T^6 were treatments of beef, chevon and mutton respectively cured in (Ogp) paste for a period of 10 hours and mildly seasoned. After curing, meat strips were dipped into a mixture of hot water (100°C) and vinegar for two hours to prevent mould growth. The strips were then sundried (from 7a.m-6p.m) under insect proven condition, they were thereafter left to dry at room temperature for two weeks (at 27°C and 75% relative humidity). The proximate, minerals, organoleptic properties, microbial counts and characterisation of biltong were investigated. It was observed that the highest preference was given to beef biltong (T^2) and chevon biltong (T^2) in terms of overall acceptability while mutton biltong was least preferred. The aerobic bacteria, coliform and lactic acid counts were more in uncured biltong meat types while the cured mutton biltong (T^6) had the least bacterial and lactic acid counts however, the highest counts was recorded in beef biltong (T^2).

Keywords: Meat types, Biltong, *Ocimum gratissimum* paste, minerals, microbes

INTRODUCTION

The value of meat and its products to the purchaser are mostly dependent on eating quality, keeping quality and nutritional values (Warner *et al.*, 2010), however, meat has a short shelf life at ambient temperature of 15-30°C and a few days at refrigerated temperature of 0–100°C. Hence, spoilage of meat occurs if untreated resulting in the meat becoming unappetizing, poisonous or infectious by bacteria and fungi which are borne by the animal itself, by the people handling the meat and by their utensils (Martinez, 2005). The demand for meat products has increased in recent years due to new recommendation in reducing saturated fat intake and consumers' desire to lose weight (Archer *et al.*, 2004, and Akesowan, 2008). Biltong is ready-to-eat dried meat products originating from beef or game meat. They are regarded in South Africa as a delicacy and are gaining international popularity (Attwell, 2003). Nearly all muscles in the carcass may be used for biltong but the largest hindquarter muscles are most suitable. *Ocimum gratissimum* commonly called African Basil, is found throughout the tropics and sub-tropics (Abdurahman *et al.*, 2012). It is a leafy vegetable and good source of dietary fibre, carotenoids, vitamin C, foliate, phytochemicals and certain minerals but has low concentration of proteins, digestible carbohydrates and lipids (Wills *et al*., 1998). It was therefore used in this study to cure different meat types in the production of biltong and the proximate composition, minerals, microbiological, organoleptic characteristics and acceptability of beef, chevon and mutton biltong cured with *Ocimum gratissimum* paste were comparatively assessed.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Department of Animal Health and Production Technology laboratory, Oyo State College of Agriculture and Technology, Igboora, the experimental area lies in savannah forest zone on latitude 7^1 43^o N and longitude 3^1 28^o E with an elevation of 140m above sea level. The average minimum temperature is about 32.5^O C. The average humidity in the study area is 58%.

Experimental Design

The treatments were allocated in a Completely randomised design (CRD) using factorial arrangement, consisting of six treatments identified as T^1, T^3, T^5 which were the controls (no *Ocimum gratissimum* paste) while T^2, T^4, T^6 (*Ocimum gratissimum* paste) were treatments of beef, chevon and mutton respectively, where uncure controls, cured treatments and meat types were factors.

Preparation of Biltong

The ingredients were purchased and processed by administering the following ingredients: Salt, Sugar, Monosodium glutamate, Pepper, coriander, Vinegar and *Ocimum gratissimum* paste on all meat types as displayed in Table 1.

Table 1: Composition of experimental ingredients

Meat Types/ Ingredients (g) and ml	Beef	Chevon	Mutton	Salt	Sugar	Coriander	Pepper	Monosodium glutamate	Vinegar	*Ocimum gratissimum* paste
Quantity	5000	5000	5000	18.8	12.2	100	55	75	400	1360

Preparation of *Ocimum gratissimum* paste

The fresh leaves of basil (1360) were harvested, weighed and rinsed with distilled water. The leaves were then finely blended into *Ocimum gratissimum* paste.

Processing of Biltong

Meat chunks (5 kg of hindquarter beef, chevon and mutton muscles) were trimmed of all visible bones and connective tissues. Meat types were stripped into approximately 8-10 x 2 x 1.5 cm along the grains, weighed and washed thoroughly with distilled water and allotted into six treatments. The samples were seasoned and left overnight in vinegar and *Ocimum gratissimum* paste under refrigeration conditions to prevent mould growth and to ensure deep penetration of the spices, After curing, the meat strips were sundried for a whole day under insect proven condition and transferred to a shade for two weeks.

Determination of proximate composition of Biltong

This was carried out to determine moisture content of biltong. Samples (2 g) from each meat type were dried in an oven at 100-105°C until constant weight was achieved as described by (AOAC, 2000), while crude protein of biltong samples were obtained using Kjeldahl method which included digestion, distillation and titration of the distillates. The values of crude protein were derived by converting nitrogen (N%) content of the distillates with a constant (6.25) thus, crude protein was obtained as (6.25xN%). Crude fat of biltong were determined with Soxhlet extraction method using petroleum ether. Biltong samples were dried in an oven for 4 hours and fat was extracted. Ash content of biltong were determined by igniting the biltong samples in a Muffle furnace at (550-600°C) for 24 hours until ashes were formed.

Mineral analysis of Biltong

Calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and sodium (Na) were obtained by dry ashing. The resulting ash was dissolved in 0.1M hydrochloric acid and the solution was used to determine the mineral content of the samples using Atomic Absorption Spectrophotometer (AAS) (AOAC, 2000).

Sensory evaluation

Biltong samples were served in plates to 40 panelists comprising of semi-trained and trained members of both sexes.with ages ranging between 25-35 years old. Each panelist was selected according to his potential based on preliminary screening. Initially panelists were asked to evaluate the samples. All treatments were served in random order. Panelists evaluated the samples seated in individual booths. Panelists used a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) to grade how much they liked or disliked each sample (Stone and Sidel, 1993). The parameters rated were colour, texture, juiciness, flavour, saltiness, hotness and overall acceptability.

Microbiological analysis of Biltong

A one in ten serial dilution was carried out for biltong samples in each treatment for the isolation and identification of microbes following the procedures of Buchanan and Gibbons (1974). Colonies were counted and expressed as 10g$_{10}$cfu/g.

Statistical analysis

Data collected from this study were analysed using SAS (2002), while means were separated using the Duncan Multiple Range Test of the same software.

Table 2: Sensory Evaluation of biltong cured with *Ocimum gratissimum* paste

Parameters/ Meat types (%)		Colour	Texture	Juiciness	Flavour	Hotness	Saltiness	Overall Acceptability
Beef	T^1	3.76 ± 0.12^{b}	3.38 ± 0.84^{ab}	4.01 ± 0.26^{a}	6.45 ± 0.57^{a}	4.07 ± 0.99	3.62 ± 0.27^{b}	5.97 ± 0.31^{b}
	T^2	4.16 ± 0.12^{a}	3.78 ± 0.14^{a}	4.81 ± 0.26^{a}	6.85 ± 0.57^{a}	4.47 ± 0.41	4.02 ± 0.40^{a}	7.37 ± 0.32^{a}
Chevon	T^3	3.93 ± 0.78^{b}	3.36 ± 0.62^{ab}	4.04 ± 0.99^{a}	6.07 ± 0.88^{a}	4.36 ± 0.81	3.71 ± 0.46^{b}	5.07 ± 0.56^{b}
	T^4	3.90 ± 0.81^{b}	3.30 ± 0.66^{ab}	3.93 ± 0.80^{b}	6.00 ± 0.71^{a}	4.22 ± 0.69	3.70 ± 0.49^{b}	7.03 ± 0.58^{a}
Mutton	T^5	2.78 ± 0.12^{c}	2.89 ± 0.77^{b}	2.96 ± 0.99^{c}	5.11 ± 0.60^{b}	4.33 ± 0.90	3.44 ± 0.25^{b}	4.81 ± 0.59^{c}
	T^6	2.86 ± 0.11^{c}	2.14 ± 0.59^{b}	3.00 ± 0.20^{b}	5.14 ± 0.58^{b}	4.46 ± 0.23	3.46 ± 0.23^{b}	4.06 ± 0.56^{c}

^{abc}**:** means on the same column with different superscripts are significantly different (P<0.05)

T^1, T^3, T^5 are controls (no *Ocimum gratissimum* paste) while T^2, T^4, T^6 were treatments were cured with *Ocimum gratissimum* paste

T1 – Beef biltong (control), T2 – Beef biltong (Ogp cured), T3 – Chevon biltong (control), Chevon biltong (Ogp cured), T5 – Mutton biltong control, T6 – Mutton biltong (Ogp cured),

Table 3: Proximate composition of biltong cured with *Ocimum gratissimum* paste (%)

Parameters/ Meat types (%)		Moisture Content	Crude Protein	Ether Extract	Ash Content	Crude Fibre
Beef	T^1	58.23 ± 0.15^{b}	21.43 ± 0.21^{c}	12.40 ± 0.10^{b}	6.13 ± 0.15^{a}	0.87 ± 0.32
	T^2	58.70 ± 0.10^{ab}	24.47 ± 0.12^{a}	11.57 ± 0.06^{c}	4.40 ± 0.10^{c}	0.43 ± 0.66
Chevon	T^3	59.00 ± 0.40^{a}	20.73 ± 0.15^{d}	12.40 ± 0.26^{b}	6.67 ± 0.15^{a}	0.57 ± 0.86
	T^4	58.20 ± 0.10^{b}	23.67 ± 0.15^{ab}	11.36 ± 0.06^{c}	4.57 ± 0.19^{c}	0.47 ± 0.16
Mutton	T^5	56.80 ± 0.16^{b}	22.53 ± 0.51^{b}	13.30 ± 0.15^{a}	6.10 ± 0.10^{a}	0.63 ± 0.76
	T^6	58.17 ± 0.15^{b}	21.60 ± 0.18^{c}	13.17 ± 0.10^{a}	5.50 ± 0.11^{b}	0.73 ± 0.06

^{abc}**:** means on the same column with different superscripts are significantly different ($P<0.05$)

T^1, T^3, T^5 are controls (no *Ocimum gratissimum* paste) while T^2, T^4, T^6 were treatments were cured with *Ocimum gratissimum* paste

T1 – Beef biltong (control)**,** T2 – Beef biltong (Ogp cured)**,** T3 – Chevon biltong (control), Chevon biltong (Ogp cured), T5 – Mutton biltong control**,** T6 – Mutton biltong (Ogp cured).

Table 4: Mineral composition of biltong cured with *Ocimum gratissimum* paste (mg/100g)

Parameters/meat types (mg)		Calcium (Ca)	Iron (Fe)	Potassium (K)	Magnesium (Mg)	Sodium (Na)
Beef	T^1	121.66 ± 0.12^{ab}	6.86 ± 0.21^{b}	260.66 ± 0.88	56.61 ± 0.76^{b}	554.21 ± 0.47
	T^2	138.33 ± 0.41^{a}	6.33 ± 0.25^{c}	264.33 ± 0.56	68.33 ± 0.89^{a}	541.68 ± 0.68
Chevon	T^3	121.60 ± 0.18^{ab}	6.73 ± 0.21^{b}	263.33 ± 0.58	45.00 ± 0.50^{d}	563.34 ± 0.58
	T^4	120.66 ± 0.57^{c}	6.47 ± 0.21^{c}	266.66 ± 0.41	56.67 ± 0.64^{b}	559.54 ± 0.41
Mutton	T^5	111.36 ± 0.41^{e}	7.67 ± 0.15^{a}	266.61 ± 0.41	53.33 ± 0.89^{c}	542.97 ± 0.64
	T^6	118.67 ± 0.89^{d}	6.99 ± 0.21^{ab}	265.44 ± 0.41	52.01 ± 0.50^{c}	556.89 ± 0.56

^{abc}**:** means on the same column with different superscripts are significantly different ($P<0.05$)

T^1, T^3, T^5 are controls (no *Ocimum gratissimum* paste) while T^2, T^4, T^6 were treatments were cured with *Ocimum gratissimum* paste

T1 – Beef biltong (control)**,** T2 – Beef biltong (Ogp cured)**,** T3 – Chevon biltong (control), Chevon biltong (Ogp cured), T5 – Mutton biltong control**,** T6 – Mutton biltong (Ogp cured).

Table 5: Mean Microbial load of beef, chevon and mutton biltong cured with *Ocimum gratissimum* paste samples ($10g_{10}$cfu/g)

	Beef		Chevon		Mutton	
Parameters	**T[1]**	**T[2]**	**T[3]**	**T[4]**	**T[5]**	**T[6]**
Aerobic count(cfus/g)	1.90±0.23[a]	0.90±0.21[c]	1.61±0.32[b]	0.82±0.17[d]	1.21±0.31[b]	0.29±0.46[e]
Coliform count(cfus/g)	1.11±0.16	1.00±0.19	1.81±0.11	1.00±0.16	1.90±0.11	1.01±0.16
Lactic acid bacteria(cfus /g)	1.32±0.31[a]	0.31±0.11?	1.22±0.29[a]	0.18±0.18?	1.11±0.31[b]	0.16±0.21

[abc]**:** means on the same column with different superscripts are significantly different (P<0.05)

T[1], T[3], T[5] are controls (no *Ocimum gratissimum* paste) while T[2], T[4], T[6] were treatments were cured with *Ocimum gratissimum* paste

T1 – Beef biltong (control)**,** T2 – Beef biltong (Ogp cured)**,** T3 – Chevon biltong (control), Chevon biltong (Ogp cured), T5 – Mutton biltong control**,** T6 – Mutton biltong (Ogp cured).

Table 6: Morphological and biochemical characterisation of bacteria isolates from biltong prepared with *Ocimum gratissmum* paste

Treatments	Citrate utilisation	Motility	Indole test	Glucose	Fructose	Maltose	Lactose	Sucrose	Galactose	Xylose	Arabinose	Raffinose	Rhamnose	Dulcitol	Mannitol	Identity
Beef T[1]	+	+	-	+G	+	+	+	+	(+)	+	+	-	-	+	+	*Bacillus megaterium*
T[2]	+	+	-	+	-	-	-	+	D	(+)	-	+	+	-	+	*Pseudomonas fluorescens*
ChevonT[3]	+	+	-	+G	-	-	+	+	+	-	-	+	+	-	+	*Bacillus subtli*
T[4]	-	+	+	+G	-	+	+	+	(+)	-	-	(+)	-	(+)	-	*Bacillus alvei*
MuttonT[5]	+	+	-	+	-	+	-	+	D	+	-	+	(+)	-	+	*Pseudomonas chlororaphis*
T[6]	-	+	-	+	-	+	+	+	+	+	+	(+)	-	+	(+)	*Bacillus pulmilus*

T1 – Beef biltong (control)**,** T2 – Beef biltong (Ogp cured)**,** T3 – Chevon biltong (control), Chevon biltong (Ogp cured), T5 – Mutton biltong control**,** T6 – Mutton biltong (Ogp cured).

N: B – R (raised) no R on table; +G (positive in glucose); (+) (weakly positive reaction); d (delayed reaction)

RESULTS AND DISCUSSION

The sensory properties of biltong samples are presented in Table 2. The results indicated that there were significant (P<0.05) differences between biltong of the three meat types. *Ocimum* cured beef biltong (T^2) had the highest and best (P<0.05) scores for colour, texture, juiciness, flavour and overall acceptability and this was closely followed by the flavour and acceptability of chevon biltong (T^3 and T^4) as reported by Anjaneyulu *et al.* (2007), most consumer's adjudge meat and meat products based first on colour and then flavour, while mutton biltong (T^6) had the least preference in all the parameters considered though numerically, it was rated higher than mutton biltong (T^5) which is in accordance with Okubanjo, (1990) who reported that most citizens of developing countries like Nigeria prefer less tender meat or meat product probably for longer chewability. The effect of curing agent used was obvious in all the organoleptic parameters assessed as these scores followed the same trend as assessed which might be attributed to the chemotherapeutics properties of *Ocimum gratissimum* paste. (Prabhu *et al.*, 2009)

It was observed that there was significant (P<0.05) difference in the proximate composition of all the biltong meat types (Table 3). There were significant (P<0.05) differences in all the variables except in fibre of biltong samples. Moisture content of cured chevon biltong control (59.00±0.40%) and beef biltong (58.70±0.10%) were higher (P<0.05) than other biltong samples, while T5 (uncured mutton biltong) had a significantly lower (P<0.05) mean (56.80±0.16%). Crude protein value was higher (P<0.05) in cured beef biltong (24.47±0.12%) and uncured chevon biltong had the least (20.73±0.15%) mean value among the three biltong meat types samples. The ether extract of uncured mutton biltong (13.30±0.15%) and cured (13.17±0.10%) were higher (P<0.05) than other treatments while cured chevon biltong (11.36±0.06%) and cured beef biltong (11.57±06) were significantly lower (P<0.05) than other biltong samples in fat content. Ash contents of biltong samples from uncured chevon (6.67±0.15%), beef (6.10±0.10%) and mutton (6.13±0.56%) were higher (P<0.05) than all samples while crude fibre values were not significant (P>0.05) but had highest (P<0.05) values in uncured beef (0.87±0.32%). This could be as a result of fibre present in plant based ingredient used in its preparation reported by Ogunsola and Omojola (2008) for kilishi, an intermediate moisture meat.

Table 4 shows the results of the mineral composition of biltong from different meat types. There were significant (P<0.05) differences in all the variables except in potassium and sodium of biltong samples. Calcium content of cured beef biltong (138.33±0.41mg/100g) was highest (P<0.05) among the product means while the least (P>0.05) was found in uncured mutton biltong control (111.36±0.41mg/100g), also iron values were higher (P<0.05) in uncured (7.67±0.15mg/100g) and cured (6.99±0.21mg/100g) mutton biltong while cured beef biltong had the least (6.33±0.25mg/100g) value among the three biltong meat type samples. The magnesium of cured beef biltong (68.33±0.89mg/100g) had the highest (P<0.05) value and uncured chevon biltong (45.00±0.50mg/100g) was significantly lower (P<0.05) in all biltong samples. High levels of potassium and sodium in this study could be as a result of salt, monosodium glutamate and coriander ingredients used in the preparation of biltong when compared with rashers, a bacon cured pork product as reported by Food Safety Authority of Ireland (2015). While, in respect of species differences the high content of iron in beef no doubt reflects the greater concentration of myoglobin in this species than in mutton or pork.

The result of the mean microbial load of biltong is presented in Table 5. The results indicated that there were significant (P<0.05) differences between the microbial loads of biltong meat types. This revealed that aerobic bacteria counts were highest (P<0.05) in all the controls of the three biltong meat types (T^1, T^3, T^5), while mutton biltong (T^6) had the least counts (P>0.05) and highest was in beef biltong (T^2) of cured samples, however, there were no significant (P>0.05) differences in the coliform counts in all the biltong meat types but numerically, cured biltong had reduction in the coliform counts. Also, the lactic acid counts of cured mutton biltong (T^6) had least scores (P<0.0), while beef biltong (T^2) recorded the highest. The results obtained from the controls of these biltong meat types could be attributed to the microbial status of the raw meat and ingredients used in processing (Wills *et al.*, 1998, Uzeh *et al.*, 2006). However, the reduction in the microbial counts observed in the cured biltong could be as a result of the presence of antibacterial activity in the ingredients and in *Ocimum gratissimum* (Uzeh *et al.*, 2006).

The preliminary identification and final characterization revealed the presence of *Bacillus megaterium, Pseudomonas fluorescens, Bacillus subtli, Bacillus alvei, Pseudomonas chlororaphis and Bacillus pulmilus* micro-organisms in the *biltong* products. The isolates were done for the storage. Microbial growthwas precisely observed in the *biltong* product after 54 days which represent the storage time except for the controls that growth was visible after 8 days of storage.

Uncured beef biltong had *Bacillus megaterium* which were citrate, and motile but indole negative, organisms utilizing fructose, maltose, lactose, sucrose and galactose for fermentation while cured beef biltong had *Pseudomonas fluorescens* being citrate, motile and also indole negative but only used sucrose, raffinose and mannitol for fermentation. Furthermore, uncured and cured chevon biltongs had *Bacillus subtli* and *Bacillus alvei* respectively. Formal were citrate, motile, indole negative and used lactose, sucrose, galactose and mannitol for fermentation but cured were citrate negative, motile, and indole positive and used glucose, maltose and lactose. However, uncured and cured mutton biltongs follow the same trend of chevon biltong but had *Pseudomonas chlororaphis and Bacillus pulmilus organisms.* Intermediate moisture meat products mostly depend on lower moisture content and consequent decrease in water activity for shelf stability.

In Nigeria, shortage of meat is not only due to absolute scarcity of animals, spoilage through microbial infestation but also to increase in deterioration and lack of meat preservation (Alonge, 1984). Obamu *et al.* (1981) observed that intermediate moisture meat (IMM) are shelf stable under the tropical climate without refrigeration and may be eaten directly with or without rehydration. The basic purpose of meat preservation is to retard or prevent microbial spoilage and other physico-chemical changes thus proper preservation safeguards the sensory quality and nutritive value of meat (Aymerich *et al.,* 2008). Various methods employed for preservation of meats are curing, smoking and radiation though, preservation appliances are hard to come by, and where they are available, erratic power supply in the country is a challenge. Various additives are employed for lowering the water activity of foods and meat products and they are known as humectants which are generally low molecular weight compounds and are chemically inert and do not modify the normal sensory qualities of the product. However, these qualities are altered when there is degradation of haemoprotein (myoglobin and haemoglobin) causing loss of colour, development of rancidity, and non-enzymatic browning resulting in loss of colour, nutritive value and possibly off –flavour occurrence.

Diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of spoilage micro-organisms (Aymerich *et al*., 2008). Among factors that affect microbial spoilage of meat include; intrinsic factors; physical-chemical properties such as water activity and pH and extrinsic factors; temperature, processing effects (hygiene, cleaning, disinfections) in which the crude paste from herb (*Ocimum gratissimum*) was used to cure *biltong* made from different meat types. In view of the activities of this medicinal plant, it was used in this study as curing agent to assess critically the curing activity of crude *Ocimum* species with special reference to *Ocimum gratissimum.* The active compounds of *Ocimum gratissimum* paste used in this study act according to their chemical structure: monoterpenes (terpenes, terpenoids, phenylpropenes) and other compounds (sesqueterpenoids). These unique chemical compounds profiles had great impact on meat profiles during processing in a number of ways. Based on this, meat products were subjected to microbiological analysis in which the cultural and cellular morphology of microbes present were studied as shown in tables 5 and 6.Which implied that *Ocimum gratissimum* paste could be use as natural phyto-preservative in the meat industry as it enhances keeping quality of biltong products but had mild effect on identified isolates with progressively reduction as increasing curing time increases and and prolonged shelf-life therefore, enhanced product acceptability.

CONCLUSION

It was observed that the highest preference was given to beef biltong (T^2) and chevon biltong (T^4) in terms of overall acceptability while mutton biltong was least preferred. Also, aerobic bacteria, coliform and lactic acid counts were more in uncured biltong meat types while the mutton biltong had the least bacterial and lactic acid bacterial counts, however, the highest counts was recorded in beef biltong.

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