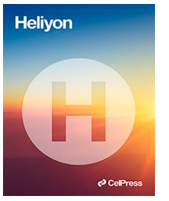
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Research article

Cholesterol oxides and quality attributes of NaCl-substituted low-fat chicken sausages prepared with different antioxidants

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ABSTRACT

This trial investigated how different salts and antioxidants influence cholesterol oxides, microbial profiles, physicochemical properties and organoleptic characteristics of low-fat chicken sausages (CS). CS were formulated with either 2 % NaCl, CS-1; 2 % NaCl +0.02 % butylated hydroxyanisole (BHA), CS-2; 1 % NaCl + 1 % KCl + 0.25 % onionskin extract (OSE), CS-3; 1 % NaCl + 1 % KCl + 0.5 % OSE, CS-4; 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE, CS-5 or 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE, CS-6, cooked, and refrigerated for 45 d. The Na content in CS-1 and CS-2 (1185 ± 21 mg/100 g) was greater than that in the other CS (640 ± 18 mg/100 g). The 19-hydroxy cholesterol, 7α-hydroxycholesterol, 25-hydroxycholesterol, 5,6β-epoxycholesterol, 7β-hydroxycholesterol and carbonyl content were greater in CS-1 than in the other sausages. The OSE-treated CS group had lower levels of 7β-hydroxycholesterol and 7α-hydroxycholesterol than did the CS-2 group. CS-1 and CS-2 were lighter than the other CS. Malondialdehyde, pH, chemical composition, textural profile, microbial counts, cook loss and sensorial quality were unaffected by additives. The partial replacement of NaCl with KCl and $K_3C_6H_5O_7$, along with the addition of BHA and OSE, decreased the Na and cholesterol oxide contents without affecting the organoleptic qualities of low-fat CS.

1. Introduction

NaCl is an essential ingredient in sausage [1,2], which is one of the most widely consumed meat products worldwide [3,4]. NaCl reduces water activity, works as a preservative, enhances flavor [5], contributes to juiciness and texture, stabilizes fat within the protein matrix and helps solubilize myofibrillar proteins [6]. However, hypertension and related cardiovascular disorders have been associated with excessive sodium intake [7–9]. A significant source of total dietary salt intake is processed meat [10,11]. Although lowering the salt content in meat products can enhance health, it can lead to poor product quality, impaired shelf life and, consequently, increased food waste [10,12,13]. Thus, salt reduction and its impact on the product quality, shelf life and acceptability of processed meat have been the subject of various investigations [14–18]. Fat is essential in the sensory and quality attributes of meat

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products [19]. However, fat-reduced meat products are gaining acceptance owing to their low cholesterol and saturated fatty acid contents, and their overall effects on human health [20]. Currently, little attention has been devoted to assessing the impact of NaCl replacement on the quality attributes of fat-reduced meat products.

A common strategy for lowering the Na content is to replace salt and Na-based additives with alternative ingredients that have a lower sodium content [2,21]. Although salt substitutes have great potential, they may have unfavorable side effects, such as bitter aftertaste and low solubility and stability [22]. While it is commonly known that the type and amount of salt can affect the extent to which meat products oxidatively deteriorate [22–25], little research has been conducted on how different types of salt can act as pro-oxidants when antioxidants are present.

Oxidative deterioration in meat products can lead to reduced nutritional value, poor sensory attributes, shorter shelf life, and potential health risks [26–28]. Muscle foods are rich in cholesterol, which can be oxidized to form cholesterol oxides depending on the processing and storage conditions [29,30]. Due to the possible harmful effects of cholesterol oxides on human health [31,32], efforts geared at preventing or limiting the formation of cholesterol oxides in meat products are crucial. Antioxidants, whether synthetic or natural, are commonly utilized to mitigate lipid oxidation and protein oxidation in meat products [33–37]. However, the ability of antioxidants to limit the formation of cholesterol oxides has been scantly investigated. Synthetic antioxidants like butylated hydroxytoluene, butylated hydroxyanisole (BHA), and propyl gallate can mitigate oxidative deterioration in meat products [33,34,38]. However, they may leave behind residues in meat products that could pose a risk to human health [39–41]. The growing antipathy among consumers and the stringent regulations surrounding the use of synthetic antioxidants have fueled the quest for suitable replacements [42–44].

Onion is one of the most popular vegetables in the world, and its output is steadily increasing [45,46]. As a result, a large amount of waste, especially onionskin, is produced and, if improperly disposed of, could cause environmental problems [47–49]. Onionskin contains numerous phytochemicals that exert antimicrobial and antioxidant effects [50–52]. The use of onionskin in food preservation could be a practical way to replace synthetic additives, whose use has recently raised concerns about world health, as well as an economical and environmentally responsible way to valorize onionskin waste [51]. We hypothesized that the partial replacement of NaCl with other salts would affect the Na content and oxidative deterioration, and the addition of antioxidants would mitigate the pro-oxidant capacity of salts in chicken sausages. Thus, this study examined how different salt varieties and antioxidant types affect the cholesterol oxides, physicochemical characteristics, microbiological profile, oxidative stability, and sensory properties of low-fat chicken sausages (CS).

2. Materials and methods

2.1. Extraction of onion skin, and determination of antioxidant properties

Red *Allium cepa* skins were obtained from a local market, sorted, air-dried for 72 h and milled into powder. The chemical composition of onion skin powder was determined using AOAC [53] methods. It was found to contain 7.45 % moisture, 0.42 % ether extract, 5.88 % crude protein, 3.23 % ash, and 7.23 % crude fiber. Pulverized onionskin (2 kg) was soaked in 10 L of 99 % ethanol for 5 d at 30 ± 2 °C. The mixture was centrifuged (6500×*g*) for 15 min, and the supernatant was evaporated in an automated rotary evaporator to obtain the concentrated onion skin extract (OSE), which was stored at −20 °C until use. The total phenolic content was quantified spectrophotometrically using Folin–Ciocalteu reagent as described by Singleton et al. [54]. The total flavonoid content was quantified using $AlCl_3$ as described by Slimestad et al. [55]. Radical scavenging activity was determined by DPPH [56]. The amount of quercetin and kaempferol in OSE was measured spectrophotometry, in accordance to the method of Pejic et al. [57] and Telange et al. [58] respectively.

Table 1
Ingredient composition of chicken sausage.

Ingredient (%)	Chicken sausage[1]					
	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6
Chicken Breast	90.00	90.00	90.00	90.00	90.00	90.00
Beef tallow	1.00	1.00	1.00	1.00	1.00	1.00
Corn starch	3.00	3.00	3.00	3.00	3.00	3.00
Piper nigrum powder	1.00	1.00	1.00	1.00	1.00	1.00
Capsicum frutescens powder	1.00	1.00	1.00	1.00	1.00	1.00
NaCl	2.00	2.00	1.00	1.00	1.00	1.00
KCl	–	–	1.00	1.00	–	–
$K_3C_6H_5O_7$	–	–	–	–	1.00	1.00
Onionskin extract (OSE)	–	–	0.25	0.50	0.25	0.50
Butylated Hydroxy anisole (BHA)	–	0.02	–	–	–	–
Cold water	2.00	2.00	1.75	1.50	1.75	1.50

CS-1, 2 % NaCl; CS-2, 2 % NaCl + 0.02 % BHA; CS-3, 1 % NaCl + 1 % KCl + 0.25 % OSE; CS-4, 1 % NaCl + 1 % KCl + 0.5 % OSE; CS-5, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE; CS-6, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE.

2.2. Preparation of chicken sausages

Six batches of chicken breast meat (5.4 ± 0.6 kg per batch) were used. Each batch constituted a replicate and was processed into sausage separately. Chicken breasts were deskinned, deboned and minced with a food processor (National MK5080M). Each batch of minced meat was divided into six portions and randomly mixed with ingredients containing 2 % NaCl, CS-1; 2 % NaCl +0.02 % BHA, CS-2; 1 % NaCl +1 % KCl +0.25 % OSE, CS-3; 1 % NaCl +1 % KCl +0.5 % OSE, CS-4; 1 % NaCl +1 % $K_3C_6H_5O_7$ + 0.25 % OSE, CS-5 or 1 % NaCl +1 % $K_3C_6H_5O_7$ + 0.5 % OSE, CS-6 (Table 1). The mixture was manually homogenized, held at 4 °C for 8 h and passed through a casing (19 mm Sausage Casings Skins Collagen (Tong master Seasonings SKU:TM00297) using a manual stuffer (Manual Sausage Maker with Funnel, X001B783QH, China). Chicken sausages were cooked in a water bath until the internal temperature reached 85 °C, cooled, packaged in Ziploc bags, and refrigerated (5 ± 1 °C) for 45 d. Each batch produced 120 sausages, each weighing 50 g. Within each batch, 20 sausages were allocated to each treatment. For each treatment, five sausages were randomly selected and analyzed during each storage period. Sodium content, cook loss and cholesterol oxides were assessed in day 1 chicken sausages, while pH, color, malondialdehyde, microbial profile, carbonyl, proximate composition, texture properties and sensory scores were assessed fortnightly for 45 days.

2.3. Determination of Na and chemical composition

Sodium content and proximate composition of CS were determined following the AOAC [53] procedures.

2.4. Determination of pH, color, cook loss, and textural profile

The pH of CS was measured with a pH meter (MW102, MILWAUKEE® instruments, Inc., NC, USA). A 1 g CS was homogenized with 5 mL of distilled H_2O after which the homogenate's pH was measured thrice. The CIE L^* a^* b^* color coordinates were measured with a colorimeter (WR-10, Shenzhen, China) having an 8 mm port size, a D_{65} illuminant and a 10° standard observer. Quadruplet readings were taken from different points on a CS and averaged. The percentage weight difference of the samples prior to and after cooking was used to calculate cook loss [59]. Textural profile of CS was measured with a texture analyzer (TA.HD plus®, Stable Micro Systems, Surrey, UK). Sliced CS samples were positioned in the middle of the platform, with the cut side up, and compressed at a speed of 5 mm/s using a 65-mm compression probe. The compression was applied with a 10-g trigger force and a 5-s recovery interval in between compressions to facilitate sample retrieval. Exponent Connect was used to analyze the force-time curve measurements to determine the texture parameters [60]. Gumminess = cohesiveness × hardness while chewiness = cohesiveness × springiness × hardness.

2.5. Determination of protein oxidation and lipid oxidation

The protein carbonyl content was assayed as per the procedure of Levine et al. [61]. Lipid oxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) as per the procedure of Varshney and Kale [62].

2.6. Analysis of cholesterol oxides

Chicken sausage (2 g) was homogenized with 4 mL of 50 % potassium hydroxide and 6 mL of ethanol. Then, the mixture was incubated for 22 h at 27 °C in the dark [63]. Following incubation, distilled H_2O (5 mL) and hexane (10 mL) were added to the sample, which was then vortexed. The hexane fraction was separated, and the 10 mL hexane-extraction was repeated thrice. A rotary evaporator was used to dry the solution. The residue was dissolved in 2.5 mL of hexane, transferred to a screw-top flask under nitrogen, and diluted with 0.5 mL of mobile phase (hexane:2-propanol (97:3, v/v)). The solution was filtered with a 22 μm filter (Millipore, Maryland, MD, USA). Cholesterol oxides were analyzed by HPLC (Shimadzu, Kyoto, Japan) equipped with a UV detector (SPD-10 AVVP). Separation was performed with a Nova Pack CN HP column (300 mm × 3.9 mm, 4 mm, Waters, Milford, MA, USA) at 32 °C. Flow rate of the mobile phase was 1 mL/min. Identification of cholesterol oxides was achieved by comparing the retention time and mass spectra of verified standards (Sigma-Aldrich, St. Louis, MO, USA) to the peaks corresponding to cholesterol oxides in the sample [18,63].

2.7. Microbial analysis

Microbial cultures and counts were performed according to AOAC [64] methods. A 9 mL of phosphate-buffered saline was put in a test tube containing 1 g of CS. The mixture was vortexed for 1 min and then serially diluted to 10^{-10}. Thereafter, sterile molten agar was added to a Petri dish that contained 1 mL of the mixture. The plates were gently rotated to ensure even mixing of the sample and agar. Total plate counts were conducted on plate count agar at 32 °C for 48 h, *Staphylococcus aureus* was cultured on mannitol salt agar at 37 °C for 48 h, coliforms were cultured on MacConkey agar at 32 °C for 48 h, and *Salmonella* spp. were cultured on *Salmonella Shigella* agar at 37 °C for 48 h.

2.8. Sensory analysis

The study was conducted as per the guidelines of the University of Ilorin Ethical Review Committee. Informed written consent was

gotten from the sensory panelists. Plastic containers with lids were coded with four-digit random numbers and contained 5 g samples of CS. The organoleptic properties of the CS were assessed using a 9-point hedonic scale, where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely [65]. Seventy-four assessors familiar with CS were recruited from the University of Ilorin students and faculty. The panel consisted of 34 males and 40 females, aged 19–48 years. Assessors were verbally recruited. One sensory evaluation session was done per storage period. Each assessor appraised six samples representing the six treatments per storage period. The same panelists assessed the CS samples throughout the storage period. Prior to the assessment, assessors were briefed on the sensory characteristics and evaluation protocols. Unsalted crackers and H_2O were provided to rinse the palate between samples.

2.9. Statistical analysis

A one-way ANOVA in a completely randomized design was used to analyze sodium, cholesterol oxide, and cook loss data. Treatments and batches were considered fixed and random effects respectively. The data for variables assessed at different time points were analyzed using repeated measures ANOVA with the PROC MIXED procedure in SAS. Additive, storage time, and their interaction were included as fixed effects, while batches and panelists (for sensory scores) were treated as random effects. Statistical significance was set at $P < 0.05$. When the F test indicated significance, the PDIFF option in SAS was used to compare least squares means.

3. Results and discussion

3.1. Antioxidant and Na contents

The OSE exhibited total phenolics, flavonoids, quercetin, kaempferol, and DPPH activity of 476.23 mg GAE/g, 245 mg QE/g, 1441.78 mg/g dry weight, 211.6 mg/g dry weight, and 87.24 %, respectively. These results are comparable to those reported for methanolic OSE [66] and ethanolic OSE [52] but exceeded those reported for aqueous OSE [34,36,52], most likely because a less polar solvent is more effective for the phytochemicals found in onion skins. *Piper nigrum* and *Capsicum frutescens*, included in the sausage formulation (Table 1), contain phytochemicals with potential antioxidant and antimicrobial properties [67,68] that may influence the sausage matrix. However, since they were added at the same levels in all sausage samples, they would not serve as a confounding factor in the current results.

The sodium content of CS containing different antioxidants and salts is presented in Fig. 1. Compared to the other sausages, CS-1 and CS-2 had greater ($P < 0.05$) Na contents. This result was anticipated due to the partial substitution of NaCl with KCl or $K_3C_6H_5O_7$. It is reasonable to confirm our hypothesis that various salts contribute differently to the Na content of CS. Overall, the Na content of the chicken sausage was reduced by almost 42 % as a result of the partial substitution of KCl or $K_3C_6H_5O_7$ for NaCl. This finding is imperative since it supports the current trend of reduced Na content in meat products. Likewise, the partial substitution of NaCl with KCl in chevon sausage reduced the Na content [23]. Moreover, partial substitution of NaCl with $CaCl_2$, KCl, and $MgCl_2$ reduced the Na content in low-fat Mortadella [14] and Italian salami [17].

3.2. Cholesterol oxides

Depending on the processing and storage conditions and meat type, cholesterols in meat products can oxidize to form cholesterol oxides [29,30], the consumption of which may impair human health [31]. Only five of the six cholesterol oxides found in this study were affected by the additives (Table 2). Compared to other sausage samples, the CS-1 sausages contained greater ($P < 0.05$) concentrations of 19-hydroxycholesterol, 7α-hydroxy cholesterol, 25-hydroxycholesterol, 7β-hydroxycholesterol, and 5,6β-epoxy cholesterol. The high NaCl level and absence of BHA or OSE in the sausage mixture may be contributing factors to the high cholesterol oxides in the CS-1 sausages. The balance of pro- and antioxidant compounds affects the oxidative stability of meat [28]. The

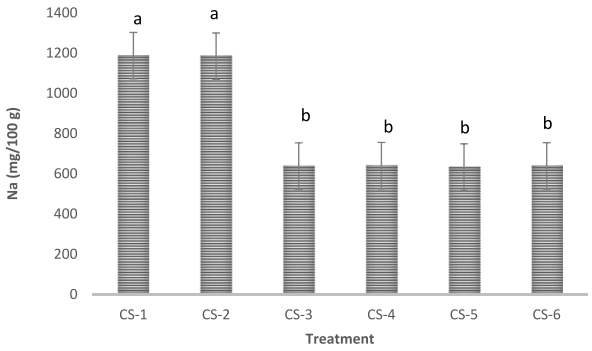


Fig. 1. Sodium content in chicken sausages containing different salts and antioxidants CS-1, 2 % NaCl; CS-2, 2 % NaCl +0.02 % BHA; CS-3, 1 % NaCl +1 % KCl +0.25 % onion skin extract (OSE); CS-4, 1 % NaCl + 1 % KCl + 0.5 % OSE; CS-5, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE; CS-6, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE.

Table 2
Cholesterol oxides in chicken sausages containing different salts and antioxidants.

Additives	Cholesterol oxides (μg/100 g)							
	7-ketocholesterol	6β-epoxide	19-hydroxy cholesterol	cholesta-3,5-dien-7-one	5,6β-epoxycholesterol	25-hydroxy cholesterol	7α-hydroxy cholesterol	7β- hydroxy cholesterol
CS-1	4.23	ND	36.32[a]	ND	5.23[a]	11.28[a]	1.52[a]	5.63[a]
CS-2	3.68	ND	10.30[b]	ND	1.23[b]	2.67[b]	0.97[b]	3.50[b]
CS-3	3.70	ND	9.56[b]	ND	1.20[b]	0.92[c]	0.62[c]	1.05[c]
CS-4	3.75	ND	11.20[b]	ND	1.21[b]	1.98[b]	0.53[c]	1.04[c]
CS-5	3.71	ND	10.44[b]	ND	1.23[b]	0.02[d]	0.51[c]	1.06[c]
CS-6	3.73	ND	11.00[b]	ND	1.22[b]	1.26[bc]	0.54[c]	1.05[c]
SEM	0.60		3.32		1.20	0.54	0.19	0.42
P value	0.09		0.024		0.023	0.045	0.029	0.024

[a, b, c] means bearing different superscripts in a column differ significantly ($P < 0.05$). CS-1, 2 % NaCl; CS-2, 2 % NaCl +0.02 % BHA; CS-3, 1 % NaCl +1 % KCl +0.25 % onionskin extract (OSE); CS-4, 1 % NaCl + 1 % KCl + 0.5 % OSE; CS-5, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE; CS-6, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE. ND, not detected; SEM, standard error of mean.

pro-oxidant action of NaCl may be due to several mechanisms, including its ability to reduce cell membrane integrity, permitting oxidizing agents easier access to lipid substrates [69], facilitate the release of iron ions from iron-containing molecules like heme proteins [70], and inhibit the activity of antioxidant enzymes [71,72]. Nonetheless, this trend needs to be investigated in high-fat CS.

Our hypothesis that the addition of antioxidants may reduce the pro-oxidant behavior of salts is supported by the decrease in cholesterol oxides in the antioxidant-treated CS. Our findings imply that OSE, owing to its phenolic and flavonoid contents, demonstrated antioxidant potential comparable to that of BHA. Similarly, adding 0.1 % dry sage leaves to chicken breast patties containing 0.5 % NaCl successfully decreased the amount of cholesterol oxides [73]. Antioxidants prevent oxidation, scavenge free radicals, and chelate metal ions to reduce cholesterol oxides in meat [43,73]. Together, these mechanisms aid in preserving the meat's cholesterol content and reducing the production of cholesterol oxides.

KCl- and $K_3C_6H_5O_7$-supplemented CS had lower concentrations of 7α-hydroxycholesterol and 7β-hydroxycholesterol than BHA-supplemented sausages. Meat oxidative status is largely influenced by the balance of pro- and antioxidant compounds [28]. Chloride ions are the main contributors to the pro-oxidant properties of salts because of either the reactive action of Cl^- on substrates or the solubilization of Fe by Cl^-. NaCl and KCl consist of approximately 60 and 48 % chloride ions, respectively, while $K_3C_6H_5O_7$ is devoid of Cl^-. Thus, the reduced Cl^- may explain the lower 7α-hydroxycholesterol and 7β-hydroxycholesterol in the KCl- and $K_3C_6H_5O_7$-treated CS. A similar reduction in cholesterol oxides was observed in beef jerky formulated with KCl and $K_3C_6H_5O_7$ [15,18]. It was surprising that despite the higher concentration of polyphenols and flavonoids in the CS treated with 0.5 % OSE, their 25-hydroxycholesterol contents were higher than those of their counterparts treated with 0.25 % OSE. These results indicate that no additional benefit was derived in treating CS with 0.5 % OSE over those treated with 0.25 % OSE. It appears that at 0.5 %, OSE exert prooxidant property. Depending on their dosage and the nature of the surrounding molecules, antioxidants can have prooxidant effects in specific situations [74]. Under certain conditions, antioxidants that are capable of reacting with molecular oxygen and are reducing agents can exert prooxidant effects [75,76]. They can produce superoxide radicals and dismutase to hydrogen peroxide under aerobic conditions [75]. For instance, when a reduced metal is present, flavonoids can act as prooxidants [75]. The concentration of 25-hydroxycholesterol in chicken sausages was influenced by the type of salt used, with the CS-5 sausages showing the lowest values. This finding underscores the antioxidant potential of $K_3C_6H_5O_7$, particularly in sausages formulated with 0.25 % OSE. It also suggests a synergistic interaction between $K_3C_6H_5O_7$ and the OSE concentration. Previous studies have highlighted the antioxidant properties of $K_3C_6H_5O_7$ in reducing cholesterol oxides in honey-treated beef jerky [15] and *Capsicum annuum*-treated beef jerky [18].

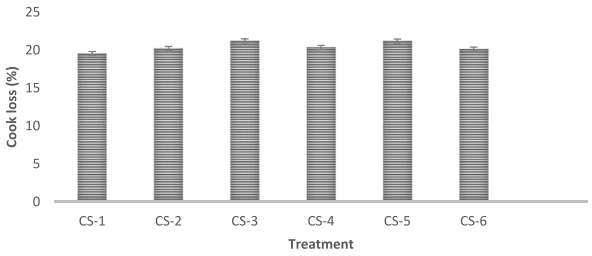


Fig. 2. Cook loss (%) in chicken sausages containing different salts and antioxidants CS-1, 2 % NaCl; CS-2, 2 % NaCl + 0.02 % BHA; CS-3, 1 % NaCl + 1 % KCl + 0.25 % onion skin extract (OSE); CS-4, 1 % NaCl + 1 % KCl + 0.5 % OSE; CS-5, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE; CS-6, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE.

3.3. Cook loss, chemical composition and textural profile

Cook loss is a key determinant of the water-holding capacity (WHC) of meat products, and it influences their quality, texture, juiciness, and overall eating experience. Reduced NaCl levels can result in decreased myofibrillar protein solubility and ionic strength, which in turn reduces gel strength and water holding capacity [77,78]. The salt and antioxidant contents did not influence the cooking loss of CS (Fig. 2). This result implies that the WHC of CS was not jeopardized by the 50 % decrease in NaCl. This result also indicates the potential of KCl and $K_3C_6H_5O_7$ as a replacement for NaCl in CS. In line with our findings, the substitution of KCl with 20 or 40 % NaCl did not affect the WHC of emulsion-type CS [79]. Furthermore, the WHC of CS was unaffected by the partial replacement (25 %) of NaCl with KCl, $K_3C_6H_5O_7$, or $C_3K_5O_3$ [4].

Assessing the chemical composition of sausage can provide valuable insights into its quality, safety, and nutritional value. No significant alterations were observed in the chemical composition of CS treated with different salts and antioxidants over the 45-d chill storage period (Table 3). The same cook loss between treatments, which implies similar water loss and, thus, similar chemical composition, may be responsible for this result. Consistent with the present findings, the ether extract, moisture content, and crude protein content of chicken nuggets were unaffected by the partial substitution of 20 or 40 % NaCl with KCl [80].

One of the most crucial sensory aspects of emulsified meat products is texture. In this study, no discernible change was found between the treatments with regard to the textural qualities (Table 3). This result was expected given the similarities in cook loss, chemical composition and pH, which can impart moisture retention and texture. These findings concur with those of earlier studies in which the partial replacement of NaCl with KCl [4,79,80], $K_3C_6H_5O_7$, and $C_3K_5O_3$ [4] did not affect the textural profile of CS. The textural profile of CS remained stable throughout the 45-day chill storage. Similarly, 30 days of chill storage had no influence on the textural profile of low-fat Bologna sausages [81] or cooked pork sausages [82].

3.4. Lipid oxidation and protein oxidation

The effects of antioxidants and salt types on the TBARS values of chicken sausages was not significant (Table 4). Considering the antioxidant properties of onionskin [34,36,52] and BHA [35,41,83], this finding was surprising. The lower fat content of the CS and the addition of 1 % beef tallow, which has a high proportion of saturated fatty acids may be responsible for this observation. This trend needs to be investigated in high-fat CS. Consistent with our findings, the TBARS value of low-fat Mortadella [14], dry-cured ham [25], chevon sausage [23], and pork sausage [24] were unaffected by partial substitution of NaCl with $CaCl_2$, KCl, and $MgCl_2$. Furthermore, in dry-cured bacon, malondialdehyde concentrations were not affected by substituting 40 % NaCl with KCl, but the malondialdehyde content increased when 70 % NaCl was substituted with KCl [16]. The lower carbonyl content in the antioxidant-supplemented CS may indicate the reduction in peptide scission and side chain oxidation of amino acid residues [84]. As expected, the oxidative stability of CS decreased during refrigerated storage, as indicated by the increase in MDA and carbonyl content. However, the MDA and carbonyl contents were all within the safe range for sausages. One important finding from our study was that during the course of the 45-day chill storage period, the malondialdehyde level ranged from 0.19 to 0.36 mg MDA/kg, which is less than the 0.6 mg MDA/kg threshold that produces an objectionable flavor in meat products [85].

Table 3
Chemical composition and textural profile of chicken sausages containing different salts and antioxidants.

Factors		Chemical composition (%)					Texture profile			
		Moisture	Crude protein	Ether extract	Ash	Hardness (kg)	Cohesiveness	Springiness (cm)	Gumminess (kg)	Chewiness (kg)
Additive (A)	CS-1	60.68	27.13	6.13	5.11	0.28	1.00	1.86	0.28	0.53
	CS-2	60.66	27.33	6.23	5.06	0.28	1.00	1.85	0.27	0.52
	CS-3	61.00	26.83	6.45	5.08	0.27	0.99	1.86	0.28	0.50
	CS-4	61.25	27.16	6.06	5.04	0.28	0.99	1.88	0.28	0.50
	CS-5	60.67	27.42	6.24	5.06	0.27	0.99	1.86	0.27	0.50
	CS-6	60.83	27.16	6.23	5.08	0.28	1.02	1.87	0.29	0.53
	SEM	2.45	0.35	0.52	0.34	0.09	0.03	0.06	0.04	0.03
	P value	0.353	0.479	0.552	0.205	0.940	0.914	0.524	0.131	0.142
Storage day (S)	1	60.24	27.33	6.28	5.33	0.28	1.01	1.88	0.28	0.53
	15	61.05	27.06	6.16	5.20	0.27	0.99	1.86	0.28	0.52
	30	61.11	27.05	6.13	5.25	0.28	1.00	1.85	0.27	0.50
	45	61.00	27.27	6.12	5.18	0.28	0.99	1.86	0.27	0.50
	SEM	0.58	0.56	0.12	0.21	0.02	0.04	0.03	0.02	0.05
	P Value	0.103	0.495	0.213	0.004	0.856	0.628	0.091	0.106	0.156
A × S	P value	0.126	0.852	0.857	0.446	0.926	0.912	0.057	0.507	0.133

[a, b, c] means bearing different superscripts in a column differ significantly ($P < 0.05$). CS-1, 2 % NaCl; CS-2, 2 % NaCl +0.02 % BHA; CS-3, 1 % NaCl +1 % KCl +0.25 % onion skin extract (OSE); CS-4, 1 % NaCl + 1 % KCl + 0.5 % OSE; CS-5, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE; CS-6, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE. SEM, standard error of mean.

Table 4
Oxidative stability and microbial profile of chicken sausages containing different salts and antioxidants.

Factors		Oxidative status		Microbe (log CFU/g)			
		Carbonyl (mmol/mg protein)	TBARS (mg MDA/kg)	TPC	Coliform	*Salmonella* spp	*Staphylococcus aureus*
Additive (A)	CS-1	0.52[a]	0.28	3.00	ND	ND	ND
	CS-2	0.45[b]	0.26	3.48	ND	ND	ND
	CS-3	0.45[b]	0.23	3.18	ND	ND	ND
	CS-4	0.45[b]	0.25	3.12	ND	ND	ND
	CS-5	0.45[b]	0.27	3.22	ND	ND	ND
	CS-6	0.46[b]	0.26	3.04	ND	ND	ND
	SEM	0.01	0.01	0.15			
	P value	0.021	0.052	0.305			
Storage day (S)	1	0.34[c]	0.19[c]	1.65[b]	ND	ND	ND
	15	0.41[b]	0.24[b]	3.58[a]	ND	ND	ND
	30	0.46[a]	0.23[b]	3.28[a]	ND	ND	ND
	45	0.47[a]	0.36[a]	3.22[a]	ND	ND	ND
	SEM	0.01	0.01	0.12			
	P Value	<0.0001	<0.0001	<0.0001			
A × S	P value	0.590	0.191	0.107			

[a, b, c] means bearing different superscripts in a column differ significantly ($P < 0.05$). CS-1, 2 % NaCl; CS-2, 2 % NaCl +0.02 % BHA; CS-3, 1 % NaCl +1 % KCl +0.25 % onion skin extract (OSE); CS-4, 1 % NaCl + 1 % KCl + 0.5 % OSE; CS-5, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE; CS-6, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE. CFU, Colony forming unit. TPC, Total plate count. ND, Not detected. SEM, standard error of mean.

3.5. Microbial profile

The microbial profile of meat products is of concern because certain microorganisms can cause foodborne illnesses if consumed. Safeguarding the microbial safety of meat products is critical for thwarting economic losses, protecting public health, complying with regulations, maintaining consumer confidence, and facilitating international trade [86,87]. The total plate count was not influenced ($P > 0.05$) by the additives (Table 4). This finding is consistent with data from other authors, which showed that salt type had no influence on microbial counts in dry-cured ham [88,89] or pork sausages [24]. During storage, no coliform bacteria, *Salmonella* spp. or *Staphylococcus aureus* were found in the CS (Table 4). The absence of these microorganisms could suggest that the CS was produced with sufficient heat treatment and hygiene. Likewise, throughout 15 days of chill storage, beef burgers treated with BHT, aqueous OSE or no additive did not contain any coliform, *Salmonella* spp., or *Staphylococcus aureus* [34]. Moreover, coliform bacteria were absent from low-fat CS that was refrigerated for 50 days [90]. The lower microbial counts on day 1 suggest successful thermal processing that rendered the majority of the bacteria inactive.

3.6. Color and pH

The color of meat products is imperative for assessing freshness, quality, safety, and cooking indications [91]. In addition, it plays a significant role in consumer perception. The OSE-treated sausages had lower lightness ($P < 0.05$) than the CS-1 and CS-2 sausages (Table 5). This observation was probably due to the red color of the OSE. Consistently, the addition of aqueous OSE reduced the

Table 5
Color and pH of chicken sausages containing different salts and antioxidants.

Factors		Lightness (L*)	Redness (a*)	Yellowness (b*)	pH
Additives (A)	CS-1	45.58[a]	4.49	15.19	6.43
	CS-2	44.34[a]	4.26	16.02	6.42
	CS-3	39.19[b]	4.29	15.02	6.38
	CS-4	37.48[b]	4.33	17.08	6.35
	CS-5	39.51[b]	4.63	16.91	6.43
	CS-6	38.13[b]	4.50	15.32	6.42
	SEM	1.94	0.36	1.24	0.02
	P value	0.019	0.973	0.753	0.110
Storage days (S)	1	44.89[a]	9.19[a]	17.49[a]	6.42
	15	43.47[a]	8.45[a]	18.14[a]	6.37
	30	41.03[a]	6.47[b]	14.43[b]	6.43
	45	33.47[b]	6.45[c]	13.63[b]	6.41
	SEM	1.59	0.29	1.01	0.02
	P value	<0.0001	<0.0001	0.008	0.181
A × S	P value	0.565	0.533	0.814	0.387

[a, b, c] means bearing different superscripts in a column differ significantly ($P < 0.05$). CS-1, 2 % NaCl; CS-2, 2 % NaCl +0.02 % BHA; CS-3, 1 % NaCl +1 % KCl +0.25 % onion skin extract (OSE); CS-4, 1 % NaCl + 1 % KCl + 0.5 % OSE; CS-5, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE; CS-6, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE. SEM, standard error of mean.

lightness of beef patties [37]. However, the redness and yellowness of CS were not influenced by the additives. The lightness of CS remained stable for the first 30 days of chilled storage and subsequently decreased ($P < 0.05$). Yellowness and redness of the CS did not change during the first 15 days of chilled storage but declined thereafter ($P < 0.05$). The changes in color coordinates could be attributed to alterations in the antioxidant-prooxidant status of the sausage samples during cold storage. A prominent mechanism is the prooxidant-induced oxidation of myoglobin to form metmyoglobin [37]. Similarly, the lightness, redness, and yellowness of beef patties decreased during cold storage [37]. The additive-storage interaction was insignificant for the color coordinates of the CS.

pH plays a crucial role in the texture, shelf life, flavor, color, and appearance of CS [90]. Controlling and monitoring the pH is indispensable to ensure the desired quality features of the final product. The pH of CS did not differ among the additives (Table 5). Similarly, the incorporation of BHT and OSE did not affect the pH of beef burgers [34]. Furthermore, da Silva Araujo et al. [23] reported that the partial replacement of NaCl with KCl did not influence the pH of chevon sausage. Chill storage had no effect on the pH of CS ($P > 0.05$). This is in tandem with the findings of Andres et al. [90], in which the pH of low-fat CS remained stable throughout 50 days of chilled storage.

3.7. Organoleptic properties

The sensory assessment of meat products has wide-ranging implications for consumer acceptance, product development and marketing, ultimately enhancing the competitiveness and overall success of meat products [92]. Additives and chill storage period did not influence ($P > 0.05$) the sensory attributes of CS (Table 6). The interaction effect between treatment and storage time was not significant. These findings indicate that the substitution of NaCl with KCl or $K_3C_6H_5O_7$ and the addition of BHA and OSE to chicken sausages did not impair the sensory attributes. Similarly, the salt type did not affect the sensorial quality of chevon sausages [23]. Bedrníček et al. [50] found no differences in the organoleptic properties of pork parties treated with water-extracted OSE. Moreover, the incorporation of BHT and OSE did not affect the sensory attributes of beef burgers [34].

4. Conclusion

The partial substitution of NaCl with $K_3C_6H_5O_7$ or KCl decreased the Na content in chicken sausage. The addition of BHA and OSE reduced the concentration of cholesterol oxides and carbonyl in the chicken sausage. Compared with BHA-treated sausages, OSE-treated chicken sausages had lower concentrations of 7α-hydroxycholesterol, 7β-hydroxycholesterol. In addition, chicken sausages treated with 0.25 % OSE had lower concentrations of 25-hydroxycholesterol than those treated with 0.5 % OSE. The salt type and antioxidant did not affect lipid oxidation, pH, total plate count, chemical composition, cooking loss, or sensory attributes of the chicken sausages. KCl and $K_3C_6H_5O_7$ could be used to replace 50 % of NaCl in chicken sausages. The 0.25 % OSE could be a potential substitute for BHA in chicken sausages. Further studies should be conducted to examine the impact of the total substitution of NaCl with other salts in the presence of antioxidants on the quality of low-fat chicken sausages. A limitation of this study is the absence of proximate composition data for the OSE. While the primary focus was on its phytochemical constituents and antioxidant potential, detailed proximate analysis of the extract could provide additional insights into its functional contributions beyond oxidative stability. Future research should include a comprehensive chemical characterization of the extract to better understand its role as a functional ingredient and its potential influence on the nutritional profile of meat products.

CRediT authorship contribution statement

Kazeem D. Adeyemi: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Olaife S. Olatunji:** Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis. **Olubunmi Atolani:** Resources, Methodology, Investigation. **Hakeem Ishola:** Resources, Methodology, Investigation. **Rafiat M. Shittu:** Resources, Methodology, Investigation. **Kehinde M. Okukpe:** Resources, Methodology, Investigation. **Victoria O. Chimezie:** Resources, Methodology, Investigation. **Muinat O. Kazeem:** Resources, Methodology, Investigation.

Data availability

Data will be made available on request.

Ethical approval and consent to participate

The study was conducted as per the guidelines of the University of Ilorin Ethical Review Committee. Informed consent was obtained from the sensory panelists.

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Table 6
Sensory attributes of chicken sausages containing different salts and antioxidants.

Factors		Sensory attributes					
		Taste	Aroma	Texture	Color	Juiciness	Overall acceptability
Additives (A)	CS-1	6	6	6	6	5	6
	CS-2	6	6	6	6	5	6
	CS-3	5	5	6	6	5	6
	CS-4	6	5	6	6	5	6
	CS-5	6	6	6	6	5	6
	CS-6	6	6	6	5	5	6
	SEM	0.29	0.27	0.29	0.29	0.30	0.28
	P value	0.530	0.376	0.790	0.397	0.831	0.648
Storage days (S)	1	5	6	6	5	5	6
	15	6	6	6	6	5	6
	30	6	5	6	6	5	6
	45	6	6	6	6	6	6
	SEM	0.20	0.19	0.20	0.20	0.22	0.20
	P value	0.198	0.307	0.669	0.152	0.405	0.140
A x S	P value	0.171	0.726	0.997	0.658	0.826	0.851

CS-1, 2 % NaCl; CS-2, 2 % NaCl + 0.02 % BHA; CS-3, 1 % NaCl + 1 % KCl + 0.25 % onion skin extract (OSE); CS-4, 1 % NaCl + 1 % KCl + 0.5 % OSE; CS-5, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.25 % OSE; CS-6, 1 % NaCl + 1 % $K_3C_6H_5O_7$ + 0.5 % OSE. SEM, standard error of mean. Sensory scores: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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