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Effect of Phyto-additives and forms of application on proximate composition and bacterial load of fried Beef

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Abstract
Plant extracts, meals or pellets are used as additives in promoting quality of meat and its products. This study investigated the influence of phyto-additives (onion, garlic, clove and ginger) and application forms (powder and extract) on proximate and bacteriological qualities of fried beef. Fifty grams (50g) of each prepared powdered additives were sprinkled on six (6) pieces of boiled beef of 35-40g/piece to the first group of meat, and six pieces were dipped in 50ml of extracts of the same additives. The samples were left for 30 minutes to equilibrate before frying. The treated beef samples were then adequately fried in vegetable oil after holding for four minutes, separately. The samples were analyzed for proximate and bacteriological load. Results showed that phyto additives significantly affected ($p<0.05$) all proximate parameters except ash and crude fibre. Similarly, forms of application significantly affected ($p<0.05$) all proximate parameters. There was no significant interaction ($p>0.05$) between natural additives and form of application on proximate parameters. The Total Bacterial Count (TBC) ranged between 2.4×10^4 to $3.5 \times 10^4 \text{cfu/g}$ across the samples. A total of five bacterial species (*Staphylococcus aureus*, *Salmonella spp*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*) belonging to different genera were isolated from the samples. It was concluded that all the additives tested, especially the powdered forms of onion and garlic with the least TBC and fewest isolates, can be used as natural additives to improve nutritional quality and safety of the product.

Keywords: Phyto-additives, application forms, proximate, bacterial load, Fried Beef

1. Introduction

Meat and its products are highly perishable and susceptible to lipid oxidation, microbial attack, physical and chemical deterioration as a result of their moisture and nutrient content. In recent times, researchers in developing world have focused on the potential role of plant materials used as extracts, meals or pellets as natural antioxidants, antibacterial and flavor enhancers in promoting the quality of meat and its products and reducing health risks which arise from the indiscriminate utilization of chemical additives [1, 2]. The presence of high phenolic content in plant materials makes them useful as important antimicrobial and antioxidant agents in food. Phenolic extracts are calculated as gallic acid equivalents (GAE) such that plant materials with more than 20mg/g (GAE > 20 mg/g) are considered to have high antioxidant and antibacterial activity levels and those with GAE < 12.1mg/g have low levels. Over the years, several research works had been carried out to analyze the phenolic contents of Phyto extracts and how they affect nutritional, aesthetic and microbial activities in meat products. Research has established that plant resources as additives in foods serve as antimicrobial agents including pathogenic microorganisms. The mode of action could be either by reducing the microbial population in the food material or inhibiting microbial activity by altering the pH or water activity of the food material, thereby creating an unfavorable condition for multiplication of microbes.

Previous studies have reported bactericidal and bacteriostatic properties of plant resources on pathogenic microorganisms, in addition to their antioxidant effects. Amin *et al.* [3] studied the possible effects of varying levels of sodium citrate and garlic paste on composition and quality parameters. Pretreated black garlic extract and various methods of cooking were investigated by Farouk *et al.* [4] to assess their synergy on quality of chicken breast. Yellow-feathered broilers meat quality was evaluated by Liao *et al.* [5] after feeding garlic straw as an unconventional feed. Lishianawati *et al.* [6] studied how black garlic powder affects the quality of spent duck meat nuggets. Meat quality of Cobb strain of broiler chickens was investigated by Kyakma *et al*, [7] after being fed diets containing cloves. Kenawi *et al.* [8] examined the aerobic plate count in order to evaluate the effect of dried rosemary and green tea on the stability of six month-frozen low fat beef products. Antimicrobial effects of papain and bromelain were investigated by

Eshamah *et al*. [9] against *E. coli* and *L. monocytogenes*. Crude bromelain extract from pineapple was utilized by Ali *et al.* [10] to assess its antimicrobial activity on microorganisms isolated from fresh and stored meat. The efficacy of water myrtle extracts (*Myrtus comunnis*) at 0.25 and 0.50% was investigated by Amenour [11] as antimicrobial agent against microbial growth in vacuum-packed chicken frankfurters. Haruna *et al*. [12] determined the quality and shelf-life of nuggets pepper, black pepper and African nutmeg extracts. All the afore-mentioned studies research proved that plant materials can be conveniently utilized as additives for various purposes on both fresh and ready-to-eat meats.

Ready-to-eat meat products are already prepared meat or poultry products that can be eaten directly with no need for additional processing. The popularity of these products worldwide is growing and consumers are opting for greater convenience. Hence, there is an intensifying demand for safe and healthy products with extended shelf life. Ready-to-eat (RTE) meat products are some of the most popular meat items in Nigeria [13]. Consumers also want an ever-widening shelf life**,** microbiological stability, sodium reduction and taste enhancement. The presence of microbial populations in meat products, especially the pathogenic bacteria exceeding acceptable limits had been reported in retail *balangu*, *kilishi* and *tsire* [14]. This research was conducted to evaluate the effects of some phyto-additives and their forms of application on the product quality of fried beef.

2. Materials and Methods

2.1 Study Area

The experiment was conducted in Aliero, located in the Southeast of Kebbi state (12^0 16’ 42” N, 4^0 27’ 6” E). The majority of the people living in Aliero are farmers, cultivating different crops such as onion, maize, rice, millet and rearing animals particularly cattle. Other business activities serving as sources of income include trading and meat selling (i.e., fresh meat, roasted meat, jerky (*kilishi*) and fried meat).

2.2 Treatments and Experimental Design

The study involved a factorial experiment consisting of four natural additives; ginger (*Zingiber officinale*), onion (*Alliums spp*), clove (*Eugenia aromatic*) and garlic (*Allium sativum*) and two application methods (powder and extract). There were eight (8) treatment combinations with one control, each replicated three times to give 27 experimental units. The experiment was laid down in a completely Randomized Design (CRD) as indicated in Table 2.1.

Table 2.1: Experimental layout

Plant materials	Appl. methods	Treatments	Rep.
Control	Control	Control	3
Garlic	Powder	Garlic powder	3
		Onion powder	3
Onion		Ginger powder	3
		Clove powder	3
Ginger	Extract	Garlic extract	3
		Onion extract	3
Clove		Ginger extract	3
		Clove extract	3
Total		9	27

2.3 Source and preparation of natural additives

Prior to the experiment, the plant materials used as natural ingredients were bought from local stores, dried and ground separately, using pestle and mortar and weighed (200g for each). Each of the ground plant material was divided into two; 100g for the two methods of application (powder and extract). The 100g of ground ingredient was soaked into 500 ml of warm distilled water of about 60ºC for three hours for the preparation of extract, for each treatment. After soaking, the mixture was filtered with a Whatman’s filter paper and funnel to obtain the phyto-extracts. The extracts were measured for density, sterilized and stored in a refrigerator at 4ºC until use.

2.4 Preparation of experimental samples

Four and a half kilograms (4.5kg) of boneless beef from an apparently healthy three-year-old bull was purchased from Birnin Kebbi central abattoir. The meat was divided into nine groups of 500g each. Each group was divided into two (250g) each and further divided into ten pieces of meat each weighing approximately 25g. The prepared powdered ingredients of 50g were sprinkled on the beef to the first group of meat and the other six pieces of meat were dipped in 50ml of phyto-extracts and the samples were allowed to equilibrate for 30 minutes before frying the samples. The treated beef was then boiled and fried in vegetable oil to an internal temperature of 100°C and held for four minutes.

2.5 Proximate Analysis

Three samples each of sprinkled and dipped meat was taken, bagged, labeled and analyzed for proximate composition. The collected samples were transported to the laboratory for further analysis of proximate composition. Moisture content, crude protein content, ether extract, ash content and total carbohydrates were determined using the procedures outlined by [15].

2.6 Microbiological analysis

2.6.1 Detection of Pathogenic Microorganisms

General and selective media were used in the determination of total bacterial count and detection of some hygiene-indicator pathogens including *Staphylococcus aureus, Escherichia coli* and *Salmonella* species from the fried meat samples. Gas production in *Escherichia coli* broth was used on suspected isolates from the counting plates. Further confirmation of gas-producing isolates was done by carrying out biochemical tests like indole, MRVP, citrate, and triple sugar iron tests. Golden yellow colonies from MSA plates were used to detect *Staphylococcus aureus* by checking for catalase and coagulase positivity. Original suspension from the fried beef was done by adding 1ml into each of 10ml tetrathionate broth (supplement with iodine) (SRL) and 10ml selenite cystine broth (HIMEDIA) to detect *Salmonella* spp. Inoculate tubes were incubated at 44°C for 48h and 37°C for 24h, respectively and observed for *salmonella* spp.

2.7 Data Analysis

Analysis of variance (ANOVA) using the procedure of SPSS was used to analyze all data obtained from proximate analysis. Non-parametric test was used for analyzing values for microbiological characteristics. TBC results were expressed as colony-forming units per gram (cfu/g) of the product.

3. Results and discussion

3.1 Proximate composition of fried beef as affected by natural additives and forms of application

Table 3.1 shows the results of proximate composition of fried beef as affected by natural additives and forms of application. Results indicated that phyto-additives had significant effect (P<0.05) on all chemical parameters measured except ash content and crude fibre content. Similarly, forms of application significantly (P<0.05) affected all proximate parameters evaluated. There was no significant interaction (P>0.05) between natural additives and forms application on all proximate parameters of the product. However, there showed significant interaction between additives and forms of application on nitrogen.

Table 3.1: Proximate composition of fried beef as affected by natural additives and forms of application

	Parameter						
Source of variation	**Moisture**	**Ash**	**Nitrogen**	**CP**	**CHO**	**Lipid**	**Crude Fibre**
Additives							
Garlic	41.42^{b}	3.00	6.60^{a}	41.26^{a}	5.27^{ab}	4.25^{b}	4.75
Onion	42.17^{a}	2.80	6.61^{a}	41.26^{a}	3.88^{b}	5.08^{a}	4.75
Clove	43.25^{a}	2.75	6.48^{b}	40.47^{b}	5.11^{ab}	3.92^{b}	4.50
Ginger	41.42^{b}	2.75	6.37^{c}	39.80^{c}	4.95^{ab}	4. 83^{a}	4.67
SE	0.28	0.13	0.02	0.09	0.47	0.11	0.12
Application							
Control	31.83^{c}	3.83^{a}	6.92^{a}	43.28^{a}	6.85^{a}	6.33^{a}	7.67^{a}
Powder	39.58^{b}	3.08^{a}	6.69^{b}	41.82^{b}	5.82^{a}	4.83^{b}	4.83^{b}
Extract	45.33^{a}	2.58^{b}	6.33^{c}	39.57^{c}	3.79^{b}	4.21^{c}	4.50^{b}
SE	0.20	0.09	0.01	0.06	0.33	0.08	0.08
Interaction							
Add × APP	NS	NS	*	NS	NS	NS	NS

abc = Means bearing different superscripts along the same column differ significantly (P<0.05), Add × APP (additives × application method)

The overall moisture content found in the current study ranged between 31.83 and 45.33%. This shows that the product is an intermediate moisture meat. It was reported that intermediate moisture meats have moisture content ranging between 35-65% moisture content. For instance, 55.5% was recorded by Oladimeji *et al.* [16] for chicken sausage, 45.00% reported by Unzil *et al.* [17] for chicken burger, Jegede *et al.* [18] recorded 51.28% and 61.59% for suya and asun, respectively. Nady *et al.* [19] also reported 62.15% for beef sausage. Ajai *et al*. [20] recorded 32.51% for roasted beef. The result of this study showed that the moisture content of the product treated with natural additives ranged between 41.42 and 43.25%, with samples treated with clove and onion having higher moisture content of 43.25 and 42.17%, respectively. This could imply that clove and onion help in retaining moisture in the product. The low moisture levels suggest that the product might be less prone to microbial attack [21]. It was reported that levels of moisture content can determine the presence of microbes and their ability to multiply in a food matrix [22]. The ash content was observed to be almost the same across all the plant-treated materials and the forms in which they were applied including the control. Onion- and ginger-treated products had higher fat contents (5.08 and 4.83%, respectively) than samples treated with the other additives. However, the samples under control had higher lipid content of 6.33% followed by 4.83 and 4.21% for powder and extract forms of application. The protein was found to be higher in garlic- and onion-treated samples with 41.26% each. However, the control samples had higher crude protein content (43.28%), followed by powdered form of application which had 41.82%. It was reported that concentration of crude protein is relative to moisture content in the food matrix [23, 24].

3.2 Microbial load in fried beef

Table 3.2 shows the results of microbial load of fried beef for both natural additives and forms of application. Results indicated that the total bacterial count (TBC) ranged from 0.55×10^{-5}cfu/g to 26.9×10^{-5}cfu/g. Onion powder had the lowest TBC and clove extract had the highest TVC. The results however showed that except for clove extract which had the highest bacterial load of 26.9×10^{-5}cfu/g, all the other phyto-additives performed better than the control samples which had 25.5×10^{-5}cfu/g. The results however indicate that microbial loads of all the samples are within the satisfactory standards according to the United State Department of Agriculture Standard (USDA) of bacterial loads in processed meats.

Table 3.2: Microbial loads of fried beef as affected by natural additives and forms of application

Treatment	Dilution factor		Viable plate count			USDA Standards
	Plate 1	Plate 2	Total	Mean	Log/cfu/g	
Clove powder	385×10^{-5}	124×10^{-6}	509	252.5	25.20×10^{-5}	Satisfactory
Garlic powder	8×10^{-5}	13×10^{-6}	21	10.5	1.05×10^{-5}	Satisfactory
Ginger extract	158×10^{-5}	16×10^{-6}	175	87.5	8.75×10^{-5}	Satisfactory
Clove extract	418×10^{-5}	120×10^{-6}	538	269.0	26.90×10^{-5}	Satisfactory
Ginger powder	35×10^{-5}	11×10^{-6}	46	23.0	2.30×10^{-5}	Satisfactory
Control	344×10^{-5}	165×10^{-6}	509	254.5	25.50×10^{-5}	Satisfactory
Onion extract	281×10^{-5}	48×10^{-6}	355	177.5	17.70×10^{-5}	Satisfactory
Garlic extract	86×10^{-5}	12×10^{-6}	98	40.0	4.00×10^{-5}	Satisfactory
Onion powder	9×10^{-5}	2×10^{-6}	11	5.5	0.55×10^{-5}	Satisfactory

The current study revealed total bacterial counts of between log0.55 to log26.9.0×10^{-5}cfu/g. This could suggest the level of hygienic processing and handling of the product as reported by [25]. The total bacterial count of up to log26.9×10^{-5}cfu/g in the current study **is** much higher than the 2.05-2.89×10^{6}cfu/g reported by [26], log8.08cfu/g [27], 0.3-0.85×10^{5} [28] and 7.17×10^{6}cfu/g [29] in meat products. The variation in the TBC could connected with the handling of the product after processing.

3.3 Prevalence of bacteria isolated and identified in fried meat samples

Prevalence of bacteria indicated that five bacteria species were isolated and identified from the meat samples. Out of ninety (90) sets of tests conducted, ten (10) for each treatment, 50(55.5%) of the samples were found to be contaminated with *S. aureus*, 21(23.3%) with *salmonella*, 9(10%) samples with *E. coli*. Similarly, *Klebsiella pneumoniae* was found in 5(5.6%) samples and *Pseudomonas aeruginosa* was found in 3(3.3%) samples.

Table 3.3: Prevalence of bacteria isolated and identified in fried meat samples

Treatment	No. of tests	*Staphylococcus aureus*	*Salmonella sp*	*Escherichia coli*	*Klebsiella Pneumoniae*	*Pseudomonasa eruginosa*	Total
Control	10	10	2	2	2	1	17
Clove powder	10	6	4	2	0	2	14
Garlic powder	10	3	3	0	0	0	6
Ginger extract	10	7	1	1	0	0	10
Clove extract	10	5	3	1	0	0	8
Ginger powder	10	4	3	0	1	0	8
Onion extract	10	5	2	2	1	0	10
Garlic extract	10	6	1	1	1	0	9
Onion powder	10	4	2	0	0	0	6
Total	90	50	21	9	5	3	
Prevalence (%)		(55.6%)	(23.3%)	(10.0%)	(5.6%)	(3.3%)	

The overall high prevalence of the isolated pathogens (*Staphylococcus aureus, Salmonella* and *Escherichia coli)* in fried meat samples in the current study could depict the poor sanitary practices employed in the slaughtering, processing and handling of the product [25, 30]. The trio are pathogenic bacteria referred to among the indicator bacteria in foods by [25] and may be associated with an increased likelihood of the presence of other pathogens. The level of *S. aureus* in the current study revealed a 55.6% incidence, representing the most prevalent among the isolated pathogens. Previous studies reported the presence of the pathogen in foods. For instance, 17.2% was reported in retail RTE meat products [31], 5.98% in RTE meat [32], 10.7% in *balangu* [33], 62% in suya [25], and 29.9% in roasted meat [34]. The consistent presence of *S. aureus* may connected not be with the fact that it survives in the environment, on handlers' body and packaging materials [35] and can hence cross-contaminate post-lethality exposed products. The presence of *Salmonella* (23.3%) in the present study nearly agrees with the 26% reported in RTE-MPs [37], 31.1% in RTE-MPs [36] and 33% in poultry meat [38]. *Escherichia coli* had been reported to be an important pollution indicator and its pathogenic strains are of a serious public health concern. *Escherichia coli* is known to be an indicator of faecal contamination, and its presence in food indicates the possible presence of other enteric pathogens [39]. The presence of *E. coli* could be as a result of inadequate processing or through cross contamination by handlers, environment or utensils. The results relatively conform to some previous reported research results on RTE meats such as the report of 25% by [40].

4. Conclusions

From the results of the study, the natural additives used and forms of application have both demonstrated positive effect on both proximate composition and microbial load of fried beef. The additives have improved the nutritional quality of the product. However, though the microbial loads were within acceptable limits, the presence of *Escherichia coli*. Salmonella and *S. aureus* shows that the handling and processing environment have hygiene issues and can pose food-borne health risks to the consuming public usually characterized by gastroenteritis, fever, typhoid, dysentery and other diseases. It can be concluded that all the additives tested, especially the powdered forms of onion and garlic, having the least TBC of 0.55×10^{-5} and 1.05×10^{-5}cfu/g, respectively, and also having low number of bacterial isolates (6 each), can be used as natural additives to improve nutritional quality and safety of the product.

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