EFFECT OF MARINATION WITH ROSELLE (HIBISCUS SABDARIFFA L.) CALYX EXTRACT AND PRESERVATION ON QUALITY OF CHICKEN TIKKA

ISAIAH A. OKERE¹

¹ Agriculture Value Addition Programme, Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, P.M.B. 5029, Ibadan, Nigeria, Tel: +234 8105275274, URL: https://iart.gov.ng/IT/

*Corresponding author: okereisaiah@gmail.com (Isaiah Annayochukwu Okere)

Abstract: The study evaluated roselle calyx extract (RCE) on quality and preservation of chicken tikka marinated by soaking meat in four concentrations of RCE excluding water only (P1) and 0.01% butylated hydroxyl anisole - BHA (P2); prepared by hot water extraction at 100°C for 10 min using 5 (P3), 10 (P4), 15 (P5) and 20 (P6) grams roselle-calyx/L. Product-yield (%), lightness-L*, redness-a*, yellowness-b* and organoleptic properties were assessed on freshly-prepared tikka and production cost were analysed. Water holding capacity - WHC (%), pH, total bacterial count - TBC and mould count - MC (log_{10} cfu/g) and lipid-oxidation (mg MDA/kg) were determined on days 0, 3, 6 and 9 of storage in refrigeration conditions $(4\pm1^{\circ}C)$. Product-yield ranged from 54.63 \pm 0.03 (P1) to 67.70 \pm 0.06 (P6). Treatments exhibited significant variations for L*, a*, b* while non-significant variations for production cost. Tikka of P6 for flavour (5.60±0.03), texture (7.20±0.01), tenderness (7.15±0.04), juiciness (6.60±0.10) were higher (p<0.05) than in P1-P3. On day 9, there were non-significant variations for WHC, pH, TBC and MC but lipid-oxidation of 0.45 ± 0.01 (P6) and 0.39 ± 0.06 (P2) though similar (p>0.05) were significantly lower than 1.31 ± 0.02 (P1), 0.70 ± 0.03 (P3), 0.65 ± 0.06 (P4) and 0.56 ± 0.01 (P5). Thus, extracts of 20 g rosellecalyx/L used for marinating chicken tikka conferred better quality and inhibited lipid oxidation during storage.

Keywords: Antioxidant, antimicrobial, chicken patties, lipid oxidation, plant extract, quality attributes

Introduction

Chicken meat is essential source of protein in human nutrition being reckoned as white meat type having unique natural endearment of bioactive compounds such as reduced low-density lipoprotein-LDL cholesterol content when compared to the red meat types (Pogorzelska-

Nowicka et al., 2018). Consumption of chicken meat has no cultural and religious restrictions and as such chicken products are in high demand globally (Naveena *et al.*, 2013; Hayat *et al.*, 2023).

Meeting the high demand for chicken products requires that products be in a form that is acceptable, tasty, safe and sustainably fit for consumption (Markoni et al., 2023; Fonti-Furnols, 2023).

Chicken tikka is one of the chicken products that have met consumers acceptability and satisfaction ever since it was innovatively developed. However, like other processed meat and meat products, chicken tikka faces the problem of oxidative changes during storage. This is due to the high content of polyunsaturated fatty acid present in chicken meat making it very prone to lipid oxidation which has negative impact on the shelf life (Huang and Ahn, 2019). Also, lipid oxidation in meat and meat products leads to reduced organoleptic, physicochemical and microbiological quality attributes (Dominguez et al., 2019). In an effort to mitigate lipid oxidation in meat and meat product, the use of synthetic antioxidants such as butylated hydroxylanisole (BHA), butylated hydroxyl toluene (BHT) and tertia butyl hydroxyl quinone (TBHQ) have been in use in the meat industry. However, there are worries about their use in meat and meat products because of health implications such as tumor promoters, lung damage, impaired blood clothing and the formation of malondialdehydes (MDA) leading to pathological changes in the digestive tract, increases peroxides and cholesterol in the blood stream as well as decreases the nutritive value of meat (Amaral et al., 2018). Thus, interventions aimed at reducing lipid oxidation while mitigating losses in the nutritive value of frozen-stored meat products using extracts from plants have been employed (Hes and Michalowska, 2016). Conversely, to curb the worries about the use of synthetic antioxidants, several studies have unveiled the potentials of natural sources of antioxidants especially from botanicals such as ginger, clove, garlic (Shah, 2014). One of such botanicals as source of natural antioxidant is the roselle calyx which possesses high content of ascorbic acid (vitamin C) and which have been reported by Islam et al., (2016). Also, researchers have reported some of the main active antioxidant compounds such as sadderetine, hisbscertine, gossypetine and flavonoids (Wong et al., 2002, Fasoyiro et al., 2005) and minerals-vitamins assays reveals that the calyx contains calcium, iron, niacin and riboflavin in rich endearment (Babalola et al., 2001).

Although a natural source of antioxidants, such as ginger, clove, garlic, pepper, rosemary, essential oils etc (Manessis *et al.*, 2020) are used in the preparation of most meat products, their use as marinades have been found to be more effective in improving the shelf life, organoleptic, physicochemical and microbiological quality attributes of meat and meat products (Kittisakulnam et al., 2016). It is on this premise that the meat pieces used in chicken tikka need to be marinated to fortify tikka against oxidative changes and microbial growth while maintaining better meat quality traits. There are different methods of marination that have been explored in the meat industry; such as injection, immersion/soaking and tumbling (Alvarado and McKee, 2007). It was reported that the immersion method of marination improved the physicochemical properties of meat products such as the pH, water holding capacity and product yield while maintaining sensory and nutritional quality (Gamage *et al.*, 2017). The positive role of antioxidants and marination in mitigating lipid oxidation and microbial growth in meat and meat products is of immense benefit to the processors and consumers. Therefore, the aim of this study was to evaluate the potential of roselle calyx extract in improving meat quality and preservation by retarding oxidative changes and microbial activities while enhancing sensory and technological properties (pH and water holding capacity) in chicken tikka.

Materials and Method

Chicken breast meat experimental treatments

Twelve breast meat (fillets) from mature chicken broiler were obtained from supermarket. Each of the fillets were cut into 16 slices of approximately the same weight (20 g) and size $(5 \text{ cm x } 3)$ cm x 1 cm) and were immersed in 1 L of water only (P1), 0.01% Butylated hydroxyl-anisole-BHA (P2) and roselle calyx aqueous extracts (grams of roselle calyx RC/L) at 5 (P3), 10 (P4), 15 (P5) and 20 g RC/L (P6) respectively for 15 min. The 4 different concentrations of the roselle calyx were prepared using hot water extraction method (Chumsri *et al.*, 2008). The six (6) sample groups of marinated chicken tikka are as follows:

 $P1 =$ Chicken tikka non-marinated without extract treatment

P2 = Chicken tikka marinated with 0.01% butylated hydroxyl anisole-BHA

- $P3 =$ Chicken tikka marinated with 5 g RC/L
- $P4 =$ Chicken tikka marinated with 10 g RC/L
- $P5 =$ Chicken tikka marinated with 15 g RC/L

 $P6 =$ Chicken tikka marinated with 20 g RC/L

Each of the sample groups were then further processed using formulation as shown in Table 1.

Product development and assessment

Chicken Tikka: The marinated boneless chicken breast consisting of 16 slices of approximately the same weight and size from each of the six treatments were mixed with spices (red chilli paste, powdered nutmeg, green pepper) and lard. The chicken pieces/slices and green capsicum pieces (used here to provide fine aroma) were stringed alternatively into skewers. The skewed chicken slices were placed into Air-fryer at 200°C for 20 min until the chicken slices were golden brown. The formulation of the experimental chicken tikka is shown in Table 1. The chicken tikka sample groups were kept for 9 days at $4\pm1^{\circ}$ C.

The assessment of the chicken tikka from each of the treatment sample groups were based on the following parameters (a) proximate composition, (b) physical attributes [cooking loss, Product yield, objective colour reading using Minolta chroma: lightness-L*, redness-a* and yellownessb*] and cost of production (iii) physicochemical properties: pH and water holding capacity (WHC), (iv) Organoleptic/ sensory qualities (v) lipid oxidation and (vi) microbiological assessment [TPC and MC in coliform unit per gram (cfu/g) of meat samples] at day 0, 3, 6 and 9 at 4 ± 1 ^oC storage.

	Sample groups						
Ingredients	P ₁	P ₂	P ₃	P4	P ₅	P6	
Breast muscle	75.00	75.00	75.00	75.00	75.00	75.00	
¹ Binder	10.00	10.00	10.00	10.00	10.00	10.00	
2 Fat	8.00	8.00	8.00	8.00	8.00	8.00	
3 Spices	4.00	4.00	4.00	4.00	4.00	4.00	
Monosodium	1.00	1.00	1.00	1.00	1.00	1.00	
glutamate							
Salt	2.00	2.00	2.00	2.00	2.00	2.00	
Total	100	100	100	100	100	100	

Table 1. Formulation of experimental chicken tikka

RC/L: Roselle calyx per liter of hot H₂O. BHA: butylated hydoxylanisole. ¹Binder: Corn flour (100%), ²Fat: Lard, ³Spices: Red chilli paste (20%), green pepper (30%), powdered nutmeg (20%), lemon juice (30%).

Proximate composition of chicken tikka

Chicken tikka samples from each of the 6 sample groups were assessed for moisture content, crude protein, crude fat and ash content.

The moisture content was assessed by weighing 10 g of tikka into a silica-dish using the procedures outlined by AOAC (2000). Afterward the weighed tikka samples at $100-105^{\circ}$ C oven temperature were dried for 24 h to a constant weight. Tikka samples from each of the 6 sample groups were cooled for 10 min. After cooling chicken tikka were reweighed and subsequently, the moisture content obtained in percentages:

$$
Percentage\ Moisture = \frac{Tikka\ weight\ before\ drying\ (g) - Tikka\ weight\ after\ drying\ (g)}{Tikka\ weight\ before\ drying\ (g)} \times 100
$$

The crude protein was assessed using 10 g of ground tikka in line with Kjeldahl method as outlined by AOAC (2000). The distillate gotten was titrated with 0.01N HCl. The derived crude protein was calculated by the conversion of nitrogen (%N) content of tikka obtained when titrated with a constant (6.25). Hence, it was expressed as (6.25 x %N).

The crude fat or ether ester was assayed in 10 g of tikka using a Soxhlet extractor with petroleum ether as solvent using both the method described by Heinz and Hautzinger (2007) and procedures outlined by AOAC (2000). The apparatus containing the solvent was heated over a bursen burner and with a siphoning movement taking place averagely 9 times in the flask. The oil released and flask itself were weighed to achieve a constant weight; the flask was dried in a preheated oven. The percentage of crude fat or ether ester was deduced from this formula:

$$
Percentage\,Crude\, Fat = \frac{Oil\, weight\, (g)}{Tikka\, weight\, (g)}\, x100
$$

The Ash content was assayed from 10 g of ground tikka in crucibles, which was placed into a muffle furnace set between $550 - 600^{\circ}$ C for 4 h using both the method described by Heinz and Hautzinger (2007) and procedures outlined by AOAC (2000). The crucibles and their contents were cooled in a desiccator at about $(270^{\circ}C)$ and reweighed.

$$
Percentage\text{ }Ash = \frac{Ash\text{ weight } (g)}{Tikka\text{ weight } (g)}\text{ }x100
$$

Physico-chemical properties and production cost of chicken tikka

The cooking loss was evaluated using freshly processed chicken tikka which was weighed before being air-fried in an air fryer for 20 min at 200° C to achieve an internal temperature of about 72^oC doneness and cooled to room temperature. The weight of the tikka after cooling was measured and recorded. The cooking loss was obtained by deducting the weight of tikka after cooking from the weight of tikka before cooking. Percentage cook loss was calculated as expressed below:

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Tikka weight before frying (g) – Tikka weight after frying

Tikka weight before frying (g) Percentage Cooking loss =

The tikka yield was obtained following the formula used by El-Nashi *et al.*, 2015. This was expressed as: Percentage Chicken Tikka Yield = (100% - % Cooking loss in Tikka).

Colour of tikka: The objective colour reading using Konica Minolta Chroma Meter CR-400 (Mainland, China) was employed to assay lightness (L^*) , redness (a^*) and yellowness (b^*) for chicken tikka from each of the 6-treatments.

Tikka pH: The pH is one the physicochemical attributes which measures the acidity and alkalinity of the tikka using the pH meter with a buffer of 4.

Water Holding Capacity (WHC) was assessed using press method adopted after Mallikarjuman and Mittal (1994). Meat samples of 10 g from chicken tikka samples of the six treatments were each placed between two previously weighed Whatman filter papers and pressed between two 10.20 x 10.20 cm² plexi-glasses using a vice for 60 sec. Weight of wet filter paper was taken and water holding capacity of meat samples were deduced as expressed:

Percentage Water Holding Capacity

Weight of wet paper (g) – Weight of dry paper (g)
Weight of tikka sample (g)

The production cost of the chicken tikka produced based on each treatment (i.e. chicken tikka marinated with non-roselle calyx extract (0g RC/L and 0.01% BHA) and roselle calyx extract (5, 10, 15 and 20g RC/L) were calculated in naira per kilogram $(\frac{N}{kg})$ of tikka. The percentage gain for each treatment of the chicken tikka were calculated from the formula and expressed in percentages as stated below:

$$
Percentage\ Gain = \frac{Product\ yield\ (g) - Cooking\ loss\ (g)}{Production\ cost} \times 100
$$

Sensorial properties/Organoleptic Evaluation

Thirty panellists were semi-trained and were tasked with evaluating the tikka samples independent of one another for colour, flavour, taste, texture, tenderness, juiciness and overall acceptability. Thus, the chicken tikka samples from each of the 6 treatments were coded and served separately in a clean saucer to the panellist who were distributed randomly to each treatment. Tikka samples were rated by panellists for colour, flavour, taste, juiciness, texture, tenderness and overall acceptability according to the method of AMSA (2015).

Table 2. Nine-point hedonic scale

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Lipid oxidative changes in chicken tikka

The changes in lipid oxidation were monitored in chicken tikka samples from each of the 6 treatments at day 0, 3, 6 and 9 of refrigeration storage. Tikka samples were assayed for lipid oxidations using 2-thiobarbituric acid (TBA). At each sampling day 10 g of tikka sample were taken twice from each treatment for values of thiobarbituric acid reactive substance (TBARS) using the methods of Gray and Monahan, (1992) and Adeyemi et al., (2013) with some modification. The 10 g of tikka sample from each of the 6 treatments was mixed with 25% Trichloroacetic acid-TCA and 20 mL of deionized water. Thereafter the mixture was homogenized for 120 sec and thoroughly filtered using Whatman filter paper. The filtrate was mixed with an equal volume of 0.02 M TBA at 100ºC for 35 min. The filtrate obtained was cooled under a running tap for 10 minutes. At the wavelength of 532 mm spectrophotometer; solution of the absorbance was measured and TBARS was deduced from reading from spectrophotometer at 532 mm x 7.8 expressed in milligram malondialdehyde per kilogram (mg MDA/kg).

Microbiological quality assessment of chicken tikka

Total bacterial count or the total bacterial load of the chicken tikka: This was assayed using Nutrient Agar as culture medium for the overall bacterial load on the tikka samples from each treatment. The growths were evaluated at day 0 on freshly chicken tikka each from the 6 treatments. Thereafter, further assessment was carried out on day 3, 6 and 9 of refrigeration storage. Homogenization of 10 g of the tikka samples from each of the 6 treatments were carried out using 90 mL of 0.1% (w/v) peptone (manufacturer, country) for 60 secs at room temperature in a blender. The appropriate prepared serial dilution was performed in 0.1% (w/v) peptone water solution. A spread on Petri plate was done using a 1mL of the mixture of each sample and was incubated at 37 ºC for 24 h as used by Heinz and Hautzinger, 2007. All colonies that appeared afterward were counted and expressed as colonies forming unit per gram (cfu/g).

The mould count of the chicken tikka was assayed using the selective culture medium of Potatoes Dextrose Agar to determine the mould count on the tikka sample from each of the 6 treatments assessed on day 3, 6 and 9 in the refrigeration storage. Chicken tikka samples of 10 g each from the 6 treatments were homogenized in 90 mL of 0.1% (w/v) peptone for 1 minute at room temperature using a blender . Serial dilution was prepared in 0.1% (W/V) peptone water in appropriation. The spread method of Heinz and Hautzinger (2007) using 1mL of mixture of each sample on petri plates was carried out and thereafter incubated for 24 hrs at 37° C. The mould colonies were counted and reported as colonies forming unit per gram (cfu/g).

Results and discussion

The proximate composition of chicken tikka in terms of moisture content (MC), crude protein (CP) and ash content (AC) concentration's levels from 0 to 20 g RC/L marinated chicken tikka ranged from 32.52 to 35.18% (MC), 22.76 to 22.81% (CP), 13.41 to 14.58% (AC) and had a direct proportional relationship to the concentration levels of the roselle calyx extract as shown in Table 3. Implying that MC, CP and AC increases as the concentration levels increases from 0 to 20 g RC/L used in marinating the chicken tikka. The MC of chicken tikka marinated in roselle calyx of $5 - 20$ g RC/L extracts had a range from 0.03 to 2.21% higher than that of non-roselle calyx (0g RC/L) marinated chicken tikka. Also, the MC chicken tikka marinated in roselle calyx of $5 - 20$ g RC/L extracts had a range from 0.26 to 2.66% higher than that of chicken tikka treated with 0.01% BHA. This percentage differences in range values for the moisture content could have been implicated by the higher water holding capacity (WHC) of 67.45 to 80.78% in

the roselle calyx marinated chicken tikka $(5 – 20 g RC/L)$ than the WHC of 56.10 to 70.59% for the chicken tikka treated with no extract (0g RC/L) and the WHC of 76.31 to 86.97% for chicken tikka treated with 0.01% BHA during the 9 days of cold $(4\pm1\degree C)$ storage.

Similarly, the CP of chicken tikka marinated in roselle calyx of $5 - 20$ g RC/L extracts had a range from 5.65 to 6.05% higher than that of non-roselle calyx (0 g RC/L) marinated chicken tikka. Also, the CP of chicken tikka marinated in roselle calyx of $5 - 20$ g RC/L extracts had a range from 0.40 to 0.80% higher than that of chicken tikka treated with 0.01% BHA. These differences in percentage crude protein content could have contributed to higher product yield that ranges from 64.21 to 67.70% in chicken tikka marinated with 5 to 20 g RC/L extracts than those of 0g RC/L (54.63%) and 0.01% BHA (55.44%) treated chicken tikka. Thus, this harmonizes with the report made by Gamage et al., 2017 that product yield improves at a higher crude protein content and that at a higher concentration of extracts used in meat marination.

The crude fat (CF) content of chicken tikka marinated with 5, 10, 15 and 20 g RC/L decreases as the level of roselle calyx extracts used in the marination of the chicken tikka increases. The least CF was in 20 g RC/L marinated chicken tikka. This implies that fat induced challenges in meat and meat products such as lipid oxidation and rancidity can be limited at a higher concentration of roselle calyx extract used in marinating chicken tikka (Possamai et al., 2018). Conversely, the ash content (AC) of chicken tikka marinated with 5, 10, 15 and 20 g RC/L was increasing as concentration of the roselle calyx extract used in marination of the chicken tikka increased. The highest AC was in 20 g RC/L marinated chicken tikka. Notably, there was similarity between chicken patties of 2.68 – 3.01% ash content treated with extracts from ginger, garlic and roselle as documented by Babatunde and Adewumi (2015) compared to ash content of 2.76 - 3.22% in chicken tikka marinated with 5, 10, 15 and 20 g RC/L.

Product yield (PY) of chicken tikka marinated at concentration levels of 5, 10, 15 and 20 g RC/L were better than those of the non-roselle calyx extract (0 g RC/L and 0.01% BHA) marinated chicken tikka (Table 4). This was implicated by appreciable product yield of 64.21% in chicken tikka marinated at a lower concentration level of 5g RC/L than those marinated with the nonroselle calyx extract [0g RC/L (PY: 55.44%) and 0.01% BHA (PY: 54.63%)]. Notably, as the concentrations of roselle calyx extract increases; this gave rise to the highest product yield of 67.70% in chicken tikka marinated with 20 g RC/L. The appreciable PY in roselle calyx extract chicken tikka was directly proportional to increased moisture content (MC) and crude protein (CP) in the roselle calyx marinated chicken tikka. Thus, as the concentration of roselle calyx extract used in marinating chicken tikka increases from 5 g RC/L with the product yield of 64.21% the MC (33.0%) and CP (28.41%) increases progressively to product yield of 67.70% in 20 g RC/L marinated chicken tikka with an increased MC (35.18%) and CP (28.81%) more than in chicken tikka of 0 g RC/L (MC: 32.97%; CP: 22.76%) and 0.01% BHA (MC: 32.52%; CP: 28.01%). The direct proportional relationships among moisture content, crude protein content and product yield of chicken marinated with roselle calyx extract was in agreement with Babatunde and Adewumi (2015); where the non-roselle calyx extract marinated chicken patties of product yield (84.39%), moisture content (32.50%) and crude protein (28.12%)] had lower product yield than in chicken patties marinated with roselle calyx extract of product yield (86.85%), moisture content (33.00%) and crude protein (28.78%).

Cooking loss (CL) in roselle calyx marinated chicken tikka decreases as the concentrations increase from 5 g RC/L (CL:44.56%) to 20 g RC/L (CL: 32.31%). Hence, the trend of cooking loss percentages was opposite to the product yield percentages in chicken tikka marinated with roselle calyx extract $(5 - 20 \text{ g RC/L})$ and the non-roselle calyx $(0\% \text{ RCE}$ and $0.01\% \text{ BHA})$ as presented in Table 4. Implying that chicken tikka marinated in 20 g RC/L with the least cooking loss (32.31%) had the highest product yield (69.70%). The relationship between cooking loss and product yield was inversely proportional as observed in this study and this was in harmony with El-Nashi et al., (2015). Notably, plant extracts employed in meat marination have been well documented and reported to have positively improved product yield or cooking yield and thereby decreasing cooking loss (Latif, 2011; Magnesis et al., 2020).

Minolta chroma colour of lightness (L*) in roselle calyx marinated chicken tikka decreases as the roselle calyx extract levels increases (Table 4). Implying that in 5 g RC/L marinated chicken

tikka the L* (51.31) was higher compared to 10 g RC/L (L*: 49.72), 15 g RC/L (L*: 47.85), 20 g RC/L (L^{*}: 46.70). The L^{*} values in roselle calyx marinated chicken tikka and non-roselle calyx treated chicken tikka were adjudged darker than normal based on the truncation values of Qiao et al., (2001) as stated herein: darker than normal (dark, L^* < 46), normal (48 < L^* < 53) and lighter than normal (lighter, $L^* > 53$). The trend of lower L^* with a range from 46.70 to 51.31 in this study was lower than L^* with a range from 81.64 to 82.89 in the report of Kim *et al.*, (2015). Notably, low lightness (L^*) values were associated with a high redness (a^*) value and low yellowness (b*) with increasing level of roselle calyx extract in the marinated chicken tikka. This observation was in harmony with Petracci et al., (2004) and Bianchi et al., (2007) where breast meat with a lower lightness-L* showed higher redness-a*. Similarly, the report of Kim et al , (2015) had the same trend with a lower redness (a*) ranging from 2.72 to 5.16 and higher yellowness (b^*) ranging from 10.15 to 13.66 which, when compared to the redness (a^*) range from 19.48 to 21.21 and yellowness (b*) range from 33.87 to 41.63 in roselle calyx marinated

Furthermore, chicken tikka marinated with and without roselle calyx extract was observed as darker than normal $(L^* < 46)$ on Minolta Chroma machine but not perceived by the sensory panellist as such since it was rated 'moderately light" (rated on average as 6.93) for colour on the 9-point hedonic scale.

chicken tikka samples were lower in both redness (a*) and the yellowness (b*) values.

Production cost per kilogram for chicken tikka marinated with 5 g, 10 g, 15 g and 20 g RC/L and 0.01% BHA was higher than non-roselle calyx (0g RC/L) marinated chicken tikka as presented in Table 4. This was owing to the fact that the extracts $(5-20 \text{ g RC/L})$ and synthetic (BHA) used in the preparation incurred more cost. Nevertheless, percentage marginal gain relative to the nonroselle calyx extract increased with increasing level of the roselle calyx extract used.

Table 4. Physical traits and cost of chicken tikka marinated with and without RCE

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Mean \pm SE on the same row with different letters differ significantly (p<0.05). Values in parenthesis represent marginal percentage increase in gain relative to the no extract samples (P1).

Sensorial properties of the chicken tikka were greatly enhanced in flavour and taste, texture, tenderness and juiciness while in colour and overall acceptability there were no significant (p>0.05) improvement observed as shown in Table 5. The panellists most preferred for flavour among the chicken tikka samples were 20 g RC/L marinated chicken tikka (rated as almost "Slightly strong") to those of 0% RC/L (rated as almost "Intermediate"), 5 g RC/L (rated as almost "Intermediate"), 10 g RC/L (rated as almost "Intermediate"), 15 g RC/L (rated as almost "Intermediate") on the 9-point hedonic scale. The positive improvement in flavour of roselle calyx marinated chicken tikka was consistent with the report of O_{moj} a *et al.*, (2007) that marinating cooked spent layers by injecting $CaCl₂$ at one-hour post-mortem improves the flavour. Also, marination improves flavour in pork chops as reported by Gao et al., (2015). The sensorial quality of taste was significantly $(p>0.05)$ preferred in chicken tikka marinated with roselle calyx extract concentration level of 20 g RC/L (rated as almost "moderately tasty") to those of chicken tikka without RCE [0% RCE (rated as almost "Slightly tasty") and 0.01% BHA (rated as "Slightly tasty")] and with roselle calyx extract marinated chicken tikka of 5 g RC/L (rated as almost "Slightly tasty"), 10 g RC/L (rated as almost "Slightly tasty") and 15 g RC/L (rated as "Slightly tasty"). The adjudgment for flavour and taste by the sensory panellists revealed that a direct proportional relationship existed between flavour and taste as seen in the trend of preference for chicken tikka marinated with 20 g RC/L roselle calyx extract to those of chicken tikka samples. Hence, this phenomenon further collaborates the description of flavour as comprising mainly of taste and aroma as reported by Jayasena et al., (2013).

Considering texture, tenderness and juiciness as sensorial quality traits of chicken tikka samples, they were significantly ($p<0.05$) preferred in roselle calyx extract marinated chicken tikka at 15 g

RC/L and 20 g RC/L to those of non-roselle calyx extract marinated chicken tikka (0 g RC/L and 0.01% BHA). The least ($p<0.05$) preferred for texture, tenderness and juiciness was in chicken tikka with non-roselle calyx extract (0 g RC/L). Notably, for texture, tenderness and juiciness could be said to be optimal at 15 g RC/L because from the adjudgments made by the sensory panellists for texture (rated as almost "Moderately fine"), tenderness (rated as almost "Moderately soft") and juiciness (rated as almost "Moderately juicy") were not significantly ($p>0.05$) different from adjudgments made at 20 g RC/L for texture (rated as "Moderately fine"), tenderness (rated as "Moderately soft") and juiciness (rated as "Moderately juicy"). The juiciness of the roselle calyx extract at 15 and 20 g RC/L could have been rated significantly higher than those of 0, 5 g RC/L and 0.01% BHA because of the higher moisture content and water holding capacity find in 15 and 20 g RC/L roselle calyx extract marinated chicken tikka than in those chicken tikka samples marinated with roselle calyx extract at 5 g RC/L, and the non-roselle calyx extract of 0 g RC/L and 0.01% BHA. This phenomenon confirms the description of juiciness in meat and meat products by Fernandez et al., (1999) and Omojola et $al.$, (2003); where juiciness in meat product had a direct proportional relationship to the moisture content and water holding capacity of the meat.

Lipid oxidation in roselle calyx marinated chicken tikka observed at three days intervals from 0 to day 9 in cold storage $(4\pm1$ ^oC) exhibited reduction in meat lipid oxidation as roselle calyx extract concentrations increased among the chicken tikka samples as shown in Table 6. This observation can be accounted for from proven scientific findings that vitamin C (ascorbic acid), is naturally abundant in roselle calyx (Wang et al., 2007; Chusmri et al., 2008). Also, flavonoids and phenols are naturally bioactive compound abundant in roselle calyx (Okereke et al., 2015; Islam et al., 2016; Jamin et al., 2019). These bioactive compounds (ascorbic acid, flavoids and phenols) in roselle calyx serve as natural antioxidants and could have positively caused the reduction of lipid oxidation in the chicken tikka as the level of roselle calyx extract concentrations increases. This possibility became evident during marination as certain proportion of ascorbic acid, flavonoids and phenols from roselle calyx extract are incorporated into chicken tikka as such conferring oxidative stability. To this end, best oxidative stability was observed in roselle calyx marinated chicken tikka at 20 g RC/L $(0.45\pm0.01$ mg MDA/kg) with similar reduction in lipid oxidation being non-significantly different from that of 0.01% BHA marinated chicken tikka (0.39 \pm 0.06 mg MDA/kg) as standard "industrial referee' in cold (4 \pm 1^oC) storage of

9 days. This observation could be compared with the report made by Hwang et al., (2013) on the use of various concentrations of extract from ganghwayakssuk with ascorbic acid in uncooked and deep-fried chicken nuggets and it was deduced that ascorbic acid and extract of ganghwayakssuk were more efficient than other antioxidant combinations in inhibiting lipid oxidation of nuggets in uncooked and deep-fried state. Similarly, Blackhurst et al., (2011) reported that South African red wine has limiting effect on lipid oxidation on beef in its cooked state.

Table 5. Sensory evaluation of chicken tikka marinated with and without RCE

Mean \pm SE on the same row with different letters differ significantly (p<0.05).

Lipid oxidation decreases as the roselle calyx extract concentration levels in marinated chicken tikka increases from 5 to 20 g RC/L resulting into increased percentage reduction relative to the non-roselle calyx extract (0 g RC/L) as follows: 5 g RC/L (46.56%) < 10 g RC/L (50.38%) < 15 g RC/L (57.25%) < 20 g RC/L (65.65%) on day 9 of cold (4 \pm 1°C) presented in Table 6. The trending in the reduction percentages in roselle calyx marinated chicken tikka at day 0, 3, 6 and 9 of cold $(4\pm1$ ^oC) storage revealed that percentage reduction increased as storage days and concentration levels of roselle calyx extract increased in the marinated chicken tikka. Consequently, the use of roselle calyx extract $(0 - 20 \text{ g RC/L})$ as an intervention in reducing

lipid oxidation in marinated chicken tikka of cold $(4\pm1°C)$ storage from day 0 to day 9 is substantially efficient in limiting lipid oxidation and mitigating the problems associated with it.

Thus, in this regard roselle calyx extract is functionally a natural antioxidant. Comparing the performance of the synthetic antioxidant used in marinating the chicken tikka (0.01% BHA) to that of roselle calyx extract at 20 g RC/L for day 0, 3, 6 and 9; it was evident that there were no significant ($p > 0.05$) differences between chicken tikka marinated with 0.01% BHA and 20 g RC/L from day 0 to day 9. Evidently, the percentage reduction in lipid oxidation was best at 20 g RC/L (65.65%) up to day 9 among chicken tikka marinated roselle calyx having similarity of percentage reduction in lipid oxidation observed for chicken tikka marinated at 0.01% BHA (70.23%) .

Lipid oxidation in cooked meat has been reported to have a minimum threshold of 0.50 to 1.0 mg MDA/kg in cold storage (Tarladgis et al., 1960). To this end, in this study the roselle calyx extract concentration levels $(5, 10, 15, 10)$ and $(20, 20)$ g RC/L) and the non-roselle calyx extract $(0, 9)$ RC/L and 0.01% BHA) marinated chicken tikka were within the minimum threshold of 0.50 to 1.0 mg MDA/kg as documented by Tarladgis et al., (1960) except on day 9 where only the non-

roselle calyx extract (0 g RC/L) chicken tikka had 1.31 mg MDA/kg. Conversely, applying the maximum permissible threshold point of 2 mg MDA/kg for lipid oxidation in meat and meat products as reported by Possamai et al., (2018), it became evident that though, the lipid oxidation in both roselle calyx marinated chicken tikka (5, 10, 15 and 20 g RC/L) and non-roselle calyx marinated chicken tikka increased with increasing cold $(4\pm1\degree C)$ storage periods but did not exceed the maximum permissible threshold point of 2 mg MDA/kg.

Table 6: Lipid oxidation (mg MDA/kg) of chicken tikka marinated with and without RCE stored

for 9 days at 4°C

Mean \pm SE on the same row with different letters differ significantly (p<0.05). Values in parenthesis represent percentage reduction in lipid oxidation relative to the no extract (P1).

Total bacterial count (TBC) of stored chicken tikka marinated with and without roselle calyx extract is shown in Table 7. No significant $(p>0.05)$ differences were observed among roselle calyx extract marinated chicken tikka (5, 10, 15 and 20 g RC/L) and non-roselle calyx extract marinated chicken tikka (0 g RC/L and 0.01% BHA) on day 0 where no microbial growths were observed among chicken tikka samples. Although, there were no microbial growths (log_{10} cfu/g) observed on day 3 in both 0.01% BHA treated chicken tikka and 20 g RC/L roselle calyx extract marinated chicken tikka samples, however, the TBC in these samples of chicken tikka were significantly ($p<0.05$) lower than those of roselle calyx extract marinated chicken tikka (5, 10) and 15 g RC/L) and the non-roselle calyx extract marinated chicken tikka (0 g RC/L) where microbial growths ranged from 0.30 to 0.48 log_{10} cfu/g. On the contrary, microbial growths were observed on day 6 and 9 in 0.01% BHA roselle calyx extract marinated chicken tikka and these were not significantly (p>0.05) lower than that of roselle calyx extract marinated chicken tikka samples of 20 g RC/L, expect for day 9 were 0.01% BHA, 15 and 20 g RC/L marinated chicken tikka had same TBC of $0.48 \log_{10} c f u/g$. This observation gave a clear indication that roselle calyx extract marinated chicken tikka at higher concentration levels of 15 g and 20 g RC/L will be efficient as 0.01% BHA in having antibacterial effect on chicken tikka as the storage days increases beyond day 9. Notably, the use of roselle calyx extract in marinating chicken tikka enhanced preservation by limiting TBC as seen in the significantly $(p<0.05)$ lower TBC than in the non-roselle calyx extract (0 g RC/L) as the storage days increases. However, the minimum threshold point of ≤ 4 log₁₀ cfu/g (Heinz and Hautzinger, 2007) was not exceeded in all the chicken tikka samples whether marinated with roselle calyx extract (5, 10 and 20 g RC/L) or not (0 g and 0.01% BHA).

Storage	Total bacterial count (log_{10} cfu/g) of chicken tikka samples					
days	P1	P2	P3	P4	P5	Р6
Ω	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00 ^a
	$0.48 \pm 0.02^{\text{a}}$	0.00 ± 0.02 °	0.30 ± 0.00^b	0.30 ± 0.00^b	0.30 ± 0.00^b	0.00 ± 0.04 ^c
6	$0.60 \pm 0.03^{\text{a}}$	0.18 ± 0.02 ^c	0.48 ± 0.02 ^{ab}	0.30 ± 0.02^b	0.30 ± 0.02^b	0.18 ± 0.02 ^c
9	$0.70 \pm 0.03^{\text{a}}$	0.48 ± 0.03^b	0.60 ± 0.03^{ab}	0.60 ± 0.03 ^{ab}	$0.48 \pm 0.03^{\rm b}$	0.48 ± 0.03^b

Table 7: Total bacterial count (\log_{10} cfu/g) of chicken tikka marinated with and without RCE

stored for 9 days at 4°C

Mean \pm SE on the same row with same letters are not statistically significantly (p <0.05).

Mould count of cold (4 ± 1) °C) stored chicken tikka marinated with roselle calyx extract (5, 10, 15 and 20 g RC/L) and without roselle calyx extract (0 g RC/L and 0.01% BHA) for 9 days are shown in Table 8. Chicken tikka samples marinated with roselle calyx extract (5, 10, 15 and 20 g RC/L) and without roselle calyx extract (0 g RC/L and 0.01% BHA) on day 0 had no significant (p>0.05) differences among them for MC with no mould growths observed. However, mould growths (log_{10} cfu/g) were observed among chicken tikka marinated with roselle calyx extracts (5 and 10 g RC/L) and without roselle calyx extract (0 g RC/L) and these were significantly (p <0.05) higher than those of 0.01% BHA, 15 g RC/L and 20 g RC/L marinated chicken tikka samples with no observable MC on day 3. Although, on day 6 there were mould growth in chicken tikka marinated with roselle calyx extract (15 and 20 g RC/L) and 0.01% BHA treated chicken tikka, both maintained same level in MC of log_{10} 0.15 cfu/g which were significantly (p <0.05) lower than chicken tikka marinated with roselle calyx extract (5 and 10 g RC/L) and 0g RC/L non-roselle calyx marinated chicken tikka. Remarkably on day 9 in chicken tikka marinated with roselle calyx extract (5, 10, 15 and 20 g RC/L) and 0.01% BHA treated chicken tikka were of similar MC with range from $log_{10} 0.30$ cfu/g to $log_{10} 0.33$ cfu/g with no significant ($p>0.05$) differences among them. Implying that from day 9 and beyond in cold ($4\pm1\degree$ C) storage the MC increases at a decreasing rate and irrespective of marination of the chicken tikka with or without roselle calyx extracts. Notably, marination with roselle calyx extract (5, 10, 15 and 20 g RC/L) and 0.01% BHA were significantly $(p>0.05)$ lower than in chicken tikka of 0g RC/L roselle calyx extract on day 9 of cold $(4\pm1°C)$ storage. However, the minimum threshold point of \leq 3 log₁₀ cfu/g (Heinz and Hautzinger, 2007) was not exceeded in all the chicken tikka samples whether marinated with roselle calyx extract (5, 10 and 20 g RC/L) or not (0 g and 0.01% BHA). **Table 8:** Mould count (log_{10} Cfu/g) of chicken tikka marinated with and without RCE stored for

Mean \pm SE on the same row with same letters are not statistically significantly (p <0.05).

The pH observed among the roselle calyx extract marinated chicken tikka (5, 10, 15 and 20 g RC/L) and non-roselle calyx extract (0 g RC/L and 0.01% BHA) marinated chicken tikka were not significantly (p>0.05) different from day 0 to 9 of cold (4° ±1) storage (Table 9). Although no significant (p>0.05) changes in pH are observed in roselle calyx extract marinated chicken tikka (5, 10, 15 and 20 g RC/L); but there is a decreasing trend in pH as the roselle calyx extract concentrations increases from 5 to 20 g RC/L on day 0, 3, 6 and 9 of cold $(4^o\pm1)$ storage. This observed trend in pH of roselle calyx extract marinated chicken tikka could be due to increasing level of ascorbic acid in the roselle calyx extract which lowers the pH value making it more acidic- since roselle calyx is abundantly endowered with ascorbic acid (Carvajal-Zarrabal et al., 2012). Similarly, the lower values of pH among the roselle calyx extract marinated chicken tikka than in 0 g RC/L and 0.01% BHA treated chicken tikka could be attributed to the acidic nature of the roselle calyx extract. Notably, the pH in meat products with range of 6.65 to 6.78 (Sallam et al., 2004), 5.91 to 5.92 (Babatunde and Adewumi, 2015), 6.37 to 6.54 (Olusola et al., 2018) were higher than 5.41 to 5.75 of roselle calyx extract marinated chicken tikka. The difference in pH values could be accounted for by variation in the nature and product formulation (Akwetey et al., 2014). Considering the acceptable threshold range for pH (4.0 to 7.0) in meat processing and product formulation as reported by Heinz and Hautzinger (2007), therefore on a positive note chicken tikka marinated with or without roselle calyx extract had pH range from 5.41 to 5.79 which did not exceed the threshold of maximum acceptable point.

Table 9. The pH of chicken tikka marinated with and without RCE stored for 9 days at 4°C

Mean \pm SE on the same row with same letters are not statistically significantly (p <0.05).

Water holding capacity (WHC) observed was best in roselle calyx extract marinated chicken tikka at 20 g RC/L on day 3 among the roselle calyx extract marinated chicken tikka samples (Table 10). But on day 0, 6 and 9 there were no significant ($p<0.05$) difference in WHC among chicken tikka marinated in roselle calyx extract of 5, 10, 15 and 20 g RC/L as well as chicken tikka treated with 0.01% BHA. Also, chicken tikka treated with 0.01% BHA had WHC that is significantly ($p<0.05$) higher than those of 0g RC/L non-roselle calyx extract treated chicken

tikka on day 0, 3, 6 and 9 of cold $(4\pm1$ ^oC) storage. Notably, is the fact that there were no significant (p>0.05) differences among roselle calyx extract marinated chicken tikka (5, 10, 15 and 20 g RC/L) and 0.01% BHA treated chicken tikka throughout storage days. The phenonema of increased WHC in roselle calyx extract marinated chicken tikka harmonizes well with the report of Hossen and Esfahani (2015) where WHC of beef patties increased in citric acid marinated beef patties.

Consequently, comparing reports of positive impact of acidic marination on increments in WHC of cooked chicken meat with range from 21.1 to 40.96% [Latif (2010)], 44.36 to 52.20% [Babatunde and Adewumi, 2015] was lower than WHC from 67.45 to 76.87% range in marinated chicken tikka marinated at 5, 10, 15 and 20 g RC/L roselle calyx extract concentrations levels. Besides, similarities between chicken tikka marinated with roselle calyx extract at 5, 10, 15 and 20 g RC/L and 0.01% BHA chicken tikka on day 0, 3, 6 and 9 is a clear indication that replacement for 0.01% BHA could be efficiently done with the use of roselle calyx extract even at the least concentration level of 5 g RC/L in the chicken tikka used in this study.

Table 10: WHC of chicken tikka marinated with and without RCE

Storage	WHC $(\%)$ of chicken tikka samples						
days	P1	P ₂	P ₃	P4	P5	P6	
θ		$70.59\pm0.37^{\circ}$ $78.31\pm0.41^{\circ}$ $76.87\pm0.17^{\circ}$ $77.26\pm0.41^{\circ}$ $77.41\pm0.38^{\circ}$ $80.78\pm0.69^{\circ}$					
3		$64.22\pm0.27^{\circ}$ 74.92 ± 0.32^{ab} 72.19 ± 0.19^b 73.18 ± 0.35^b 73.67 ± 0.32^b 76.26 ± 0.56^a					
-6		61.15 ± 0.27^b 70.50 ± 0.32^a 68.06 ± 0.18^a 69.06 ± 0.35^a 69.38 ± 0.35^a 70.56 ± 0.52^a					
9		56.10 \pm 0.27 ^b 66.97 \pm 0.27 ^a 66.36 \pm 0.19 ^a 66.78 \pm 0.28 ^a 66.97 \pm 0.32 ^a 67.45 \pm 0.37 ^a					

Mean \pm SE on the same row with same letters are not statistically significantly (p <0.05).

Conclusions

The sensory or eating qualities, product yield, lipid oxidation in chicken tikka marinated with roselle calyx extract $(5, 10, 15 \text{ and } 20 \text{ g RC/L})$ were more desirable than the non-roselle calyx extract (0 g RC/L) marinated chicken tikka. Roselle calyx extract marinated chicken tikka samples had higher percentage reduction in lipid oxidation with better oxidative stability than non-roselle calyx extract of 0 g RC/L while reducing total bacterial count and mould count in chicken tikkas. The best oxidative stability conferred to chicken tikka among the roselle calyx extract was at 20 g roselle calyx/L and this natural source of antioxidant compares well to Butylated hydroxyl anisole in chicken tikka as a synthetic source of antioxidant. Hence, on a

positive note roselle calyx extract as a botanical with antioxidative properties can be recommended for use to improve sensorial and physiochemical properties in chicken tikka while securing a stable shelf-life product devoid of anxieties for food safety.

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