



## Effect of *Gongronema latifolium* and *Ocimum basilicum* Extracts on Antioxidant and Physicochemical Characteristics of Smoked Beef Stored under Room Temperature

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### Authors' contributions

This work was carried out in collaboration among all authors. Author APB designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author OOO supervised the study while authors JFA and AA managed the analysis of the study and read through the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

**Introduction:** Incessant health risks associated with chemical preservatives have resorted to the need of exploring natural alternatives with antioxidant potentials in meat processing. In this study, the effect of *Ocimum basilicum* (OBE) and *Gongronema latifolium* (GLE) extracts were evaluated in smoked beef during 9 days of room storage ( $25\pm 1^\circ\text{C}$ ).

**Methodology:** Beef samples were prepared from freshly cut rounds (2 kg each) allotted to four groups containing Nitrite, OBE, GLE and OBE+GLE respectively. Prepared beef samples were subjected to physicochemical (cooking loss, yield and colour), sensory characteristics, pH and lipid oxidative analyses. Data were analysed using descriptive statistics and ANOVA at  $\alpha_{0.05}$ .

**Results:** No significant change was observed in cooking yield and colour although higher values occurred in control samples. OBE and GLE inclusions exhibited a marked potential in sensory

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characteristics such as flavour, texture, tenderness, juiciness with the panelists rating the latter as the samples with overall acceptability. Results in storage period showed a significant progression in TBARS and pH values in all treatments with the highest and lowest values observed in control and OBE treatments.

**Conclusion:** GLE treated beef showed a marked efficacy throughout the storage period than the other extract-treated samples. With the need to provide natural alternatives against chemical preservatives, exploring its synergistic effects with other cheap, accessible and rich bioactive sources will be imperative in shelf life extension of meat.

**Keywords:** *Gongronema latifolium*; *Ocimum basilicum*; smoked beef; antioxidant properties; physicochemical characteristics.

## 1. INTRODUCTION

Beef, from time immemorial, has made positive contributions to the diet of many, including those of lower income. It is a relatively cheap and a major source of food, providing a significant portion of the protein intake in the diet of a large proportion of people, particularly in developing countries. It is of consistently high quality, lower in saturated fats, enriched with essential nutrients and is demanded worldwide. However, due to its rich nutritive content, susceptibility to microbial contamination, chemical and lipid deteriorative changes are usually high [1]. These nutritive benefits encourage increasing problems of spoilage and low shelf life associated with poor storage and preservation methods in developing countries including Nigeria. FAO [2] reported that high temperatures in tropical regions, lack of basic infrastructures and unsanitary production conditions prevailing in most developing nations have resulted to decreasing shelf life of foods.

Considering the rise in economic development, quality of life, improvement and increasing concern about the health of modern life, the palatability and functionality of food are of utmost importance in consumers' safety and sustenance [3]. Following these concerns, meat/meat products are becoming high-quality product with intake convenience and high functional materials in addition to their existing acceptance as common protein foods [4]. According to Fowler et al. [5], reduction of water during cooking of intact muscles increase nutrient concentration. Thus, to produce meat products with functionality and safety, researchers [6,7] affirmed the need for environment-friendly natural preservatives and food additives with superior cell function regulating effect. In the food industry, antioxidants which are essential properties in food preservation, are categorized into two groups: one is synthetic antioxidants such as

nitrite and second includes natural antioxidants such as plant extracts possessing antioxidant characteristics. Although synthetic antioxidants are being used worldwide, some recent studies have reported their toxic effects in human health [8-10], thus the need for natural antioxidants has tremendously increased in recent times [11,12].

Natural spices of interest such as *Ocimum basilicum* (sweet basil) and *Gongronema latifolium* (bush buck) commonly called Utazi/Utasi and Aroreke in Southeastern and Southwestern Nigeria respectively [13], are among the important plants known for their nutritive and aromatic properties. Although these extracts are reported to possess antibacterial, antifungal, antioxidative and antihelminthic properties according to Odukoya et al. [14], there is little information on their effect (singly and in combination) on physicochemical and antioxidant characteristics on smoked beef stored under room temperature especially for those with lack of cold storage. This lacuna further obliged the study.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study (including preliminary investigations) was carried out between July, 2018 – September, 2018 at the Animal Products and Processing Laboratory, Department of Animal Science, University of Ibadan, situated 6 kilometres to the North of the city of Ibadan (Lat. 7°26'N and Long 3°54'E) at a mean altitude of 277 meters above sea level (Fig. 1).

### 2.2 Source of Study Materials

The two test materials used in the study were *Ocimum basilicum* and *Gongronema latifolium*. The leaves of both plants were pruned in a farm in Ojoo area of Ibadan, Oyo State, thereafter

leaves were harvested fresh after 3 weeks of regrowth.

### 2.3 Preparation of Aqueous Extracts

*Ocimum basilicum* and *Gongronema latifolium* leaves were extracted by aqueous solution. Filtration was done using a muslin cloth to separate the extracts from the leaves. The fresh extracts obtained were used according to their percentages in composition for the study.

### 2.4 Preparation of Meat Samples

Ten (10 kg) fresh beef round (24 hr postmortem) was purchased from the abattoir of University of Ibadan Research Farm. They were stored in a cooler containing crushed ice and transported immediately to Animal Products and Processing Laboratory, Department of Animal Science, University of Ibadan, Ibadan. Fat tissues were trimmed off from the samples, followed by cleaning and storage at 4°C until use.

### 2.5 Preparation of Curing Solution

Every ingredient (on dry basis) including distilled water was weighed into a labelled container per treatment. The method adopted by Bassey [15] was used to prepare the curing solution. In a 100

g composition of ingredients, Control contained 0.5 g of curing salt (nitrite) while OBE, GLE and OBE+GLE had extracts included. 100 ml of distilled water was used to extract 10 g leaves each of *O. basilicum* and *G. latifolium* leaves used for OBE and GLE respectively, while 5 g each of both leaves were also used in 100 ml of distilled water for OBE+GLE. After the aqueous extraction, 27 g of water was weighed and added in the mixture as Control, 27.5 g each of extracts was weighed and included in the mixture of OBE and GLE while 13.75 g each of OBE and GLE was used for OBE+GLE treatment. The test ingredient used for each treatment was added to the spice mixture (ground), thoroughly shaken to ensure an even mix before curing. Curing of the beef samples was done using a single-hole injector. This procedure lasted for 30 minutes with the injection administered at various spots across muscle areas to facilitate even distribution of ingredients throughout the muscle parts. After injection, the beef samples were immersed in the remaining solution for 20 minutes, thereafter removed to drain for 10 minutes before being smoked. The beef samples were smoked at a cooking temperature of 165±5°C until a core temperature of 72°C was attained. After smoking, the products were removed, placed on a clean dry board and allowed to cool. For the study, a 2000 g weight of composition was used for each treatment.



Fig. 1. Map of Nigeria showing the experimental area (University of Ibadan, Ibadan, Oyo State)

**Table 1. Composition of smoked beef [15]**

Ingredients (g)	Control	OBE	GLE	OBE+GLE
Beef	65.00	65.00	65.00	65.00
Water	27.00	-	-	-
OBE	-	27.50	-	-
GLE	-	-	27.50	-
OBE+GLE	-	-	-	27.50
Curing salt (Nitrite)	0.50			
Spice mixture (ground)	7.50	7.50	7.50	7.50
Total	100.00	100.00	100.00	100.00

## 2.6 Physicochemical Characteristics of Smoked Beef Treated with GLE and OBE

### 2.6.1 Cooking loss and yield

Cooking loss was estimated by recording the difference between pre and post cooking weights of beef samples and expressed as a percentage.

*Cooking loss % =*

$$\frac{\text{weight of the sample before cooking} - \text{weight after cooking}}{\text{weight before cooking}} \times 100$$

The cooking yield was obtained by recording the difference between 100% and the percentage cooking loss of each meat samples [16].

$$\text{Cooking yield \%} = \frac{\text{weight of cooked product}}{\text{weight of raw sample}} \times 100$$

### 2.6.2 Colour reading

The method of O' Neill et al. [17] was adopted to determine the colour differences in the meat samples using a Colorimeter (CR 400, Konica Minolta Co., Japan). Before using, the colorimeter was standardized with a standard observer (aperture =  $\phi$ 8 mm/ $\phi$ 11 mm, illuminant = D<sub>65</sub>, Y= 85.5, X= 0.319, y = 0.337). Colour of each beef sample was measured at three different areas perpendicular to the surface and the mean values for each sample (taken in triplicates) were analyzed.

## 2.7 Sensory Evaluation

Sensory evaluation of the treated samples was done after smoking to determine the general acceptability of respective treatments. A 20-man semi-trained panel cutting across the ages of 22-29 was used for the evaluation. The samples

were cut into small but uniform sizes and served with random codes representing the treatments and served to the panelists. Water and biscuits (crackers) were served for mouth rinsing before subsequent evaluation to avoid bias. For the evaluation, a 9-point Hedonic scale was used for the measurement of the following parameters (colour, flavour, tenderness, taste, juiciness and overall acceptability) as adopted by Cross et al. [19]. The overall acceptability of the samples was measured from Dislike extremely (1) to Like extremely (9).

**Table 2. Composition of dry spices for smoked beef [18]**

Spices (dry basis)	Inclusion level (%)
Onions	25
Salt	20
Red pepper (chilli)	20
Monosodium glutamate	12
Thyme	10
Nutmeg	8
Curry	5
Total	100

## 2.8 Determination of pH

The pH was determined by using a digital pH meter model (PHS- 25 Micro field instrument England) as described by AOAC [20]. The pH value of smoked samples was determined by weighing 10 g of the sample into a blender with 90 mL of distilled water and homogenised until smooth slurry was achieved. The digital pH meter was placed in a buffer solution for 2 min to allow equilibrium before placing it into a prepared slurry. All readings were taken in triplicates.

## 2.9 Lipid Oxidation Using Thiobarbituric Acid-reactive Substances (TBARS)

The method described by Buege and Aust [21] was adopted for this determination with slight

modifications. 3 ml each of glacial acid and 1% TBA solution were added to test tubes appropriately labelled blank and tests. 0.6 ml of distilled water was added to the blank, while 0.6 ml of the homogenised sample was added to each of the test tubes. These were thoroughly mixed, incubated in a boiling water bath for 15 minutes, then allowed to cool, followed by centrifugation and collection of supernatants. The supernatant from the blank was used to reset the spectrophotometer (Jenway 6305 Spectrophotometer, UK), preset at 532 nm before reading the absorbance of the supernatant from the test solutions.

$$TBA = \frac{O.D \times V \times 1000}{A \times V \times I \times Y}$$

Where:

O.D = Absorbance of the test at 532 nm.

V = Total volume of the reaction mixture = 6.6 mL

A = Molar extinction coefficient of the product, which according to Buege and Aust [21], is equal to  $1.56 \times 10^5$

I = Length of light path = 1 cm.

Y = mg of tissue in the volume of the sample used.

V = volume of tissue extract used = 0.6 ml

## 2.10 Statistical Analysis

All analyses were performed in triplicate and two trials were conducted for the study. For pH and lipid oxidation in smoked beef, factorial design with two-factor ANOVA (treatment with four levels: control, OBE, GLE and OBE+GLE, and storage time with four different days: (0, 3, 6 and 9 days) was applied for each parameter using the SPSS statistical software, version 25 (SPSS Inc., Armonk, NY, USA). To determine significance in mean values of multiple comparisons, Duncan's multiple range test was performed ( $P < 0.05$ ).

## 3. RESULTS AND DISCUSSION

### 3.1. Physicochemical Characteristics of Smoked Beef Treated with *O. basilicum* and *G. latifolium* Extracts

Percentage of physicochemical characteristics of smoked beef (Table 3) shows the highest and lowest yield percentages occurring in control (49.62%) and OBE (47.70%) treated sample respectively. Changes, however, were in tandem with the findings of AOAC [20] who reported that chilling, ageing, injecting non-meat ingredients and tumbling are related causes leading to a decline in yield.

Researches have shown that addition of non-meat ingredients can cause colour changes during thermal processing of comminuted and non-comminuted meat products and their derivatives. Cheng and Sun [22] reported that the most common of these probable causes are pigment denaturation, especially browning reactions such as Maillard condensation of hexoses and amino components. As observed in the study, control sample had the highest colour values with the least occurrence in  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) was recorded in OBE+GLE and GLE samples respectively due to the inclusion of nitrite, known to improve and stabilize colour of product. The values were not in accordance with the report of Kumar and Kumar [23] on reduced-fat meat batter containing encapsulated *Murraya koenigii* berries extract. According to Hunt [24], lightness is related to the thin aqueous layer on the muscle's surface and depends on the water content (moisture) and water movement (dehydration) towards the surface [25]. The higher  $b^*$  values may be attributed to the bioactive compositions of test ingredients as was also observed by Xiong et al. [26] for fresh pork samples incorporated with nisin and grape seed grapes.

**Table 3. Physical characteristics of smoked beef treated with *O. basilicum* and *G. latifolium* extracts**

	Cooking loss (%)	Cooking yield (%)	Colour		
			$L^*$	$a^*$	$b^*$
Control	50.38	49.62	31.33	14.31	46.31
OBE	52.30	47.70	20.00	12.68	40.15
GLE	50.64	49.36	23.62	11.60	34.88
OBE + GLE	50.66	49.34	17.95	13.27	35.92
SEM	0.56	0.56	2.81	1.00	3.43

OBE = *Ocimum basilicum* extract; GLE = *Gongronema latifolium* extract; SEM = Standard Error Mean;  $L^*$  = lightness;  $a^*$  = redness;  $b^*$  = yellowness

### 3.2 Sensory Characteristics of Smoked Beef Treated with *O. basilicum* and *G. latifolium* Extracts

The organoleptic properties of the cured smoked were analysed using a 9-point Hedonic scale to range of Likeness (9) and Dislikeness (1) of the products (Table 4). From the results, the control sample showed the highest values for colour (6.15) and taste (6.45). This difference was attributed due to the incorporation of nitrite, which is a known colour stable, flavour improving and preservative ingredient. OBE treated samples which showed highest flavour value (6.10) compared to other treatments, may be attributed to the high concentration of flavonoid and other essential chemical components in *Ocimum*, which resulted to the deposition of an intense flavour and aroma in the meat layers mostly during the curing process [27]. GLE treated samples were rated above other groups in tenderness, juiciness, texture and overall acceptability. The scores reported were within the findings of Pires et al. [28] and Vidal et al. [29] for bologna-type sausages and low-sodium salted meat treated with Echium (*Echium plantagineum* L.) oil and chia (*Salvia hispanica* L) flour, and lysine and yeast extracts respectively. Although Khan et al. [30] reported higher organoleptic values in goat patties treated with *Chrysanthemum morifolium* extract (CME) and Butylated Hydroxytoluene (BHT), no distinct change occurred in experimental samples as observed in the study. Turgut et al. [31] also reported similar findings on beef meatballs formulated with pomegranate extract (PE) at the beginning of storage period. Resultant effect may be attributed to the cooking method adopted and bioactive composition of the test ingredients used.

### 3.3 Effect of Extracts on Lipid Oxidation and pH of Smoked Beef during Storage

Lipid oxidation (Fig. 3a) is the deteriorative factor that contributes to the development of undesirable flavours, rancid odour, colour change and formation of toxin compounds in foods that pose risks to man [32,33]. In the study, the TBA values showed a significant ( $p < 0.05$ ) increase as days progressed with the highest and lowest values observed in OBE and control samples during storage respectively. This was lower with the observations of Oshibanjo et al. [34] for breakfast sausage treated with selected oils in day 0 as increase could be attributed to

the comminution process known to induce higher oxidative breakdown than in intact muscles. The values, however, correlated with the report of Torres et al. [35] on cured camel meat that increase in oxidative indices occurred during storage days. High TBA values indicates a higher degree of lipid oxidation [26]. According to Campo et al. [36], Suman et al. [37], lipid oxidation is likely to be detected by sensory panelists as rancid when the MDA content is higher than 2 mg/kg, although Utrilla et al. [38] asserted that values up to 5 mg/kg are acceptable for some fermented sausages such as *salchichon*. Values above 2 mg/kg were observed in all samples in day 9, hence the termination of experiment. Kumar [39] reported that higher TBA values is due to relatively higher content of polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) in comparison to animal fat while Sukumaran et al. [40] reported that high temperature can increase the proliferation of spoilage microflora, especially lactic acid bacteria and negatively affect the oxidative stability of meat, thereby inducing oxidative breakdown [41]. However, adding an adequate amount of an antioxidant can slow down oxidative processes [42].



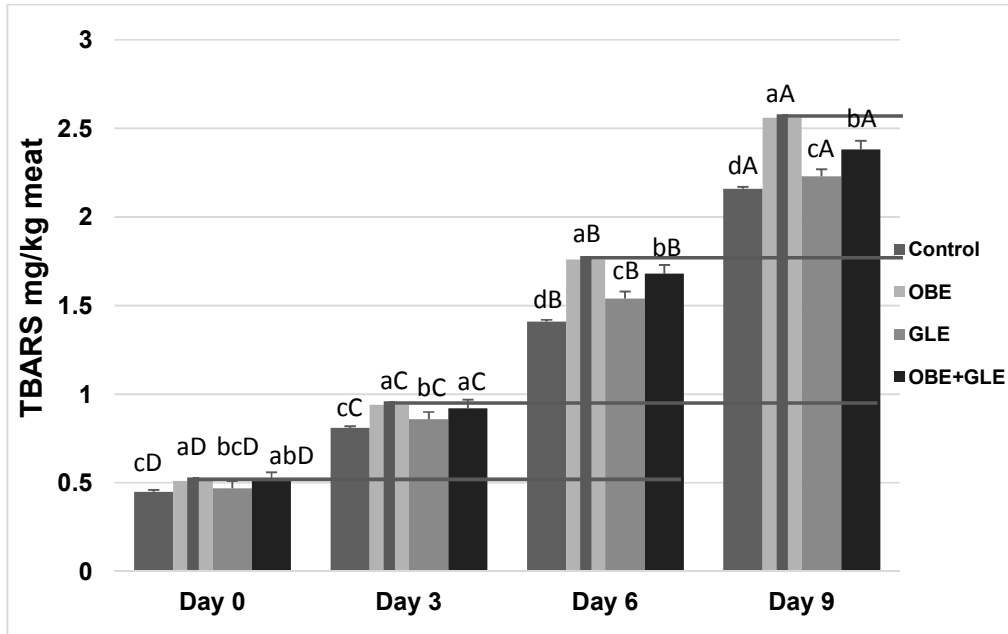
**Fig. 2. Colour variations among treatment groups after smoking**

A = Control 2 = OBE 3 = GLE 4 = OBE + GLE OBE = *Ocimum basilicum* extract; GLE = *Gongronema latifolium* extract

The pH on the other hand (Fig. 3b), is known to have a direct effect on lipid oxidation, colour and vice versa. Although the result showed an increasing trend throughout the storage period in treated samples, a slight decline was observed in day 3 for control samples. Ghesari and Eskandari [43] reported that decrease in pH value in meat may be attributed to the breakdown of glycogen with the formation of lactic acid while its increase may be as a result of partial proteolysis leading to the increase of free

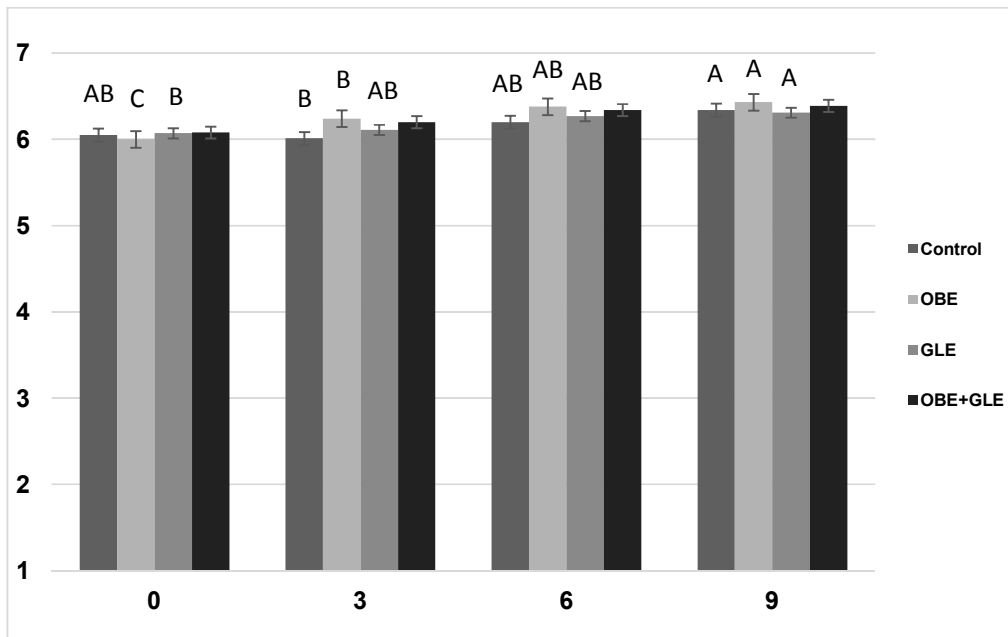
alkaline groups depending on the conditions of such changes. This result tallied within the range of 5.2 – 6.4 reported by Zhang et al. [44] on cured buffalo meat stored under refrigerated

conditions. This fell in line with the study of Nazir et al. [45] that pH value of meat under any condition should not exceed 6.4, otherwise be considered unfit for human consumption.



**Fig. 3a. TBARS content of smoked beef during the storage period**

Means followed by different small letters (treatment effect) and means followed by different capital letters (effect of storage days) are significantly different



**Fig. 3b. The pH of smoked beef during the storage period**

Means followed by different capital letters (effect of storage days) are significantly different

**Table 4. Sensory characteristics of smoked beef treated with *O. basilicum* and *G. latifolium* extracts**

Parameters	Control	OBE	GLE	OBE+GLE	SEM
Colour	6.15 <sup>a</sup>	5.35 <sup>b</sup>	5.75 <sup>a</sup>	5.60 <sup>a</sup>	0.11
Flavour	5.75 <sup>ab</sup>	6.10 <sup>a</sup>	5.10 <sup>b</sup>	5.15 <sup>ab</sup>	0.16
Texture	5.10	5.65	5.95	5.15	0.16
Tenderness	5.85	5.80	6.00	5.70	0.12
Taste	6.45 <sup>a</sup>	6.30 <sup>ab</sup>	6.30 <sup>ab</sup>	5.55 <sup>b</sup>	0.13
Juiciness	5.25	5.40	5.55	5.10	0.16
Overall acceptability	6.15 <sup>ab</sup>	6.05 <sup>ab</sup>	6.45 <sup>a</sup>	5.55 <sup>b</sup>	0.13

<sup>abc</sup> Means on the same row with different superscripts are significantly different ( $p < 0.05$ )

OBE = *Ocimum basilicum* extract; GLE = *Gongronema latifolium* extract; SEM = Standard Error Mean

#### 4. CONCLUSION

In the study, it can be concluded that OBE and GLE are promising plants with vast secondary metabolites which can serve as natural preservative alternatives to synthetic types used. GLE, on the other hand, showed a marked improvement among the extracts against nitrite from organoleptic properties to antioxidative potential. These satisfactory observations during the storage period call for innovative technologies to explore their rich bioactive properties singly or synergistically in meat processing and preservation.

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#### COMPETING INTERESTS

Authors have declared no existence of competing interests.

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