

Effects of Different Natural Antioxidants and Storage Time on Nutritional and Microbiological Qualities of Chicken Sausage

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Abstract: *One of the challenges of food industry is to formulate healthy sausage that can provide physiological benefit and reduce cardiovascular risk through natural antioxidants and preservatives. This experiment was carried out to determine the quality attributes of chicken sausage with different natural antioxidants. Seven different sausages spiced with natural antioxidants (Garlic alone, Ginger alone, Clove alone, Garlic + Ginger, Ginger + Clove, Garlic + Clove) and the control were prepared and analysed for chemical compositions and microbial assay. They were preserved for thirty days and samples collected at 0th and 30th day. Data collected were subjected to analysis of variance in a completely randomize design. The antioxidants used had no ($p>0.05$) influence on the crude protein but the ash and the ether extract were influenced ($p>0.05$). The single inclusion of Garlic, Ginger and Clove positively ($p<0.05$) reduced the total cholesterol, HDL and LDL with increase values ($p<0.05$) in their combinations respectively. Storage time had no ($p>0.05$) on the proximate composition but Total Cholesterol, lipid peroxidation and Triglyceride were increased ($p<0.05$). Control gave the highest ($p<0.05$) value in Mean Total Bacteria Count (9.11×10^6), Mean Total Coliform Count (7.75×10^4) and Mean Total Yeast and Mould Count (7.45×10^3) respectively. While the lowest ($p<0.05$) for all the parameters were found in clove sausage (3.83×10^6) (4.20×10^4) except for Yeast and Mould Count (2.15×10^3) which the lowest value were found in garlic sausage. *Escherichia coli* occurs frequently across the chicken sausage samples except for the sample with ginger alone, *Salmonella typhosa* occurred only in chicken sausage sample with garlic alone, *Shigelladysentariae* occurred only in samples with ginger alone, garlic + ginger and ginger + clove, *Pseudomonas aeruginosa* occurred only in the sample with garlic + clove, *Proteus vulgaris* occurred only in the chicken sausage sample with garlic alone. The use of natural antioxidants singly greatly reduced the microbial load, improved the nutrient quality with lower Total cholesterol and its hereby advocated*

Keywords: Antioxidants, Ginger, Garlic, Clove, Proximate, Lipid profile and Microbial assay.

1. Introduction

Meat and meat products play an important role in human diet by providing a large variety of micro and macro nutrients. Poultry meat is accepted worldwide and its consumption has grown considerably from less than half a kilogram per person in 1990 to 2.2kg per annum person in 2013 (1).

Meat being a highly perishable commodity undergoes spoilage from time of slaughter till consumption. There has been increasing concern about the presence of food borne, pathogens and their effect on shelf life of the product (2).

Lipid oxidation and growth of undesirable micro-organisms in food products results in the development of spoilage, off flavor, rancidity and deterioration, rendering such products unacceptable for human consumption (3; 4), and yielding many compounds that contribute to the pathogenesis of cancer, arteriosclerosis, heart and allergic diseases (5). Development of lipid oxidation in meat products during processing, distribution and storage adversely affects critical quality attributes such as flavor, color and nutritional value and has a major negative economic impact (6). In an effort to retard this process, several synthetic food additives have been widely used in the meat industry to extend food shelf-life, inhibit lipid oxidation and delay or inhibit the growth of pathogenic microorganisms. The most efficient and practical way to prevent oxidation and color deterioration of meat products is to incorporate antioxidant into formulations which could be natural or artificial (7).

Antioxidants are substance or compounds that can retard lipid oxidation and prolong product shelf-life (8). In industrial processing, mainly synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are used to prolong the storage stability of meat products (9). Adverse health effects of chemical preservatives have led to the decreased preference for chemically treated food stuffs. Currently, food scientists are focusing their interest in discovering new natural antimicrobial and preservatives (10).

Natural antioxidants are various substances with different chemical characteristics which are widely present in plant. Examples are honey, turmeric, moringa, clove, ginger, garlic etc. Spices like clove, ginger and garlic, generally are used in culinary to provide distinctive flavor to the food. They have also been reported to exhibit inhibitory effect on many food borne pathogens (3; 11). The inhibitory effect of spice on microbes is due to the presence essential oils and active ingredient in them such as eugenol in clove, gingerol in ginger and allicin in garlic (12).

One of the most important fundamentals for the development of civilization is food preservation and storage. The transformation of raw materials to more-or-less stable foods by drying and fermentation was well known in many ancient cultures and used for different foods such as meat. Sausage is one of the oldest forms of processed meat product known through historical evidence. It is considered as one of

the most appetizing, nutritious, enjoyable and convenient meat products (13).

Sausage is one of the oldest forms of processed meat products known through historical evidence. It is considered one of the most nutritious, appetizing, enjoyable and convenient meat products (13). It can be defined as meat products that are manufactured by selecting, chopping and mincing lean and fat, with or without offal, adding condiments, spices, additives, and starter culture. The ingredients are stuffed in casings, ripened, cured and in some cases smoked (13).

However, this present study is to assess the effect of different natural antioxidants (clove, ginger, garlic and their combinations) and storage time on nutritional and microbiological quality of chicken sausage.

2. Materials and Methods

Site of Experiment

The experiment was carried out at the Animal Products and Processing Technology Laboratory of the Department of Animal Nutrition and Biotechnology, LAUTECH, Ogbomosho, Oyo State.

Experimental Materials

Eight weeks old broiler chicken (Arbor acre strain) was purchased from the poultry unit of the Teaching and Research farm, LAUTECH, Ogbomosho. Ingredients such as spices (nut meg, thyme, onions, curry, Monosodium), vegetable oil, and different natural antioxidants (clove, ginger and garlic) were purchased from local market in Ogbomosho, Oyo state. Sausage casing (ruminant intestine) was also be purchased at the main centralslaughter house in Ogbomosho, Oyo state.

Sausage formulation

The birds were slaughtered scalded, de-feathered and eviscerated as described by Akinwumi and Odunsi (14). The carcasses were deboned and trimmed off visible fat to reduce the fat content before grinding through 3 mm plate of Breville® stainless steel meat grinder. The ground chicken mixed with several ingredients in a food processor mixer for 3-5 minutes at 4-6°C. The sausage batter was then prepared as shown in Table 1 and stuffed in a casing (ruminant casing) with an electrical operated stuffer (Breville® stainless steel meat grinder) to have 10 sausages with diameter 1.6 cm and length 10 cm. The sausages were then labelled from T1 to T7 for control, Garlic alone, Ginger alone, Clove alone, Garlic + Ginger, Ginger + Clove and Garlic + Clove respectively. The sausages were then held 4 hours at 4°C to allow for the ingredients to equilibrate. Afterward, the sausages were boiled at 85°C for 20 minutes, and the products were cooled down at ambient temperature.

Chemical Composition

The proximate composition was determined in triplicate according to the method described by AOAC (15) for Crude protein, ether extract, moisture content and ash. The lipid profile was determined using the method of Allain *et al.*, (16) while the Malondialdehyde (MDA) method stated by

Raharjo and Sofos (17) was used for assessing the extent of lipid peroxidation.

Statistical analysis

Data collected were subjected to Analysis of Variance using the General Linear Model for factorial within a completely randomized design of SAS (18). Means were separated by Duncan's range option of the same statistical software.

Table 1: Ingredients composition for the preparation of sausage

Ingredients	Control	Garlic alone	Ginger alone	Clove alone	Ginger + Garlic	Ginger + Clove	Garlic + Clove
Chicken	65	65	65	65	65	65	65
Extender (flour)	22	20	20	20	20	20	20
Spices	3.0	2.5	2.5	2.5	2.5	2.5	2.5
Vegetable oil	5	5	5	5	5	5	5
Water	5	5	5	5	5	5	5
Antioxidants		2.5	2.5	2.5	2.5	2.5	2.5
Total	100	100	100	100	100	100	100

3. Results

Effects of different natural antioxidants and their combinations on proximate composition of chicken sausage

Table 2 presented the main effect of different natural antioxidants and their combinations on proximate composition of chicken sausage. No significant differences ($p > 0.05$) were observed on the effects of all antioxidants under study on moisture content and crude protein of chicken sausage. Chicken sausage with garlic inclusion had the highest ($p < 0.05$) level of ether extract (29.21%).

Effect storage time on proximate composition of chicken sausage

Table 3 presented the effect of storage time on proximate composition of chicken sausage. There was no significant difference ($p > 0.05$) recorded on storage time for proximate composition across the experiment.

Effect of different natural antioxidants and their combinations on lipid profile of chicken Sausage

Table 4 showed the main effect of different natural antioxidants on lipid profile composition of chicken meat sausage. There were significant differences ($p < 0.05$) among the natural antioxidants used on lipid profile of chicken sausage. Chicken sausage with inclusion of clove has lowest ($p < 0.05$) level of cholesterol (51.52mg/dl) closely followed by sample with inclusion of ginger (52.72mg/dl) while cholesterol level was highly significant ($p < 0.05$) in the control sample (92.11mg/dl) and ginger + clove treated chicken sausage (90.01mg/dl).

Effect of Natural Antioxidant on storage time for lipid profile of chicken sausage

Table 5 showed the effect of Natural Antioxidants on storage time for lipid profile of chicken sausage. It showed that the antioxidants at 30th day did not have positive effect in reducing the level of cholesterol, triglyceride and malonadealdehyde (MDA). The values on 30th day were significantly ($p < 0.05$) higher in cholesterol (78.14),

Triglyceride (137.38) and MDA (10.06) when compared with the 0th day (61.06, 59.12 and 5.32 respectively). No significant ($p>0.05$) difference were observed in the 0th and 30th for HDL and LDL.

Microbial load of chicken sausage with different natural antioxidants

The microbial load of chicken sausage with different natural antioxidant is presented in Table 6. The Total Bacteria Count (TBC) showed that the highest count ($p<0.05$) was recorded for control (9.11×10^6), followed by the combination of garlic + ginger (8.58×10^6), ginger alone (5.53×10^6), the combination of ginger + clove with (4.71×10^6) count, the combination of garlic + clove (4.58×10^6), the values in garlic alone and clove alone were (3.91×10^6) and (3.83×10^6) respectively. It was observed that clove alone has the least ($p<0.05$) value (3.83×10^6) of TBC. No significant ($p>0.05$) differences was observed on the effect of Garlic (3.91×10^6) and Clove (3.83×10^6) on the TBC but both have the least value when compared to control (9.11×10^6) and the combination of garlic + ginger (8.58×10^6) which had the highest value of Bacteria Count on chicken sausage.

Table 6 also revealed the Total Coliform Counts (TCC) for the chicken sausage. The highest count ($p<0.05$) was recorded for control with 7.75×10^4 , followed by the combination of garlic + clove (5.71×10^4). It is observed that clove alone has the least ($p<0.05$) coliform count (4.20×10^4). No significant ($p>0.05$) difference was observed on the effect of the combination of Ginger + Clove (4.34×10^4), Garlic (4.25×10^4) and Clove (4.20×10^4) on the TCC but they have the least value when compared to control (7.75×10^4) which had the highest value of Coliform Count on chicken sausage.

The highest ($p<0.05$) for the Yeast and Mould count was recorded for control (7.45×10^3), followed by the combination of garlic + ginger (5.73×10^3), ginger + clove (4.53×10^3), clove alone (4.30×10^3), garlic + clove (4.22×10^3), ginger alone (3.23×10^3) and garlic alone (2.15×10^3). No significant difference ($p>0.05$) was observed on the effect of Ginger (3.23×10^3) and Garlic (2.15×10^3) on the Total Yeast and Mould Count but both have the least values when compared to control (7.45×10^3) which had the highest ($p<0.05$) value of Yeast and Mould Count on chicken sausage.

Microbial Characterization and Identification of Bacteria in chicken sausage with different natural antioxidants

Table 7 presented the characterization of bacterial in chicken sausage prepared with different natural antioxidants. *Escherichia coli* occurs frequently across the chicken sausage samples except for the sample with ginger alone. *Staphylococcus epidermidis* also occurred in all the chicken sausage samples except for the samples with garlic alone and control (sample without antioxidant). *Citrobacterfreundii* occurred in chicken sausage samples with garlic alone, ginger alone and control. *Salmonella typhosa* occurred only in chicken sausage sample with garlic alone. *Shigelladysenteriae* occurred only in samples with ginger alone, garlic + ginger and ginger + clove. *Pseudomonas*

aeruginosa occurred only in the sample with garlic + clove. *Proteus vulgaris* occurred only in the chicken sausage sample with garlic alone.

4. Discussion

The percentage composition for moisture, crude protein and ash content were not affected by the inclusion of natural antioxidants as there were no significant differences between their averages and the control. Addition of different extracts did not cause any significant change in the nutritional content of the products. Ether extract levels were lower in all treatments except in garlic compared to the control. Levels of Nitrogen free extract were increased in all treatments except in the inclusion containing garlic.

Addition of examined antioxidants shows significant ($p<0.05$) improvement compared to control sample. These results were in accordance with GeOrganielset *al.*, (19) who observed lower TBARS (Thiobarbituric acid reactive substances) values for fresh pork sausage treated with natural antioxidants. The samples treated with natural antioxidants will delay or lower the lipid oxidation in chicken sausage. These results were in agreement with previously published studies of Rhee *et al.*, (20) which reported higher effectiveness of natural antioxidants products and suggesting the possibility of using these extracts as replacer of commercial compound. The susceptibility of meat to lipid peroxidation is depending on animal species, muscle type and anatomical location (20). In addition, various processing factors can influence the rate of lipid peroxidation in meat and meat products: composition of raw meat, aging time, cooking or heating, size reduction processes such as grinding, flaking, and emulsification, deboning, especially mechanical deboning, additives such as salt, nitrite, spices, and antioxidants, temperature abuse during handling and distribution, oxygen availability, and prolonged storage (21;22).

Triglycerides are the main constituents of body fats which have to be kept in low levels; addition of the antioxidants increased the triglyceride levels in the garlic and clove treatment while there was a decrease in the levels of triglyceride in the other treatments. The levels of high density lipoprotein (HDL), the good type of cholesterol in the garlic and a mixture of garlic and clove compared to the control were increased while the levels of low density lipoprotein (LDL) were significantly reduced in all of the treatments except in the combination of ginger and clove. Malondialdehyde is an indicator of oxidative stress, its level was significantly increased in the mixture of ginger and clove followed by the mixture of ginger and garlic, then the garlic treatment but lower in other treatments. Lipid oxidation is one of the main reasons for foods deteriorating and causes a significant reduction in their nutritional value as well as taste (23). Clove was effective in reducing the levels of cholesterol, low density lipoprotein and Malondialdehyde lipid peroxidation. This finding was similar to the report of several authors; Clove extracts can retard the lipid oxidation of meat products (23, 24, 25, 26).

Increased bacteria count was observed in control since it has no inclusion of natural antioxidants while the reduced count

was observed in clove because its chemical component has a significant potential effect to reduce the number of microorganisms in the sausage. The high bacteria count is also an indication of potential microbial contamination during processing, distribution and storage (27). The sample with clove has the lowest bacterial count. These result agreed with that reported by Mandeeet *al.*, (28) who stated that *Escherichia coli* was sensitive to clove, and other antimicrobial activity. The results were also in accordance with those reported by researchers (29,30,31) who showed that clove and its essential oil was among the most capable antioxidants for controlling bacteria such as *Escherichia coli*. In general, results in this study showed that the TVC for the sausage ranged between (9.11×10^6 and 4.58×10^6). The contamination that increases the microbes comes from different sources mainly flesh(skin), water, equipment, intestinal content and slaughtering floor as reported by Empey and Scott, (32).

It was observed from the table that *E. coli* was present in all the chicken sausage samples even with the inclusion of natural antioxidants. This is because Madappa, (33) reported that enterobacteriaceae occurs as normal flora and they are widely distributed in nature and this account for their presence in sausage. *E. coli* are rod shaped, gram-negative bacilli that commonly inhabit the large intestine and are naturally excreted in faeces (33). The presence of *E. coli* in food of animal origin is considered as indicator of faults during preparation, handling and storage (34). *E. coli* is considered as an indicator of fecal contamination, besides it may induce severe diarrhea in infants and young children, as well as food poisoning and gastroenteritis among the adults (35).

The result indicate contamination of chicken sausage samples with various bacterial species including *Citrobacterfreundii*, *Escherichia coli*, *Salmonella typhosa*, *Staphylococcus epidermidis*, *Shigellaceyclonesis* as most common bacterial. There were variation in the bacteria identified on chicken sausage, the variation in the microbial characterization and identification of bacteria in chicken sausage could be either from the status of the animal used in its production or processing technology as reported by

Ogbonnaet *al.*, (36). Suree and Pana (37) reported that the isolated bacteria found in control (sample without natural antioxidant) was more than in the samples with natural antioxidant which contradicts the result presented in this study whereby samples with natural antioxidants showed presence of more isolated bacteria than the sample without natural antioxidants this could be due to contamination during processing, equipments, storage and improper handling.

Their presence in large numbers in food indicates inadequate processing/or recontamination due to cross contamination by raw materials, dirty equipment or poor hygienic handling (27). Enterobacteriaceae are the most common bacteria form of acute infective diarrhea and are the most commonly reported bacterial cause of food-borne infections (38,39). Shehu and Adesiyun (40) reported *Escherichia coli* in fermented Nigerian milk. *Enterobacteriaceae* occur as normal flora of the intestinal tract. They are widely distributed in nature and this account for their presence in sausage. A number of foods in Nigeria have been reported to have high incidence of microbial contamination of sausages sold in Nigerian supermarkets (40). Very few bacteria can thrive under freezing conditions (41). Results of the characterization and identification of the isolated bacteria obtained in the present study were in agreement with standard suggested by Bergey's Manual for Determinative Bacteriology (42).

5. Conclusion

It was observed from the result of this study that the three Natural antioxidants (clove ginger and garlic) in chicken sausage had impact on level of cholesterol, low density lipoprotein, lipid peroxidation (MDA) and microbial loads. Inclusion of clove however had better on the quality attributes of chicken sausage. The storage time did not have much impact on the nutrient composition since the antioxidants sustained the nutrients. This study therefore stresses the use of natural antioxidants in enhancing the quality and shelf life of meat and meat products.

Table 2: Effect of Different Natural Antioxidant on the Proximate Composition of Chicken Sausage

Treatments (%)	Control	Garlic	Ginger	Clove	Garlic + Ginger	Ginger + Clove	Garlic + Clove	SEM
Moisture content	61.86	58.78	60.85	57.44	60.50	59.06	62.47	2.18
Crude protein;	47.02	46.73	47.91	45.05	46.98	45.57	46.57	2.34
Ether extract	26.28 ^b	27.61 ^a	22.08 ^c	25.30 ^b	25.99 ^b	23.18 ^c	24.03 ^c	1.67
Ash	2.83 ^a	2.63 ^c	2.73 ^b	2.81 ^a	2.74 ^b	2.80 ^a	2.80 ^a	0.21
Nitrogen free extract	23.89 ^c	23.03 ^c	24.45 ^c	27.58 ^a	24.30 ^c	28.46 ^a	26.62 ^b	1.17

^{abcd} means having different superscript on the same row are significantly different ($p < 0.05$).

Table 3: Effect of selected Natural Antioxidants on storage time for Proximate Composition of Chicken Sausage

Treatments (%)	0 Day	30 Days	SEM
Moisture content	59.80	60.46	3.12
Crude protein;	47.03	46.05	2.04
Ether extract	25.05	24.79	1.56
Ash	2.79	2.73	0.23
Nitrogen free extract	25.33	26.47	1.12

Table 4: Effect of different natural antioxidants of lipid profile on quality attributed of chicken sausage

Parameters (mg/dl)	Control	Garlic	Ginger	Clove	Ginger + Garlic	Ginger + Clove	Garlic + Clove	SEM
Total Cholesterol	92.11 ^a	58.06 ^{bc}	52.72 ^c	51.52 ^c	63.68 ^{bc}	90.01 ^a	79.11 ^b	2.43
Triglyceride	97.51 ^c	125.16 ^a	94.99 ^c	102.92 ^b	82.71 ^d	87.18 ^d	97.30 ^c	2.02
High density Lipoprotein	10.97 ^a	11.53 ^a	5.42 ^b	10.42 ^a	6.25 ^b	9.59 ^a	11.25 ^a	1.32
Low density lipoprotein	61.63 ^a	21.50 ^c	28.30 ^c	20.52 ^c	40.89 ^b	62.99 ^a	48.40 ^b	2.13
Malondialdehyde (nmol/g tissue)	8.60 ^b	8.68 ^b	5.32 ^c	4.91 ^c	9.11 ^b	11.39 ^a	6.93 ^c	1.04

^{abcd} means having different superscript on the same row are significantly different (p<0.05).

Table5: Effect of storage time on Lipid profile of treated chicken sausage

Parameters	0 Day	30 Days	SEM
Total Cholesterol	61.06 ^b	78.14 ^a	3.23
Triglyceride	59.12 ^b	137.38 ^a	5.34
High density Lipoprotein	9.09	9.60	2.01
Low density lipoprotein	40.15	41.06	2.22
Malondialdehydenmol/g tissue	5.32 ^b	10.06 ^a	1.34

^{ab} means having different superscript on the same row are significantly different (p<0.05).

Table 6: Microbial Loads of chicken sausage with different natural antioxidants

Parameters	Control	Garlic alone	Ginger alone	Clove alone	Garlic + Ginger	Ginger + Clove	Garlic + Clove	SEM
Mean Total Bacteria Count cfu/g x 10 ⁶	9.11 ^a	3.91 ^d	5.53 ^b	3.83 ^d	8.58 ^a	4.74 ^c	4.58 ^c	1.52
Mean Total Coliform Count cfu/g x 10 ⁴	7.75 ^a	4.25 ^c	5.23 ^b	4.20 ^c	5.33 ^b	4.34 ^c	5.71 ^b	1.23
Meat Total Yeast and Mould Count cfu/g x 10 ³	7.45 ^a	2.15 ^d	3.23 ^d	4.30 ^c	5.73 ^b	4.53 ^c	4.22 ^c	1.20

Table 7: Microbial Characterization and Identification of Bacteria in chicken sausage with different natural antioxidants

Parameters	Probable Organisms
Control	<i>Citrobacterfreundi</i> <i>Citrobacterfreundi</i> <i>Escherichia coli</i>
Garlic alone	<i>Salmonella typhosa</i> <i>Citrobacterfreundi</i> <i>Proteus vulgaris</i> <i>Escherichia coli</i>
Ginger alone	<i>Citrobacterfreundi</i> <i>Staphylococcus epidermidis</i> <i>Citrobacterfreundi</i> <i>Shigelladysentariae</i>
Clove alone	<i>Proteus vulgaris</i> <i>Escherichia coli</i> <i>Staphylococcus epidermidis</i>
Garlic + Ginger	<i>Shigellaceyclonesis</i> <i>Escherichia coli</i>
Ginger + Clove	<i>Shigelladysentariae</i> <i>Staphylococcus epidermidis</i> <i>Escherichia coli</i>
Garlic + Clove	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus epidermidis</i> <i>Escherichia coli</i>

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