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Full Length Research

Antibacterial effects of some selected spices used in Nigerian pepper soup on cooked broiler meat on days 3 and 6 of refrigeration storage

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ABSTRACT: Food deterioration with subsequent spoilage has been attributed to the presence of micro-organisms, in which bacteria play a prominent role in this regard, especially in the tropics. However, study of this nature was designed to help boost existing progress made in line of curtailing the activities of this micro-organism. Research studies have shown that spices have anti-microbial properties, hence the ability to inhibit the activities of bacteria. The experiment was conducted in phases: phase one investigated the antibacterial effects of some selected individual spices used in Nigerian pepper soup, phase two investigated the effects of mixture of the spices in an unformed manner, (as prepared in the open market), while phase three, investigated the effects in an informed manner, that is, preparation based on the results from phases one and two of the study. The selected spices were *Tetrapleura tetraptera* (Aidon fruit), *Zingiber officinale* (Ginger), *Piper gueneense* (Guinea pepper), *Aframomum melegueta* (Grain of paradise), *Xylopia aethiopica* (Ethiopian pepper), *Allium sativum* (Garlic), *Ocimum gratissimum* (big leaf basil), *Ocimum basilicum* (small leaf basil) and *Monodora myristica* (African nutmeg). The experiments investigated the effects of the spices on days three and six of refrigerated storage using serial dilution method. The selected spices significantly ($p<0.05$) reduced bacterial load on the two separate days of refrigerated storage. This could have been due to the presence of phytochemical in them.

Keywords: Anti-bacterial, food, micro-organisms, spices, spoilage.

INTRODUCTION

Ravindran et al. (2002) reiterated the importance of spices as food additives which contain active antimicrobial compounds that helps to preserve food by delaying spoilage. Adamson (2004) defines spices as being used in nutritionally insignificant quantities as a food additive for flavour and sometimes as a preservative by killing or preserving the growth of harmful bacteria.

Micro-organisms play important roles in the quality of meat and meat products before, during and after processing by initiating many undesirable biological changes (Mikami, 1994). Forrest et al. (1995) reported that micro-organisms that are found on or in meat consists of fungi and bacteria, the fungi include moulds and yeasts. Microorganisms or microbes are microscopic organisms that exist as unicellular, multicellular, or cell clusters. Microorganisms are widespread in nature and are beneficial to life, but some can cause serious harm. They can be divided into six major types: bacteria, archaea, fungi, protozoa, algae and viruses (Biology LibreTexts, 2020). Aerobes require oxygen for growth and as such many species of bacteria, yeasts and moulds can reproduce in the presence of varying amount of oxygen (Ozbas et al., 1996). Therefore, vacuum packaging is conceived primarily to inhibit the growth of these organisms. Anaerobes do not require oxygen for growth because oxygen can be toxic to them. Most of the aerobes

important to meats are bacteria species, though yeasts are also important. Facultative anaerobes are organisms that will grow with or without oxygen. Micro-organisms have an optimum temperature of which they best develop and bacteria that are important to meat can flourish over a wide range of temperatures which are classified as psychrophiles; these are organisms that like cold condition and grow well at temperature below 20°C. Many species in this category thrive at refrigeration temperature of 3 to 7°C and are common in packaging houses. Mesophiles are organisms that prefer warmer temperatures of 21 to 37°C, majority of bacteria fall into this category, while thermophiles are organisms that prefer hot temperatures around 54 to 60°C or even higher. In their reports, Guillon and Guespsin (1996) and Hornick et al. (1999) did mentioned some microbial contaminations of fresh meat to include psychrophiles; *pseudomonas sp., Lactobacillus sp. Moraxella, Acimetobacter, Microbacterium, Thermosphactum, Brochthrix thermosphacta, Klebsiella* and *vibro*. The mesophiles include; *Salmonella sp, Escherichia coli, Clostridium botulinum, Clostridium perfringens*. The thermophiles are; *Streptococcus faecalis, Thermus aquaticus, Thermococcus litoralis* and *Pyrococcus furiosus* among others. Bacteria invasion exhibits two forms of characteristics, extrinsic and intrinsic, however, the exposed surfaces are usually prone to bacteria invasion in carcasses from healthy animals and such type of bacteria are referred to as extrinsic bacteria, but where they are intrinsic, they are usually the proteolytic species. Extrinsic bacteria include almost all mesophillic organisms, while the most common intrinsic bacteria include *Clostridium perfringens*, *Salmonella typhimurium* and *Escherichia coli* among others (Lawrie, 1991).

This research was aimed at investigating the effects of usage of selected spices basically as individual (singly), uninformed and informed manner on bacteriological status of cooked meat from broiler bird, with a view to help curtail the activities of bacteria especially in the area of meat preservations, thereby increasing the shelf life of the meat.

MATERIALS AND METHODS

Preparation of the spices and meat samples

The spices [*Tetrapleura traptera* (Aidon fruit), *Zingiber officinale* (Ginger), *Piper gueneense* (Guinea pepper), *Aframomum melegueta* (Grain of paradise), *Xylopia aethiopica* (Ethiopian pepper), *Allium sativum* (Garlic), *Ocimum gratissimum* (big leaf basil), *Ocimum basilicum* (small leaf basil) and *Monodora myristica* (African nutmeg)] were purchased from the local herbal market in Akure, Ondo State, Nigeria having been duly identified by a renowned scholar in the area of plant taxonomy in the Department of Crop, Pest and Control, Federal University of Technology, Akure and thereafter air-dried, pulverized and kept in labeled airtight containers for use as spice. The study was carried out at The University Teaching and Research Farm, Federal University of Technology, Akure, Nigeria. Five (5) grams of each spice was added to 1 kg of the broiler chicken (thigh). Thereafter, 500 ml of water was added, and the meat cooked for 25 minutes as described by Fakolade et al. (2014). At the end of 25 minutes of cooking, the meat samples were removed from the broth and allowed to cool for 20 minutes. Thereafter, the cooked meat was processed for bacteriological status.

Microbiological analysis

Cooked thigh (2 pieces) for each treatment was kept in the refrigerator (at 4 to 6°C) for 3 and 6 days. At each period, the samples were removed for bacteriological analysis using serial dilution method as described by Yangming et al. (1996). Nine milliliters (9 ml) of water was pipetted into sample bottles, covered and sterilized in the autoclave at 121°C for 15 minutes. The sterile water was allowed to cool, 120 sample bottles were divided into four portions with each portion having 30 bottles arranged serially. Petri-dishes (30), 30 syringes as well as nutrient agar were also provided. Small portion of the thigh from each treatment which was replicated three times (10 treatments × 3 replicates = 30) was cut with a sterilized scissors and put in the first bottle, it was mixed thoroughly, then a syringe was used to measure 1 ml of the thoroughly mixed swab sample from the first bottle, into the second bottle, which was done serially for the second, third and the fourth bottle (10^4). Mixed swab sample (2 ml) was measured out from the fourth bottle into each sterile labeled petri-dishes. The media used (nutrient agar) was prepared from commercially dehydrated products, reconstituted according to the manufacturer's directives. The nutrient agar powder (28 g) was dissolved in 1 litre of water and mixed properly in a conical flask, corked with cotton wool and autoclaved at 121°C for 15 minutes. The sterile media were allowed to cool before pouring into petri dishes containing the 1 ml portion of the aliquout from the fourth dilution. These were gently mixed for even distribution of the inoculum. This was done for all the 10 treatments (replicated thrice). The division of the sample bottles into four represented the dilution factor (10^{-4}). The inoculated plates were allowed to cool and set properly before it was transferred into the incubator at 37°C for 24 hours. Slant sub-culture of microorganisms from the old plate was prepared inside the universal bottles by selecting a microbial colony per slant culture. This preserved the organisms for longer time and enabled pure isolate to be recovered from the old colonies. An inoculating wire loop was flamed red hot, allowed to cool, and then used to collect a loopful of the bottles containing the agar by streaking two or three times in different direction to obtain the pure isolates. The sub-cultures were incubated in inverted positions at 37°C for 24 hours. Cultural characterization was used as a presumptive test for the

isolates. This includes; colour, morphological characterization, surface, edge and elevation of the colony. Grams' staining was carried out as described by Baker et al. (1999) and the slides viewed under the electric microscope with oil immersion (X 100 magnification). Organisms seen on each slide was recorded as suspected, as this gave information on the type of sugar test to be carried out. The ability of the organisms to produce oxidase, catalase and to mobilize glucose by both fermentation and oxidation were tested. Sugar fermentation assay and indo-methyl red tests were also carried out as stated by Olutiola et al. (1999).

Phase 1: Possible effects of individual usage of selected spices used in Nigerian pepper soup on quality characteristics of broiler-chicken meat

Experimental broiler chickens

Day-old Arbor Acre broiler chicks (100) were purchased from a reputable hatchery in Ibadan, Oyo State, Nigeria. On arrival, the birds were removed from the chick boxes, counted into the brooding pen and were immediately offered water containing glucose and vitamins. Broiler starter diet (23% CP) and broiler finisher diet (19% CP) were provided (1 to 28 days – starter phase and 29 to 56 days – finisher phase). Feeds and water were given to the birds *ad-libitum*. Adequate brooding process was observed in addition to normal vaccination, medication and routine management. At the end of the eighth week, 30 birds (all males) were randomly selected and sacrificed by humanely severing the jugular vein. Thereafter, the slaughtered birds were scalded at 65^0C, dressed and eviscerated. The carcasses were dissected as described for Turkey by Harn and Spindler (2002)

Experimental procedure

Three chicken carcasses were assigned to each of the following spices representing individual treatment *Tetrapleura tetraptera* (Aidon fruit), *Zingiber officinale* (Ginger), *Piper gueneense* (Guinea pepper), *Aframomum melegueta* (Grain of paradise), *Xylopia aethiopica* (Ethiopian pepper), *Allium sativum* (Garlic), *Ocimum gratissimum* (big leaf basil), *Ocimum basilicum* (small leaf basil) and *Monodora myristica* (African nutmeg). In addition to the control (sample without spice inclusion), making a total of 10 treatments. Thighs from the carcasses assigned to each treatment were kept refrigerated for 6 hours before cooking to allow the muscle to set. The individual spices (5 g) was added to 1 kg of meat and 500 ml of water was added. The meat samples were cooked for 25 minutes. Thereafter, the cooked meat and broth were allowed to cool for 20 minutes, this was then followed by the bacteriological status determination of the refrigerated meat samples for days 3 and 6 respectively, following the method described above.

Phase 2: Possible effects of un-informed mixture of spices (combinations of spices) used in Nigerian pepper soup, on quality characteristics of broiler chicken meat

Experimental animals

Day-old broiler chicks (50) were purchased from a reputable hatchery in Ibadan, Oyo State, Nigeria. Other management practices (feeding, medication and vaccination), slaughtering and processing of the birds were as described in the first phase of the experiment.

Preparation of un-informed spices

The individual spices in the expected mixture of pepper soup ingredients were purchased from the local herbal market, Arakale road, Akure, Ondo State, Nigeria. The weight of the edible portion of the different spices in each group of the spice mixture in an un-informed manner (as prepared in the open market) was taken and thereafter pulverized together as a mixture and kept in an air tight container for adequate preservation before the next stage of the study. The different spice mixtures identified as groups in this study were purchased from different sellers and tagged A, B, C, D and E (Table 1). Thereafter, the percentage weight of individual spice in the un-informed mixture was calculated to determine the ratios of individual spices in each mixture as:

$$\text{Percentage of inclusion of spices} = \frac{\text{Wi}}{\text{Wu}} \times \frac{100}{1}$$

Where: Wi = Weight of individual spices and Wu = Weight of total uninformed spices

Experimental procedure

Three chicken carcasses were assigned to the treatments with each of the spice mixture representing a treatment, in addition to the control which did not contain any spice mixture (treatment F), making a total of 6 treatments. Thighs from the three carcasses assigned to each treatment were carefully separated and cooked with the spice mixture (un-informed). The cooking procedure and bacteriological status determination were as described in phase one of the experiment.

Phase 3: Possible effects of informed mixture of spices used for pepper soup on quality characteristics of broiler chicken meat

Experimental animals

Day-old broiler chicks (50) were purchased from a reputable hatchery in Ibadan, Oyo State. Other management

Table 1. Percentage inclusion level of individual spice in un-informed mixture of Spices in Nigerian pepper soup.

No.	Spices	Un-informed mixtures				
		A	B	C	D	E
1	*Tetrapleura tetraptera*	18.84	39.00	26.00	10.00	28.15
2	*Zingiber officinale*	31.88	12.00	16.00	14.00	11.65
3	*Allium sativum*	6.28	9.00	8.00	34.00	10.68
4	*Monodora myristica*	14.50	10.00	14.00	10.00	7.77
5	*Piper guineense*	8.69	6.00	9.00	9.00	10.68
6	*Xylopia aethiopica*	9.18	13.00	12.00	7.00	12.62
7	*Aframomum melequeta*	2.42	1.00	2.00	2.00	1.94
8	*Ocimum gratissimum*	3.86	5.00	6.00	8.00	6.80
9	*Ocimum basilicum*	4.35	5.00	7.00	6.00	9.71

Table 2. Proportion of *Tetrapleura tetraptera* (aidon), *Zingiber officinale* (ginger) and *Monodora myristica* (African nutmeg) in the informed spice mixture of Nigerian pepper soup.

Treatments	Spices		
	T. tetraptera	***Z. officinale***	***M. myristica***
I (control)	0	0	0
II	50	25	25
III	25	50	25
IV	25	25	50
	Spice mixtures		
Treatments	**II**	**III**	**IV**
V	50	25	25
VI	25	50	25
VII	25	25	50

practices (feeding, medication and vaccination), slaughtering and processing of the birds were as described in the phase one and two of the experiments.

Three chicken carcasses were assigned to the treatments with each of the informed mixtures of spices (formulated spices) representing a treatment, in addition to the control making a total of 7 treatments. The thighs from the three carcasses were assigned to each treatment, after all the procedural exercises as described in phases 1 and 2.

Preparation of informed mixture of spices

Based on their positive attributes in terms of bacteriological status of the meat from individual and un-informed mixture of spices, three (3) of the pepper soup spices were selected (*T. tetraptera, Z. officinale and M. myristica*) and used in different combinations in the informed mixtures. The spices were used in ratio for formulating the inform spices as described in Table 2.

Experimental procedures

Similar to the experimental procedures in Phases 1 and 2 (Individual and un-informed spices respectively), three chicken carcasses were assigned to a treatment tagged; i, ii, iii, iv, v, vi and vii. Treatment i was the control. Thighs from the three carcasses were carefully separated and cooked with the informed spice mixtures. Five grams (5 g) from the informed spices was added to 1 kg of meat, along with 500 ml of water and cooked for 25 minutes. Bacteriological status of the meat was carried out as described in phases 2 and 3.

RESULTS

Isolated bacteria and bacterial load of broiler meat cooked with individual spices on day 3 and 6 of refrigerated storage

Table 3 shows the various isolated bacteria from the cooked and refrigerated meat samples on days 3 and 6 of refrigeration storage (*Staphylococcus aureus, Staphylococcus edidermidis, Salmonella sp, Serratia marcesces, Lactobasillus sp, Micrococcus luteus, Proteus vulgaris, Pseudomonas aureginosa and Bacillus sp*.). During the first three days of refrigeration of the cooked

Table 3. Isolated bacteria and bacterial load of broiler meat cooked with some spices used in Nigerian pepper soup on day 3 of refrigerated storage.

Spices	Isolated bacteria									Bacterial load (x10^{4}cfu/ml)
	S.aureus	*S. epidermidis*	*Salmonella sp.*	*Serratia marcesces*	*Lactobacillus sp.*	*M. luteus*	*Proteus vulgaris*	*P. aureginosa*	*Bacillus sp.*	
Control	+	-	+	-	+	-	-	-	-	4.00±0.00^{ab}
Tetrapleura tetraptera	+	-	+	-	+	-	-	-	-	3.00±1.16^{ab}
Zingiber officinale	+	-	+	-	+	-	-	-	-	4.00±1.53^{ab}
Allium sativum	-	-	-	-	-	-	-	-	-	0.00±0.00^{c}
Monodora myristica	-	-	-	-	-	-	-	-	-	0.00±0.00^{c}
Piper guineense	+	-	-	-	-	-	-	-	-	5.00±0.58^{a}
Xylopia aethiopica	+	-	-	-	+	-	-	-	-	2.00±1.00^{bc}
Aframomum melequeta	+	-	-	-	-	-	-	-	-	2.00±1.73^{bc}
Ocimum gratissimum	-	-	-	-	-	-	-	-	-	0.00±0.00^{c}
Ocimum basilicum	+	-	-	-	-	-	-	-	-	2.00±1.00^{bc}

Means, n=3. Means with different superscripts in the same column for bacterial load differed significantly ($p < 0.05$).
Key: - = Not present and + = Present.

Table 4. Isolated bacteria and bacterial load of broiler meat cooked with some spices used in Nigerian pepper soup on day 6 of refrigerated storage.

Spices	Isolated bacteria									Bacterial load (x10^{4}cfu/ml)
	S. aureus	*S. epidermidis*	*Salmonella sp.*	*Serratia marcesces*	*Lactobacillus sp*	*Micrococeus Leteus*	*Proteus vulgaris*	*Pseudomonas aureginose*	*Bacillus sp.*	
Control	+	-	+	+	+	+	+	-	-	162.00±10.97^{ab}
Tetrapleura tetraptera	+	-	+	+	+	-	-	-	-	68.00±2.29^{d}
Zingiber officinale	-	+	+	-	+	+	-	-	-	84.00±6.93^{d}
Allium sativum	+	-	+	-	-	+	-	+	+	28.67±4.33^{e}
Monodora myristica	+	-	+	-	-	-	+	-	-	24.67±0.88^{e}
Piper guineense	+	-	+	+	+	-	-	-	-	8.67±0.33^{f}
Xylopia aethiopica	+	-	+	+	+	-	-	+	-	168.67±2.60^{ab}
Aframmum melequeta	+	-	+	-	-	+	-	+	-	178.00±2.31^{a}
Ocimum gratissimum	+	-	+	-	+	-	-	+	+	157.67±9.52^{b}
Ocimum basilicum	-	-	+	+	-	+	+	-	-	127.67±2.03^{c}

Mean±SE, n=3. Means with different superscripts in the same column for bacterial load differed significantly ($p < 0.05$).
Key: - = Not present and + = Present.

meat, the spices significantly ($p<0.05$) influenced the bacterial load in the cooked meat samples kept for 3 days under refrigerated conditions (Table 3). No bacteria growth was detected in meat cooked with *M. monodora, P. gueneense* and *O. gratissimum.* However, the chicken meat cooked with *Piper gueneense* (5.00±0.58x10^4 cfu/ml) recorded the highest bacterial load followed by those cooked with *Zingiber officinale* (4.00±1.53x10^4 cfu/ml) and the control group (without spices) (4.00±0.00x10^4 cfu/ml).

Table 4 shows the isolated bacteria and bacterial

Table 5. Isolated bacteria and bacterial load of broiler meat cooked with un-informed mixture of some spices used in Nigerian pepper soup on day 3 of refrigerated storage.

Un-informed spice mixtures	Isolated bacteria									Bacterial load ($x10^4$cfu/ml)
	S. aureus	*S. epidermidis*	*Salmonella sp*	*Serratia marcesces*	*Lactobacillus sp*	*Micrococcus luteus*	*Proteus vulgaris*	*Pseudomonas aureginose*	*Bacillus sp*	
Control	-	-	+	-	-	-	-	-	-	2.00±0.20[a]
A	+	-	+	-	+	-	-	-	-	2.00±0.23[a]
B	-	-	-	-	-	-	-	-	-	0.00±0.00[b]
C	-	+	+	-	-	-	-	-	-	2.00±0.16[a]
D	+	-	-	-	-	-	-	-	-	2.00±0.01[a]
E	-	-	-	-	-	-	-	-	-	0.00±0.00[b]

Mean±SE, n=3. Means with different superscripts in the same column for bacterial load differed significantly ($p < 0.05$).
Key: - = Not Present and + = Present.

load of broiler meat cooked with individual spices on day 6 of refrigerated storage. Similar to the result obtained in day 3, the following bacteria were isolated; *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella sp., Serratia marcesces, Lactobacillus sp., Micrococcus leteus, Proteus vulgaris, Pseudomonas aureginose* and *Bacillus sp.* The *Piper gueneense, Monodora myristica*, *Allium sativum, Tetrapleura tetraptera* and *Zingiber officinale* significantly ($p<0.05$) reduced the bacterial load, when compared with other spices group. Meat cooked with *Aframmum melequeta* recorded the highest bacterial load of 178.00±2.31 cfu/ml but not significantly ($p>0.05$) different from values recorded for *X. aethiopica* and the control. However, meat cooked with *P. gueneense* had the least bacterial load ($8.67±0.33x10^4$ cfu/ml) followed by those cooked with *M. myristica* ($24.67±0.88x10^4$ cfu/ml), *A. Sativum* ($28.67±4.33x10^4$ cfu/ml), *T. tetraptera* ($68.00±2.29x10^4$ cfu/ml) and *Z. officinale* ($84.00±6.93x10^4$ cfu/ml) (Table 4).

Isolated bacteria and bacteria load of broiler meat cooked with un-informed mixture of spices on days 3 and 6 of refrigeration storage

The result of isolated bacteria and bacterial load of broiler meat cooked with un-informed mixture of spices used in Nigerian pepper soup on day 3 of refrigerated storage is presented in Table 5. The result shows some isolated bacteria like *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella sp, Serratia mascesces, Lactobacillus sp, Micrococcus luteus, Proteus vulgaris, Pseudomonas aureginosa,* and *Bacillus sp*. after 3 days of refrigeration in treatments A, C, D and F. However, no bacteria was found in meat cooked with treatments B and E showing that the used spices significantly ($p<0.05$) reduced the bacterial load. However, meat cooked with spice mixtures in treatments A, C, D and F (control) showed similar bacterial load ($2.00±0.23x10^4$ cfu/ml, 2.00±0.16 cfu/ml, $2.00±0.01x10^4$ cfu/ml and $2.00±0.20x10^4$ cfu/ml) respectively.

Table 6 depicts the isolated bacteria and bacterial load of broiler meat cooked with un-informed spices on day 6 of refrigerated storage. Similar to the isolated organisms in day 3, *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella sp, Serratia marcesces, Lactobacillus sp, Micrococcus Luteus, Proteus vulgaris, Pseudomonas aureginosa, and Bacillus sp.* were isolated. *Staphylococcus epidermidis* and *Serratia marcesces* were absent in all the samples in day 6. The presence of *Salmonella sp.* was noticed in all the meat samples. Similarly, all the samples tested positive to *Staphylococcus aureus* except sample B. The highest load of bacteria ($218.67±35.35 \times 10^4$ cfu/ml) was recorded in chicken meat cooked without spice mixtures (control) and the meat samples were significantly ($p<0.05$) influenced by the spice mixtures. Meat cooked with spice mixture in Treatment C recorded the least value of bacterial load ($13.50 ±0.87X\ 10^4$ cfu/ml).

Isolated bacteria and bacterial load of broiler meat cooked with informed mixture of spices in Nigerian pepper soup on day 3 and 6 of refrigerated storage

Isolated bacteria and bacterial load of broiler meat cooked with informed mixture of spices on 3 days of refrigerated storage is described in Table 7. The following bacteria were isolated from the meat cooked with the informed spice mixture; *Staphylococcus aureus, Micrococcus luteus, Salmonella sp, Serratia mascesces* and *Staphylococcus epidermidis. Staphylococcus aureus* was noticed in all the treatments. *Micrococcus luteus* was discovered in all the treatments except in Treatment III. *Salmonella sp.*

Table 6. Isolated bacteria and bacterial load of broiler meat cooked with un-informed mixture of somespices in Nigerian pepper soup on day 6 of refrigerated storage.

Un-informed Spice mixture	Isolated bacteria									Bacterial load (x10^{4}cfu/ml)
	S. aureus	*S. epidermidis*	*Salmonella sp*	*Serratia Marcesces*	*Lactobacillus sp*	*Micrococeus Leteus*	*Proteus vulgaris*	*Pseudomonas aureginose*	*Bacillus sp*	
Control	+	-	+	-	+	+	+	+	+	218.67±35.35^a
A	+	-	+	-	+	-	+	-	+	155.50±4.33^b
B	-	-	+	-	+	-	-	-	-	79.00±4.04^c
C	+	-	+	-	-	-	-	-	-	13.50±0.87^e
D	+	-	+	-	-	-	-	-	-	28.50±1.44^d
E	+	-	+	-	-	+	+	-	+	21.50±3.75^d

Mean±SE; n=3. Means with different superscripts in the same column for bacterial load differed significantly ($p < 0.05$).
Key: - = Not present and + = present.

Table 7. Isolated bacteria and bacterial load of broiler meat cooked with informed mixture of spices used in Nigerian pepper soup on day 3 of refrigerated storage.

Informed spice Mixture	Isolated bacteria					Bacterial load (x10^{4}cfu/ml)
	Staphylococcus aureus	*Micrococcus leteus*	*Salmonella SP*	*Serratia Marcesces*	*Staphylococcus epidermidis*	
I (control)	+	+	+	-	-	92.00±5.78^a
II	+	+	+	-	-	81.00±9.24^a
III	+	-	+	+	-	74.00±3.46^{ab}
IV	+	+	+	-	+	68.00±6.93^b
V	+	+	-	+	-	72.00±8.03^{ab}
VI	+	+	+	-	-	82.00±8.66^a
VII	+	+	+	-	+	74.00±2.89^{ab}

Mean±SE, n=3. Means with different superscripts in the same column for bacterial load differed significantly ($p < 0.05$) .
Key: - =Not Present and + = Present.

was absent in Treatment V. The presence of *Serratia marcesces* was discovered in samples from Treatments III and V, while Treatments IV and VII showed the presence of *Staphylococcus epidermidis*. The bacterial load in meat samples from Treatment V was least (72.00±8.08 cfu/ml), while meat from Treatment F (control) had the highest bacterial load (92.00±5.78 cfu/ml).

Table 8 shows isolated bacteria and bacterial load of broiler meat cooked with informed mixture of spices on day 6 of refrigerated storage. *Staphylococcus aureus* were discovered in all the samples. The absence of *Micrococcus luteus* and *Salmonella sp* were noticed in Treatments III and V respectively which were noticed in other treatments. Many more bacteria were discovered on day 6 of refrigerated storage as against what was observed in day 3, which were *Enterobacter aerogenes*, *Klebsiella sp., Proteus vulgaris* and *Pseudomonas aureginosa,* for example Treatments ii & vii had *Enterobacter aerogenes*, Treatments iii, iv & vi had *Klebsiella sp.,* Treatments i, v & vii had *Proteus vulgaris* and Treatments i, v &vi had *Pseudomonas aureginosa.* Similar to results from day 3, samples from treatment III recorded the least bacteria load (94±4.04 cfu/ml) while Treatment II had the highest bacteria loads (146.00±3.08 cfu/ml). However, there was no significant ($p>0.05$) difference between treatments I (control), V, VI and VII.

Table 8. Isolated bacteria and bacterial load of broiler meat cooked with informed mixture of spices used in Nigerian pepper soup on day 6 refrigerated storage.

Informed spice mixture	Isolated bacteria									Bacterial load (x10^{4}cfu/ml)
	S. aureus	*Micrococus Leteus*	*Salmonella sp*	*Serratia marcesces*	*Enterobacter aerogenes*	*Klebsiella sp*	*Proteus vulgaris*	*Staphylococcus epidermidis*	*Pseudomonas aureginosa*	
I (control)	+	+	+	-	-	-	+	-	+	141.00±4.62^a
II	+	+	+	-	+	-	-	-	-	146.00±3.08^a
III	+	-	+	+	-	+	-	-	-	94.00±4.04c
IV	+	+	+	-	-	+	$^+$	+	-	121.00±6.93b
V	+	+	-	+	-	-	+	-	+	136.00±3.48^{ab}
VI	+	+	+	-	-	+	-	-	+	142.00±6.35^a
VII	+	+	+	-	+	-	+	+	-	139.00±4.04^a

Mean±SE, n=3. Means with different superscripts in the same column for bacterial load differed significantly ($p < 0.05$).

The influence of the informed spice mixture in Treatment III brought about reduction in bacterial load (94.00 ± 4.04 cfu/ml).

DISCUSSION

Nigeria is under-nourished looking at the level of protein intake by the populace as yardstick (FAO, 2009). This trend was still observed in FAO (2014) report, noting that there is high percentage of under-nourishment prevalence among sub-sahara Africa populace. Poultry especially chickens have the potential to bridge the gap between supply and demand of animal protein in developing countries like Nigeria (Bashar et al., 2010). For this to be fully achieved, meat preservation will have to be brought into focus and one of the ways to achieve this is to look for an alternative source of meat preservation due to the irregular supply of electricity in Nigeria, which is the major means of powering refrigeration equipment. Renaud and Fisher (1997) defined meat quality as the measure of its palatability and acceptability to the consumers, and in order to achieve a very high level of acceptance in term of meat quality, a study of this nature seems relevant.

The efficacy of antimicrobial spices and their derivatives has been assessed against different type of microorganism (Gottardi et al., 2016). Basically, spices contain different bioactive elements present in variable amounts and can be grouped into volatile and non-volatile compounds. For instance, the saponins and tannins were reported to have antibiotics potentials in treating common pathogenic strains (Kubmarawa et al.,2007 and Gupta et al., 2009). In the current study, the isolated bacteria and bacterial load of broiler meat cooked with the individual pepper soup ingredients on day 3 of refrigerated storage showed the absence of *S. epidermidis, S. marcesces, M. luteus, P. vulgaris, P. aureginosa* and *Bacillus sp*. Noticeable quantities of *S. aureus, Salmonella sp, Lactobacillus sp* were identified. Meat cooked with *A. sativum*, *M. myristica* and *O. gratissimum* showed no presence of any bacteria which explains the antibacterial effects of these spices on the cooked meat samples as supported by Shelef (1983), who described sulphur containing compounds such as allicin, isolated from garlic oil (*Z. officinale*) as an inhibitor to the growth of both gram positive and gram negative bacteria. Similarly, quinone isolated from *Pergularia daemia* leaves was reported to effectively inhibit the growth of food-contaminating pathogens like *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* (Ignacimuthu et al., 2009). The inhibitory effects notice in the current study could be due to the bioactive constituent's ability to bind to the protein receptor and make substrate unavailable to the microorganism (Hintz et al., 2015). According to Tajkarimi et al. (2010) and Gottardi et al. (2016), spices has the potency to exert antimicrobial action by disallowing the growth of spoilage microorganism and or regulate the growth of pathogenic microorganism in food. For example, thymol, a terpenoid, exert their antimicrobial action by interacting with the membrane of the proteins, determining an accumulation of misfolded structures, or with the polar head-group region of the lipid layer, affecting the permeability (Hyldgaard et al., 2012; Marchese et al., 2016).

Isolated bacteria and bacterial loads of broiler meat cooked with individual spices on 6 days of refrigerated storage showed an increase in population of bacteria when compared with the quantity of bacterial load in day 3 of refrigerated

storage. This is supported by the findings of Kandeepan and Biswas (2005), who reported an increased bacterial growth in meat preserved with refrigeration for 7 days, confirming the earlier findings of Lawrie (1991) who attributed such result to the growth promoting effect of moisture on bacteria in meat preserved in chiller. Furthermore, Gill and Ahvenainen (2003) stated that spoilage bacteria will grow on meat that is not frozen at temperatures down to –3°C under both aerobic and anaerobic conditions. Hence, storage at chiller temperatures would not prevent the ultimate onset of microbial spoilage, but can delay the onset of spoilage. Therefore, the bacterial load which was highest in the control ($162.00\pm10.97x10^4$ cfu/ml) and subsequently reduced in other treatments could be due to the antibacterial properties of the spices used on the meat samples.

In conclusion, the spices used in this study helped in reducing the growth of spoilage microorganism and prolong the shelf life of the cooked meat. The spices like *Allium sativum, Monodora myristica,* and *Ocimum gratissimum* were seen to inhibit the growth of bacteria such as *S. aureus, S. epidermidis, Salmonella sp., Serratia marcesces, Lactobacillus sp., M. luteus, Proteus vulgaris, P. aureginosa* and *Bacillus sp*. in the batch subjected to 3 days refrigeration and also reduce the growth of the microorganism in day 6.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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