Quality of Meat and Sausage from Broiler Chickens Fed Dietary White and Cayenne Pepper Powders Subjected to Refrigeration Storage

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Abstract: Oxidative and microbial spoilage affect the nutritional quality of poultry products. White pepper (WP) and cayenne pepper (CP) powders possess antioxidative and antimicrobial properties, therefore, prompting an investigation into the activity of dietary peppers on proximate, oxidative and microbial profile of broiler chicken meat and sausage subjected to refrigeration storage. At the expiration of feeding 336 randomly allotted chickens seven diets (Control (C) and six other diets), fifty-six (56) birds (two per replicate) were selected and feed-fasted for 12 hours before slaughter. Post-slaughter, meat and sausage proximate were immediately analysed, but aseptically collected meat samples and raw sausage stored in sterile plastic bags were transferred to the Microbiology Laboratory of the Department of Veterinary Medicine and analysed for malondialdehyde (MDA) and microbial [*Escherichia* sp, *Salmonella* sp, *Enterococcus* sp, *Lactobacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp and *Saccharomyces* sp] assessments following standard laboratory procedure. Proximate composition of Meat and sausage were not influenced ($p>0.05$) by additives, but MDA and microbial counts were affected ($p<0.05$). C+250WP diet lowered meat MDA. Groups fed with WP diets were as effective as the Control against *Escherichia coli* and *E. faecalis*. *Salmonella typhimurium* was repressed by all groups excluding C+250WP and C+100WP+100CP. On day (D) 0, all pepper-fed groups had lower *Staphylococcus aureus* than the Control, while C+200CP had lower total bacterial count than the Control. C+200CP and C+250CP groups had no *Saccharomyces cerevisiae* like the Control, while Sausage from Control and C+250WP groups exhibited identical impact against *Saccharomyces cerevisiae* on D 28. Chicken sausage from the Control, C+200WP, C+200CP and C+250CP groups inhibited fungi growth.

Keywords: white + cayenne pepper powder; malondialdehyde count; microbial load; refrigeration storage, Meat quality, Sausage

INTRODUCTION

Consumption of safe products enhance healthy living especially when farm to fork culture is imbibed in the absence of extensive or elaborate storage facilities (Estevez, 2015). Food spoilage by microbial colonies involves many complex mechanisms that precede food rejection. Inadequate storage facilities in many developing countries contribute to microbial proliferation in meat and its products thereby contributing to food deterioration, illnesses and death in extreme cases (Adeyeye, 2017). Refrigeration storage is designed to suppress bacterial proliferation and extend storability, but a shortfall in constant cold storage with temperature kept between 35 and 40 ^{0}F translates into product spoilage within hours (Shaltout *et al*., 2016; Lorenzo, 2018). Considerable environmental and internal factors influence the potency of natural antimicrobials against microbial growth on meat and its products. The bacterial load, strain and biochemical composition of meat and meat products interact, thereby, influencing product during spoilage. Also, the diet fed birds, water activity, pH, and fat content of livestock products can either enhance or stifle microorganism proliferation (Siddig, 2012).

Food spoilage constitute a challenge to food security with economic consequences if ignored. Storage affect nutritional quality and definite precautionary and preventive measures must be employed to prevent spoilage during storage. At the point of purchase, meat customers or consumers are limited at detecting the extent of microbial invasion on the product post-slaughter, implying meat and meat products could have acceptable organoleptic properties, yet, be declared unsafe for consumption. (Moschopoulou *et al*., 2019). This has resulted in the application of natural preservatives, refrigeration storage and active packaging material to minimize spoilage in meat.

An analysis of chicken meat reveal it has similar cholesterol content as beef and pork, but in relation to its protein, total fat and calorie content, it offers superior nutrients (Marangoni *et al*. 2015). Sausages are one of the oldest products formulated from processed meat using minced meat, salt, spices and other seasoning ingredients (Jayawardana, 2015). An excellent mix of specific ingredients in appropriate quantities with a structured design and controlled process gives sought-after sausages.

Recent studies reveal meat quality can be improved by incorporating plant principles into animal diet (Delles *et al*., 2014). Botanical principles added are retained within the tissues, consequently impacting the shelf-life of livestock products (Martinez *et al*., 2006; Tazi-Safa *et al*., 2014). Pepper species such as *Piper nigrum* and *Capsicum frutescens* have been explored for their beneficial role in feed utilization in broiler chickens (Safa and El-Tazi *et al*., 2014; Adegoke *et al*., 2018), however limited information exists on the role white pepper (*Piper nigrum* in its ripe state) and cayenne pepper] powders and shelf-life of chicken meat and sausage. On this premise, this experiment was designed to investigate the quality of meat and sausage from broiler chickens fed dietary white and cayenne pepper powders subjected to refrigeration storage .

MATERIALS AND METHODS

Layout prior to experiment

Birds were selected for microbial assessment from the field layout comprising the Control (C) – Treatment (T) 1; T2 – Control diet + 200 g White Pepper (WP) (C+200WP); T3 – Control diet + 250 g White Pepper (C+250WP); T4 – Control diet + 200 g Cayenne Pepper (CP) (C+200CP); T5 – Control diet + 250 g Cayenne Pepper (CP) (C + 250CP); T6 – Control diet + 100 g WP + 100 g CP (C+100WP+100CP) and T7 – Control diet + 125 g WP + 125 g CP (C+125WP+125CP) groups.

Chickens were fasted for 12 hours prior to experiment. Twenty-eight broiler chickens (Cobb) weighing between 1.9 – 2.0 kg were slaughtered and dressed following ethical procedure outlined by the Animal Welfare Board of the College of Animal Production and Livestock Science, Federal University of Agriculture, Abeokuta (FUNAAB, 2013). Carcasses were chilled and breast muscles extracted aseptically. Ninety (90) grams of meat from the breast muscles was cut-out per replicate. Each sample was further divided into 3 portions of 30 g each.

Another twenty-eight broiler birds were slaughtered following procedure detailed above to prepare chicken sausage. Dressed carcasses were partitioned into primal cuts and meat from the breast muscles was cut-out to prepare chicken sausage. Other ingredients were added until 500 grams of sausage per batch was attained. Each batch was divided into four portions of hundred grams each; with each further subdivided into four portions. All samples were stored in the refrigerator at 4^{o} C ± 1 throughout the storage phase. Assessment was carried out on days 0, 7 and 14; as well as 0, 14 and 28 for meat and sausage respectively, while cut-portions were initially assessed immediately to obtain the microbial load (Ercolini *et al*., 2009; Ziomek *et al*., 2021).

Sausage preparation

Sausage formulated composed of meat – 48%, wheat flour – 25%, vegetable oil – 10%, *additives (spice and seasoning) – 2%, cold water – 13% and salt – 10%. * Additives comprise of white pepper – 0.3 g, cayenne pepper – 0.3 g, ginger – 0.25 g, turmeric – 0.90 g, coriander – 0.23 g and seasoning – 0.02 g. Prepared meat was run through a 5 mm plate in the grinder unit of Kenwood (Hampshire U. K) food processor. Subsequently, ingredients were added at defined portions, then uniformly mixed using the K-Beta. Lastly, wheat flour was added prior to stuffing and subsequent analysis.

Determination of proximate composition of chicken meat and sausage

Proximate composition of the Control diet, chicken meat and sausage were obtained following AOAC, (2005) method. The Control diet had 22.90% crude protein; 12.05% metabolizable energy; 3.80% crude fibre; 3.98% ether extract; 1.36% calcium; 0.58% Phosphorus; 1.61% lysine and 0.54% methionine.

Storage of samples

All labelled samples were wrapped in polythene bags and stored in the dark at refrigeration temperature of 4 ± 1 °C.

Thiobarbituric acid reactive substance (TBARs) value in chicken meat and sausage

Modification of procedure described by Witte *et al*. (1970) was employed. Two (2) grams of meat and sausage samples were separately weighed into test tubes, each uniformly homogenized with 4 ml of distilled water. Thereafter, centrifugation was performed at 2000 revolutions per minute for 10 mins after the addition of 5 ml of Trichloroacetic acid to the samples. Each sample after centrifugation was decanted into test tubes with thiobarbituric acid (TBA) added at 1:2 % into the test tubes. Mixture was boiled for 35 minutes into a curd. Afterwards, a Jenway 6305 spectrophotometer was used to obtain the concentration of absorbance of samples at 532 nm and reading expressed as mg malondialdehyde/kg sample.

Identification and Count of bacteria and fungi colonies on meat and sausage

Twenty-five (25) grams of meat and sausage were separately ground with 225 mL of sterile 0.1% sterilized buffer peptone water (Food and D. Administration, 2012) respectively. Homogenate sample was serially diluted in 9 ml of 0.1% sterilized buffer peptone water, then plated using standard pour plate method. Colonies of *Escherichia* sp*, Salmonella* sp*, Pseudomonas* sp *Enterococcus* sp. *Staphylococcus* sp, *Lactobacillus* sp and *Saccharomyces sp* were enumerated on Plate count agar (PCA, Himedia, India), *Salmonella-Shigella* agar (Hi-media, India), MacConkey agar (SRL) Mannitol Salt agar (Himedia, India), Man, Rogosa and Sharpe (MRS) Agar and Potato Dextrose agar (PDA, (Biolife, Italy). Diluted meat samples spread onto these plates were incubated at 37 °C for 24 - 48 hrs, excluding the detection of fungi, which was incubated at 25 °C for 5 days according to colour of colonies published by International Commission on Microbiological Specifications for Foods – ICMSF (2002) and El-Nash *et al.* (2015). All isolates were characterized on cultural and cellular morphology (Benjamin *et al.,* 2018), while identification of pure colonies of *P. aeruginosa*, *S. aureus, E. coli, E. faecalis*, *S. typhimurium, L. acidophilus, and S. cerevisiae* was carried out by biochemical tests (Methyl Red, Coagulase, Voges Proskauer, Oxidase, Catalase, Sulphide Motility test and molecular technique – PCR and NCBI Blast (Bergy *et al*., 1994; Forbes *et al*., 2007; Prathab and Lalitha, 2012). Calculations for colonies forming unit per gram was derived as follows:

$$\text{CFU} = \frac{colonies\ counted\ x\ \ dilution\ factor}{Amount\ plated}$$

Result obtained was recorded and expressed as mean Log_{10} CFU/g

Statistical analysis of data

Data obtained were analysed using SPSS version 22.0. One-way Analysis of Variance was employed for the analysis of proximate data while interactions were arranged in 2 x 7 and 3 x 7 factorial arrangements for TBARs and microbial counts assessments respectively, with p value < 0.05 declared significant at 95% of confidence level and comparisons of means performed at 95% confidence using Duncan Multiple Range Test of the same statistical package.

RESULTS

Proximate composition (%) of meat from chickens fed pepper powders

Proximate analysis of meat of birds fed dietary pepper powders is presented in Table 1.

Moisture, protein, fat, ash and crude fibre were not influenced ($p<0.05$), however, the Control group had highest numerical values for all parameters, excluding moisture, which was more in C+100WP+100CP group.

Proximate composition (%) of chicken sausage constituted from meat of chickens fed *Piper nigrum and Capsicum frutescens* powders fed as additives

Proximate analysis of sausage produced from meat of chickens fed dietary additives was documented in Table 2 with no significant effect ($p>0.05$) observed.

Interaction effect of dietary pepper powders and storage days on meat malondialdehyde value

Effect of interaction of dietary pepper powders and storage days (D) on meat malondialdehyde (MDA) value is presented in Table 3. Meat from chickens fed C+250CP diet on D 7 of refrigeration storage had the least ($p<0.05$) MDA value. Meat MDA of birds supplied the Control, C+200WP and C+200CP diets on D 7 was similar ($p>0.05$) as content of birds offered C+250WP diet on D 7 of storage. Highest MDA measured was present in groups offered C+200WP diet on D 14 of storage with identical count as meat of chickens offered C+250CP and C+100WP+100CP diets on D 14 of storage.

Interaction effect of additives (pepper powders) and storage days on sausage 2-thiobarbituric acid value

Combined effect of additives and storage days on chicken sausage formulated with the meat of birds fed dietary additives is presented in Table 4. Sausage MDA count was affected by additives and storage days. Sausage from birds fed with the Control diet had the overall least TBARs value as opposed to the highest amount in meat of birds fed C+200CP diet on D 28 days of refrigeration storage. All groups on D 14 had identical ($p>0.05$) count as the Control, except sausage from meat of birds fed C+200CP and C+100WP+100CP diets.

Combined influence of dietary pepper powders and refrigeration storage days on microbial count on chicken meat

Combined impact of additives and storage days on bacterial activity in refrigerated chicken meat is reported in Table 5. Meat *E. coli* count was reduced in meat from group fed C+250CP diet on D-0. Least *E. faecalis* prevalence was observed in meat of groups fed additives on D-0, though C+250CP and C+125WP+125CP count were comparable ($p>0.05$) as well as Control and C+250WP groups on D-7 of storage. Prevalence of *S. aureus* count on meat was suppressed ($p<0.05$) among group offered C+250WP diet on days 0 and 7 of refrigeration storage; C+125WP+125CP diet on D-7 as well as the Control group on D-14. Low ($p<0.05$) *L. acidophilus* count was recorded on meat of groups offered the Control and C+200WP diets; dietary additive combinations on D-0 of storage; C+200CP and C+200WP diets on D-7 of storage as well as the Control and C+200WP diet on D-14 of storage.

P. aeruginosa was more ($p<0.05$) widespread on meat of chickens fed C+200CP diet on D-14 of storage, followed by prevalence on meat from C+125WP+125CP group on D-14 of storage, then C+250CP and C+100WP+100CP groups on days 7 and 14 respectively. All other groups had least ($p<0.05$) count, except count on meat of chickens fed C+125WP+125CP diet that was similar ($p>0.05$) as groups with peak *P. aeruginosa* count. Meat *S. typhi* count was highest among birds supplied C+250WP diet on D-0 of storage, followed by count on meat from groups offered C+100WP+100CP on D-0, while other groups had least ($p<0.05$) *S. typhimurium* count. Meat from the Control group stored for 14 days had least ($p<0.05$) count. Effect of interaction of pepper powders and refrigeration storage days reveal *Saccharomyces* count was widespread in meat from birds fed C+200WP diet on D-0 than D-7, with both groups having increased count than C+100WP+100CP group on D-0, but others

had least ($p<0.05$) population across storage days.

Impact of interaction of dietary pepper powders and storage days on sausage microbial prevalence

Details of micro-organisms present in sausages subjected to the interactive influence of diet and storage days is documented in Table 6. *E. coli, E. faecalis*, *S. aureus, L. acidophilus*, *P. aeruginosa, S. typhimurium* and Total Bacterial Count (TBC) were influenced ($p<0.05$). *E. coli* count was highest on D-0 of storage for meat from chickens fed C+250WP diet.

No microbial count was documented for sausage from chickens offered the control and C+125WP+125CP diets on D-0; C+200WP and C+125WP+125CP diets on D-14 of storage as well as groups fed the Control, C+200WP, C+250WP, and C+100WP+100CP diets on D-28 of storage. No *E. faecalis* growth in sausage from meat of chickens fed the Control diet on Days 0 and 28; C+200WP diet on days 0 and 14; C+250WP diet on days 0 and 28; C+250CP diet on D-28, C+100WP+100CP diet on D-28 and C+100WP+100CP diet on D-0. *S. aureus* population was highest ($p<0.05$) on D-14 in sausage from meat of chickens fed the Control diet but higher in meat from birds supplied C+200CP diet at D-0 of storage. The latter had similar count as sausage from meat of chickens fed C+125WP+125CP diet on D-0 of storage but was higher than the microbe population in sausage from meat of chickens fed C+100WP+100CP diet on D-0 of storage; sausages from meat of chickens fed C+200WP, C+250CP and C+125WP+125CP diets on storage D-14 as well as sausages from chickens fed C+100WP+100CP diet on storage D-28 while no presence of *S. aureus* was documented for the remaining groups. *L. acidophilus* count was reduced ($p<0.05$) in sausage from meat of birds fed the Control and C+250WP diet on D-0 of storage as well as the Control, C+200WP, C+250WP and C+100WP+100CP groups on D-28 of storage. All sausages had no *P. aeruginosa* except those from meat of chickens offered C+200CP diet on D-0, the Control diet on D-14 and C+250CP diet on D-28 of storage. For *S. typhimurium,* C+200CP and C+100WP+100CP diets on D-0 had comparable ($p>0.05$) count as population on sausage from meat of birds supplied C+250CP diet on D-0 of storage and C+200WP diet on D-14 of storage. Sausage TBC reveal no growth ($p<0.05$) on sausage formulated from meat of birds fed the Control diet at days 0 and 28 as well as C+250WP diet stored for 28 days; followed by sausage from meat of birds offered C+100WP+100CP diet on D-14 of storage as well as C+200WP and C+100WP+100CP diets on D-28 of preservation. However, sausage from meat of C+250WP and C+250CP groups was highest for TBC. Sausage *S. cerevisiae* count was affected ($p<0.05$) by combined activity of additives (cayenne and white pepper powders) and storage days. Sausage formed from C+250WP group at the initial day as well as C+125WP+125CP group for all assessed days of refrigeration storage had higher ($p<0.05$) *S. cerevisiae* population than other groups that had no growth, except C+100WP+100CP on D-0 and C+250WP group on days 14 and 28 of refrigeration storage that had identical count as groups that had no microbial growth.

Table 1. Proximate composition (%) of meat from chickens fed *Capsicum frutescens and Piper nigrum* powders as additives

Parameters (%)	Control (C)	C+200WP	C+250WP	C+200CP	C+250CP	C+100WP +100CP	C+125WP +125CP	SEM
Moisture	71.99	73.53	79.23	72.98	76.27	78.05	73.16	4.41
Protein	18.12	17.14	13.67	17.81	15.58	14.38	17.33	0.94
CHO	4.04	4.11	3.01	3.60	3.35	3.00	4.20	0.32
Fat	1.72	1.52	1.13	1.61	1.38	1.16	1.56	0.11
Ash	3.41	3.08	2.46	3.32	2.84	2.68	3.11	0.20
Crude fibre	0.72	0.62	0.50	0.68	0.58	0.53	0.64	0.06

CHO – carbohydrate CP – cayenne pepper WP – white pepper

Table 2. Proximate composition (%) of chicken sausage constituted from meat of chickens fed *Piper nigrum* and *Capsicum frutescens* powders as additives

Parameters (%)	Control (C)	C+200WP	C+250WP	C+200CP	C+250CP	C+100WP +100CP	C+125WP +125CP	SEM
Moisture	46.83	52.86	53.53	48.64	54.21	49.55	50.40	2.36
C. P	29.11	26.68	26.10	27.70	25.49	27.48	26.98	1.80
CHO	14.51	12.58	12.76	14.79	13.26	14.27	14.21	1.20
Fat	2.91	2.42	2.31	2.74	2.26	2.68	2.54	0.16
C. F	1.20	1.04	0.96	1.15	0.92	1.12	1.10	0.10
Ash	5.41	4.42	4.34	4.98	3.86	4.90	4.77	0.30

WP – white pepper CP – Cayenne Pepper C. P – Crude Protein CHO – Carbohydrate C. F – Crude Fibre

Table 3. Interactive effect of dietary additives (white and cayenne powders) and storage days on meat thiobarbituric acid count

Diet	**Control**		**C+200WP**		**C+250WP**		**C+200CP**		**C+250CP**		**C+100WP+100CP**		**C+125WP+125CP**		**SEM**
Days	7	14	7	14	7	14	7	14	7	14	7	14	7	14	
TBARs (MDA/g tissue)	0.093^{gh}	0.200^{de}	0.089^{gh}	0.310^{a}	0.058^{h}	0.150^{ef}	0.087^{gh}	0.210^{cd}	0.130^{fg}	0.290^{ab}	0.150^{ef}	0.260^{abc}	0.151^{ef}	0.240^{bcd}	0.017

CP – Cayenne pepper powder WP- white pepper powder TBARs – 2-thiobarbituric acid reactive substance values MDA – malondialdehyde

Table 4. Effect of interaction of additives (pepper powders) and storage days on sausage malondialdehyde content

Diet	**Control**		**C+200WP**		**C+250WP**		**C+200CP**		**C+250CP**		**C+100WP+100CP**		**C+125WP+125CP**		**SEM**
Days	14	28	14	28	14	28	14	28	14	28	14	28	14	28	
TBARs (MDA/g tissue)	0.24^{e}	0.32^{de}	0.46^{bcde}	0.55^{abcd}	0.49^{bcde}	0.58^{abcd}	0.75^{ab}	0.80^{a}	0.45^{bcde}	0.60^{abcd}	0.57^{abcd}	0.70^{abc}	0.42^{cde}	0.63^{abcd}	0.017

$^{a, b, c, d, e}$ – Means on the same row with different superscripts differ significantly (p <0.05).

Table 5. Mean Log count of meat microbe subject to the influence of dietary pepper powders and refrigeration storage days

Diet	Storage Days	*E. coli*	*E. faecalis*	*St. aureus*	*L. acidophilus*	*P. aeruginosa*	*S. typhi*	TBC	*Sacc. cerevisiae*
					Mean $\log_{10}$ CFU/g				
Control	0	1.00^{efg}	0.00^{d}	3.70^{a}	0.00^{f}	0.00^{d}	0.00^{c}	4.50^{bcdef}	0^{d}
C+200WP		5.20^{a}	0.00^{d}	0.60^{def}	0.00^{f}	0.00^{d}	0.00^{c}	5.80^{abc}	0.74^{a}
C+250WP		4.00^{abc}	0.00^{d}	0.00^{f}	2.60^{ab}	0.00^{d}	2.73^{a}	9.00^{a}	0^{d}
C+200CP		1.00^{efg}	0.00^{d}	1.50^{bcd}	0.80^{ef}	0.00^{d}	0.40^{c}	3.70^{cdefg}	0^{d}
C+250CP		0.00^{g}	0.50^{cd}	1.00^{cdef}	3.40^{a}	0.03^{d}	0.00^{c}	5.00^{bcd}	0^{d}
C+100WP+100CP		5.50^{a}	0.00^{d}	0.50^{def}	0.20^{f}	0.00^{d}	1.60^{b}	7.80^{ab}	0.25^{c}
C+125WP+125CP		6.00^{a}	0.83^{cd}	1.00^{cdef}	0.00^{f}	0.00^{d}	0.70^{c}	8.50^{a}	0^{d}
Control	7	0.60^{fg}	0.00^{d}	0.30^{ef}	0.50^{ef}	0.00^{d}	0.00^{c}	1.40^{efg}	0^{d}
C+200WP		0.50^{fg}	0.20^{cd}	0.20^{ef}	0.20^{f}	0.00^{d}	0.20^{c}	1.20^{fg}	0.58^{b}
C+250WP		0.90^{efg}	0.00^{d}	0.00^{f}	0.30^{ef}	0.00^{d}	0.60^{c}	1.80^{defg}	0^{d}
C+200CP		1.10^{efg}	0.63^{cd}	0.30^{ef}	0.00^{f}	0.00^{d}	0.00^{c}	2.10^{defg}	0^{d}
C+250CP		1.60^{defg}	0.50^{cd}	$1.50b^{cd}$	0.50^{ef}	0.40^{b}	0.10^{c}	4.60^{bcde}	0^{d}
C+100WP+100CP		2.80^{bcd}	1.00^{c}	2.00^{bc}	2.10^{bc}	0.20^{c}	0.40^{c}	8.50^{a}	0.10^{d}
C+125WP+125CP		2.50^{cde}	3.20^{a}	0.00^{f}	2.00^{bcd}	0.00^{d}	0.00^{c}	7.70^{ab}	0^{d}
Control	14	0.40^{fg}	0.30^{cd}	0.00^{f}	0.00^{f}	0.00^{d}	0.30^{c}	0.70^{g}	0^{d}
C+200WP		0.60^{fg}	0.40^{cd}	0.50^{def}	0.00^{f}	0.20^{c}	0.40^{c}	1.47^{efg}	0.10^{d}
C+250WP		5.10^{a}	2.00^{b}	0.50^{def}	1.30^{cde}	0.00^{d}	0.10^{c}	7.00^{abc}	0^{d}
C+200CP		4.20^{ab}	0.50^{cd}	2.40^{b}	1.10^{cd}	0.60^{a}	0.20^{c}	6.33^{abc}	0^{d}
C+250CP		0.80^{fg}	0.30^{cd}	2.10^{b}	1.00^{ef}	0.00^{d}	0.20^{c}	4.20^{cdsf}	0^{d}
C+100WP+100CP		1.00^{efg}	2.00^{b}	1.10^{cde}	3.10^{a}	0.40^{b}	0.00^{c}	7.60^{ab}	0^{d}
C+125WP+125CP		1.80^{def}	2.00^{b}	0.20^{ef}	0.40^{ef}	0.50^{ab}	0.00^{c}	5.00^{bcd}	0^{d}
	SEM	0.51	0.26	0.31	0.32	0.05	0.22	1.02	0.04

$^{a, b, c, d, e}$ Means on the same row with different superscript differ significantly ($p<0.05$)

CP – Cayenne pepper powder WP- white pepper powder *Escherichia coli - E coli* *E – Enterococcus*

St. – Staphylococcus *L - Lactobacillus* *P - Pseudomonas* *S – Salmonella*

TBC – Total Bacteria Count CFU/g – Colonies forming unit per gram *Sacc – Saccromyces*

Table 6. Mean log count of microbes on sausage subject to interaction effect of dietary pepper powders and storage days

Diet	Storage Days	*Esc. coli*	*E. faecalis*	*St. aureus*	*L. acidophilus*	*P. aeruginosa*	*S. typhi*	TBC	*Sacc. cerevisiae*
		Mean $\log_{10}$ CFU/g							
Control	0	0.00^{g}	0.00^{c}	0.00^{d}	0.00^{e}	0.00^{b}	0.00^{d}	0.00^{g}	0^{b}
C+200WP		0.30^{cde}	0.00^{c}	0.00^{d}	0.20^{cde}	0.00^{b}	0.00^{d}	0.50^{ef}	0^{b}
C+250WP		1.20^{a}	0.00^{c}	0.00^{d}	0.00^{e}	0.00^{b}	0.50^{b}	1.70^{a}	0.58^{a}
C+200CP		0.20^{def}	0.20^{ab}	0.30^{b}	0.10^{de}	0.10^{a}	0.50^{a}	1.40^{ab}	0^{b}
C+250CP		0.80^{b}	0.20^{ab}	0.00^{d}	0.50^{b}	0.00^{b}	0.40^{ab}	1.70^{a}	0^{b}
C+100WP+100CP		0.40^{cd}	0.00^{c}	0.10^{cd}	0.30^{bcd}	0.00^{b}	0.00^{d}	0.80^{de}	0.15^{b}
C+125WP+125CP		0.00^{g}	0.20^{ab}	0.20^{bc}	0.40^{bc}	0.00^{b}	0.50^{a}	1.30^{bc}	0.89^{a}
Control	14	0.40^{cd}	0.10^{bc}	0.50^{a}	0.40^{bc}	0.10^{a}	0.10^{cd}	1.60^{ab}	0^{b}
C+200WP		0.00^{g}	0.00^{c}	0.10^{cd}	0.20^{cde}	0.00^{b}	0.30^{abc}	0.60^{ef}	0^{b}
C+250WP		0.20^{def}	0.10^{bc}	0.00^{d}	0.30^{bcd}	0.00^{b}	0.00^{d}	0.60^{ef}	0.20
C+200CP		0.40^{cd}	0.10^{bc}	0.00^{d}	0.40^{bc}	0.00^{b}	0.10^{cd}	1.00^{cd}	0^{b}
C+250CP		0.50^{c}	0.10^{bc}	0.10^{cd}	0.10^{de}	0.00^{b}	0.20^{bcd}	1.00^{cd}	0^{b}
C+100WP+100CP		0.10^{ef}	0.10^{bc}	0.00^{d}	0.20^{cde}	0.00^{b}	0.00^{d}	0.40^{f}	0^{b}
C+125WP+125CP		0.00^{g}	0.20^{ab}	0.10^{cd}	0.80^{a}	0.00^{b}	0.00^{d}	1.50^{ab}	0.70^{a}
Control	28	0.00^{g}	0.00^{c}	0.00^{d}	0.00^{e}	0.00^{b}	0.30^{d}	0.00^{g}	0^{b}
C+200WP		0.00^{g}	0.30^{a}	0.00^{d}	0.00^{e}	0.00^{b}	0.00^{d}	0.30^{f}	0^{b}
C+250WP		0.00^{g}	0.00^{c}	0.00^{d}	0.00^{e}	0.00^{b}	0.00^{d}	0.00^{g}	0.06^{b}
C+200CP		0.10^{ef}	0.20^{ab}	0.00^{d}	0.30^{bcd}	0.00^{b}	0.00^{d}	0.60^{ef}	0^{b}
C+250CP		0.30^{cde}	0.00^{c}	0.00^{d}	0.40^{bc}	0.10^{a}	0.00^{d}	0.80^{de}	0^{b}
C+100WP+100CP		0.00^{g}	0.20^{ab}	0.10^{cd}	0.00^{e}	0.00^{b}	0.10^{cd}	0.40^{f}	0^{b}
C+125WP+125CP		0.50^{c}	0.10^{bc}	0.00^{d}	0.20^{cde}	0.00^{b}	0.00^{d}	0.80^{de}	0.65^{a}
	SEM	0.08	0.06	0.04	0.07	0.02	0.07	0.11	0.11

$^{a, b, c, d, e, f, g}$ – means on the same row with different superscripts differ significantly (p <0.05).

CP – Cayenne pepper powder WP- white pepper powder *Escherichia coli – E. coli* *E - Enterococcus*
St. – Staphylococcus *L - Lactobacillus* *P - Pseudomonas* *S – Salmonella*
TBC – Total Bacteria Count CFU/g – Colonies forming unit per gram *Sacc – Saccromyces*

DISCUSSION

Meat from birds offered C+250WP had preferably lowered TBARs on D-7 of refrigeration storage (4 ^{o}C). Piperine – a bioactive alkaloid in white pepper protects against oxidative damage by inhibiting or quenching reactive oxygen species. Minimized count on D-7 was extended to D-14 signifying white pepper (*Piper nigrum*) effectively delayed off-odour formation owing to its richness in flavonoids (Martinez *et al.* (2006). Expectedly, TBARs increased as storage progressed, while combinations of peppers at both designated storage days did not suppress malondialdehyde values than the Control, though all groups had values lower than 1 mg malonaldehyde/kg per sample. Meat incorporated with botanical principles in white peppers encourages shelf-life extension and storability of refrigerated meat as antioxidative and antimicrobial principles of white pepper (Singh *et al*., 2013) deposited in meat positively correlates with preservation.

Control, C+200WP, C+250WP, C+250CP and C+125WP+125CP sausages with identical TBARs content on D-14 allude to the higher content of nutrient prone to oxidative spoilage, possibly the unsaturated fatty acids in sausages, but the Control group on D-28 had numerically highest fat proportion. Highly unsaturated phospholipid fractions in meat of birds supplied dietary antioxidants may have triggered loosely controlled peroxidation with texture modification, loss of essential fatty acids or formation of toxic compounds in products. This implies that meat from broiler chickens fed dietary botanical additives have timebound impact post-slaughter. Although sausage from meat of the Control group seemingly exhibit better storage potential, all sausages notably had less than 1 mg malonaldehyde/kg sample, thus inferring that the unsaturated fatty acids in sausages formulated from meat of birds fed additives should be harnessed prior to exhaustion of essential fatty acids, considering TBARs value when equal to 1 is the limit of off-odour perception (Djenane *et al*., 2002).

Interaction between additive and storage days affected bacterial species. Deposition of active substances of cayenne pepper in 250 g 100 kg^{-1} diet resulted in inhibitory activity against *E. coli* on D-0 of storage but not 200 g 100 Kg^{-1} diet. This result contradicts the report of Karleigh *et al.* (2017) on fractionated jalapeño pepper (*Capsicum annuum* var. *annuum*) extract, as further High-Performance Liquid Chromatography fractionation of jalapeno pepper and antibacterial analysis using different methods showed clear inhibition of *L. monocytogenes* but not *Escherichia coli* O157:H7 and *Salmonella enterica* growth. This imply that the incorporation of cayenne pepper at 250 g 100 kg^{-1} diet resulted in increased bactericidal activity. No *Staphylococcus* sp count was recorded on days 0 and 7, though minimal on D 14. A report on the activity of aqueous extract of black pepper and turmeric against *B. subtilis and Staphylococcus aureus* stated that black pepper did not exhibit antibacterial activity against *B. subtilis* but had inhibitory action against *Staphylococcus aureus* according to Ram and Prana, (2010). White pepper contains similar biochemical and bioactive constituents as black pepper and its inclusion at 250 g 100 kg^{-1} diet likely ruptured bacterial cells by plasmolysis induction, leading to subsequent metabolic dysfunction in cells prior to energy build up on days 0 and 7 of refrigeration storage. A systematic review by Takooree *et al.* (2019) revealed the antimicrobial impact of *Piper nigrum* against various pathogens via biofilm inhibition, bacterial efflux pumps, bacterial swarming, and swimming motility with similar inhibitory action observed against *Lactobacillus* (*L*) *sp* on meat from chickens supplied C+200WP diet on days 0 and 14. Noticeable from the data analysed for *L. acidophilus* on D-7 is the optimum potency both peppers exhibit above 200 g addition to the Control Diet. However, when storage days were extended up till D-14, the increased bactericidal potency was observed for the Control and C+200WP groups. *Lactobacillus* sp, precisely psychotropic

Lactobacillus is beneficial for meat preservation since it usually ensures that shelf-life is extended. When *L.* sp spoil meats, it occurs by souring, however other specific types of spoilage can exist. Some strains reportedly cause slime formation and greening on meats, and others may generate hydrogen sulphide during growth on vacuum-packaged beef (Silvina *et al*., 2010). Hence, considering the significance of *L.* sp, which may be positive or negative depending on the strain and count, its presence on meat in this study is noteworthy, though within acceptable limits. Feeding 250 g 100 kg^{-1} white pepper diet resulted in comparably better overall potency by positively inhibiting growth and proliferation of other microbes which may be attributed in part to the initial *L.* sp count on meat on D-0.

The efficacy of piperine on D 14 against *P. aeruginosa* for groups given 250 g 100 kg^{-1} white pepper diet agrees with the outcome declared by Careaga *et al.* (2003), whose study indicated that the antimicrobial activity of Capsicum extract on *P. aeruginosa* inoculated in minced beef was highly potent (bacteriostatic to drastic bactericidal effect) as concentration increased. Also, the bacteriostatic power of piperine against different micro-pathogens according to Aldaly (2010) and Shityakov (2019) affirm its inhibitory power at minimal dosage against *Pseudomonas* sp. For *S. typhimurium*, Careaga *et al.* (2003) investigation on capsicum extract on *S. typhimurium* inoculated in minced beef showed it exhibited minimal lethal effect as inclusion increased. In this study, cayenne pepper fed was incorporated up to 250 g, resulting in significant impact across all storage days. The impact of white pepper on meat *Salmonella typhimurium* population disagrees with the report published by Senthil Kumar (2012) – that white and black pepper at 0.5% inclusion had no deleterious action against *Salmonella typhimurium.* Such significance impacts the possibility of microbial borne poisoning. Meat *S. cerevisiae* count in this study was lower than the 0.1 – 2.9 CFU/g count declared by Ismail *et al.* (2000) on fresh and processed poultry product. *S. cerevisiae* count for all treatments contradicts the report of Ismail *et al.* (2000) that reported a yeast population greater than 1 CFU/g in raw chicken breast, wings, and ground chicken. A study by Veloso *et al.* (2014) reveal capsaicin exhibited fungicidal activity against seven isolates of five species of fungi. Further examination showed that the lateral chain of capsaicinoids had higher inhibitory activity than the phenolic part, with capsaicin and N-vanillylnonanamide conferring protection against pathogenic fungus. Similarly, a study by Genesis *et al.* (2018) showed that extracts and bioactive compounds of *Capsicum chinense* and *Piper nigrum* demonstrated optimal anti-aflatoxigenic activity at 150 µg/mL of extracts incorporation.

For sausage, the proliferation of *E. coli* may be attributed to nutrient availability and favourable nutrient profile of the product (Olaimat and Holley, 2012). Cellular adaptation to oxidative spoilage involves suppression of radical activity via decreased oxygen availability. This impact (decreases) enzymatic expressions needed for reactive oxygen or nitrogen species production (Surai *et al*., 2019). Meat *E. coli* population was likely affected by limited oxygen present in meat of birds offered the Control diet. That additive incorporation across all storage days did not show bactericidal or inhibitory effect on *E. coli* might have been as a result of the rich nutrient profile of the meat, supported by outcome of study of Karsha and Lakshmi, (2010). Inhibitory activity of *E. coli* in sausage of groups offered white pepper at 200 g 100 kg^{-1} signify bactericidal power of *Piper nigrum* against *E coli*, supported by findings published by Yona *et al.* (2013) that had minimum inhibitory concentration (MIC) of 10% documented for white pepper extract while the minimum bactericidal concentration (MBC) was declared at 12.5% against *Streptococcus mutans*).

Pepper powders lowered *E. faecalis* growth than the Control on D-14 of storage attesting to the efficacy of pepper containing bactericidal ingredients. Research conducted by Aldalhy (2010) and Sergey *et al*. (2019) identify piperine – a bioactive alkaloid as a potent inhibitor of *S. aureus*. Consumption of *S. aureus* may cause food poisoning if the species present on meat produce enterotoxins. In addition, it has been suggested that human handling of poultry meat may lead to the colonization of the skin of the meat leading to mucosae development (Kadariya *et al*. 2014) with consequence on health when consumed beyond tolerable levels. The Control group likewise had higher *S. aureus* count than other groups on D 0, likely indicating that bioactive components such as piperine and capsaicinoid were potent to suppress microbial growth. This is of significance especially in regions practicing farm to fork feeding system. This outcome agrees with Liu (2017), that piperine in black pepper is potent against positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*. Inhibition of *E. faecalis* is of importance to humans considering *Enterococci* can modify the consumer's digestive tract by acting as opportunistic pathogens and donors of antimicrobial resistance determinants to the endogenous microbiota (Lebreton *et al*., 2014).

Also, Capsaicinoids (Capsaicin, dihydrocapsaicin and nordihydrocapsaicin) are potent bioactive alkaloids in cayenne pepper that modify bacterial growth. Cayenne pepper powder in 200 g 100 kg^{-1} diet inhibited *S. typhimurium* proliferation across storage days than at 250 g 100 kg^{-1} inclusion. Optimum inclusion at 200 g 100 kg^{-1} on days 14 and 28 can be labelled as protagonist against *S. typhi* proliferation. Additionally, sausage from meat of birds given C+100CP+100WP additive inhibited *L.* sp growth on days 14 and 28 of refrigeration storage. Synergism between both pepper powders inhibited *L.* sp multiplication. Higher dosage of such combinations at 125:125 did not elicit the same response on D-28 of storage, suggesting optimum synergistic activity at defined levels (100:100) across observed days of storage. Piperine content in 200 and 250 g 100 kg^{-1} diets resulted in bactericidal action on *P. aeruginosa*. This finding disagrees with study reported by Karsha and Lakshmi (2010) where gram-negative bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli* were less susceptible to piperine in black pepper. Likely, higher piperine content in white pepper powder and its bactericidal potency over black pepper powder may be attributed to this response. Chicken sausage from groups fed the Control diet effectively stifled *S. typhi* growth on days 0 and 28. A similar outcome was reported in sausage from meat of chickens offered C+100WP+100CP diet on days 0 and 14. *Piper nigrum* can overcome microbial lipid membranes of bacterial strains at certain concentrations (Khan and Siddiqui, 2007 and Bryan *et al*., 2015) evidenced from all groups fed white pepper powders exhibiting bactericidal action in sausage on D-28. Highest Sausage *S. cerevisiae* count in C+125WP+125CP group across all storage days may be attributed to the high oxygen content in sausage, while no growth was observed for the Control group. Graeme and Graham (2016) explained that oxygen is absolutely essential as growth factor for membrane fatty acid (for example, oleic acid) and sterol (for example, ergosterol) biosynthesis in yeast, and it agrees with the outcome reported. On the contrary, the high ash or salt content in cayenne pepper likely repressed *S. cerevisiae* growth. Noticeably, fungicidal activity was noticed for sausage formulated with meat of birds offered 200 and 250 g cayenne pepper, but not C+100WP+100CP and C+125WP+125CP groups for all days of refrigeration storage. Across all storage days, yeast population in all treatment groups was lower than the 5.1log10 CFU/g declared for poultry products stored at 5 ^{o}C (Deak, 2001).

CONCLUSION

With no deleterious impact on proximate composition of meat and sausage of birds fed dietary white and cayenne pepper powders, it is safe to conclude that the incorporation of bio-active compounds in these peppers into animal tissues via diet can minimize oxidative spoilage and positively impact product quality against specific microbes, especially when certain days are targeted for meat and sausage respectively. On D-0, meat from all birds fed dietary pepper powders had lower *S. aureus* population than the Control, while C+200CP diet lowered total bacterial count than the Control. Meat of birds fed WP diets had similar effectiveness as the Control against *E. coli* and *E. faecalis* while *S. typhimurium* growth was effectively repressed by dietary applications excluding C+250WP and C+100WP+100CP diets. C+200CP and C+250CP diets exhibited fungicidal activity as the Control. Sausage from meat of birds given the Control and C+250WP diets exhibited identical microbial impact for all microbes assessed on D 28, with beneficial potential quality obtainable beyond D 28 of refrigeration storage.

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