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A TWO-WAY STABILIZATION OF CHICKEN NUGGET USING ETHANOLIC EXTRACTS OF SOME COMMON SPICES IN NIGERIA

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ABSTRACT

Manually deboned and comminuted broiler meat was used to determine the antioxidant and antimicrobial activities of cloves (*Eugenia caryophyllata*), ginger (*Zingiber officinale*) and pepper (*Capsicum annuum*) extracts. The synergetic effect of combined spice extracts (1:1 w/w) on the quality and shelf-life of nuggets stored for 12 days at 4°C was also determined using a completely randomized design. The results revealed that lipid oxidation of chicken nuggets was improved by the inclusion of spice extracts, with ginger and pepper (GP) combination performing better ($P<0.05$) than the other treatments. Total plate, coliform and fungal counts were significantly ($P<0.05$) reduced following the addition of spice extracts, but the combined extracts of clove and ginger (CG) better suppressed the counts. Our results demonstrated that, spice extracts especially when combined are highly effective against microbial growth and lipid oxidation, highlighting their potential as natural stabilizers in chicken nuggets preservation.

Keywords: Stabilization, Antioxidant, Antimicrobial, Spice extracts, Shelf life

INTRODUCTION

The progressive demand for convenient food has led to increased production of ready-to-eat (RTE) products. RTE meat products, especially those of chicken origin have high unsaturated fatty acids, leading to quality deterioration under oxidative stress (Cortinas *et al.*, 2006; Nkukwana *et al.*, 2014). Studies have also shown the high susceptibility of processed meat products, which are minced, mixed and/or heated like chicken nugget to lipid oxidation and off flavor development during storage (Cortinas *et al.*, 2005; Samouris *et al.*, 2007). Oxidative rancidity and microbial invasion are the major problems causing shelf life quality deterioration in stored meat and meat products (Ahmed *et al.*, 2017; Si *et al.*, 2018).

Non-thermal and bio-preservative techniques are gaining attention in the food industry (Zhou *et al.*, 2010) due to increasing consumer's quest for healthy food, devoid of conventional chemical preservatives (Mulla *et al.*, 2017; Weerakkody *et al.*, 2010). Spices which have been used to improve the sensory characteristics of food over centuries, are currently being explored in the form of powder and extracts or essential oils as reliable bio-preservatives for the control of microbes and oxidative rancidity in meat products (Baker *et al.*, 2012; Ene-Obong *et al.*, 2016; Zhang *et al.*, 2009).

Studies on the potency of extracts from spices such as cloves (Cortés-Rojas *et al.,* 2014), ginger (Si *et al.,* 2018) and pepper (Gurnani *et al.,* 2015) as efficient antioxidant and antimicrobial agents have been reported. Clove in particular has been reported to have outstanding antimicrobial and antioxidant activity (Baljeet *et al.,* 2015; Gurnani *et al.,* 2015; Shan *et al.,* 2005; Zhao *et al.,* 2011). Combination of spice extracts have also revealed stronger preservative potentials in *in vitro* studies due to their synergetic effects (Bag and Chattopadhyay, 2015; Baljeet *et al.,* 2015; Reji and Rajasekaran, 2015; Shaaban *et al.,* 2013; Zhang *et al.,* 2009). However, there is little or no information on *in vivo* comparative study to determine the synergic preservative effect of clove, ginger and pepper extracts, their combination and their simultaneous antioxidant and antimicrobial potency on processed comminuted meat product as chicken nugget. Thus, the objective of this work was to determine the effects of cloves, ginger and pepper, applied in chicken nugget individually and/or in combination, on pH, thiobarbituric acid reactive substances (TBARS) and microbial analysis during storage at 4°C.

MATERIALS AND METHODS

Preparation of spice extracts

Clove (*Eugenia caryophyllata*), ginger (*Zingiber officinale*) and pepper (*Capsicum annum*) were purchased from Kurmi market in Kano State, Nigeria. The spices were washed with water, spread on a clean table and air dried at room temperature overnight. Dried spices were ground into powder using a hammer mill. The spice extracts were prepared as follows: Aliquots (50 g each) of pulverized spices were mixed into 400 ml of 95% (v/v) ethanol for 12 h in enclosed flasks with constant shaking (100 rpm) using a flask (Stuart: SF1). After filtration with a Whatman No. 2 filter paper, 200 ml of 95% ethanol were added to re-extract the residue for 12 h and then the extracts were filtered again. The combined filtrates were concentrated on a rotary evaporator (RE- 100, Bibby Sterilin Co., Ltd., U.K.) at 50°C with a vacuum pump. The extracts were further evaporated on water bath using Petri dishes. Dried extracts were placed in sealed bottles and stored at 4°C before use (Zhang *et al.,* 2009).

Chicken nugget preparation

Meat was deboned manually, cut into small pieces and minced in a meat mincer (Breville®, UK) with a 3 mm plate. Emulsion of each formulation was prepared using bowl chopper (Breville®, UK). The percentage weight of all nugget formulations were: broiler meat, 60%; soy bean oil, 10%; ice flakes, 10%; refined wheat flour, 2.5%; skim milk powder, 2%; whole egg liquid, 5%; table salt, 2%; sugar, 1%; sodium tri-polyphosphate, 0.2%; condiments/spice mix, 2.5%; and spice extract,4.8%. The formulation obtained was carefully cut in nugget size pieces (4×1.5×1.5 cm) and baked at 160°C for 15 minutes. The cooked product was cooled and weighed. The nugget were packed in sterilized aluminum foil plates and stored at refrigerated temperature (4±1°C) prior to analysis (Kumar and Tanwar, 2011).

pH

The pH of the nugget was determined according to the method described by Alakali *et al*. (2010). Briefly, five grams of sample was homogenized with 50 ml of distilled water. The pH reading was then taken by inserting the glass electrode of the pH meter into the homogenate.

Thiobarbituric Acid

Thiobarbituric acid (TBA) was determined according to the method proposed by Kirk and Sawyer (1991). Ten grams of nugget were macerated with 50 ml of water for 2 minutes and transferred to a distillation flask with 47 ml of water. Thereafter, 2.5 ml of 4 M hydrochloric acid (HCL) were added to adjust the pH to 1.5. After the addition of a few glass beads, the flask was heated so as to collect 50 ml of distillate in 10 min from the time boiling commenced. Five

milliliters of the distillate were pipetted into a beaker, and 5 ml TBA reagent was added. The tube was stoppered, shaken and heated on a boiling water bath for 35 min. A blank was prepared in a similar way using 5 ml of water with 5 ml reagent. The beaker was cooled in water for 10 minutes and the absorbance (D) was measured with the aid of a spectrophotometer (Spectro 20D, Pec medicals U.S.A) against the blank at the wavelength of 538 nm using 1 cm cells. The TBA was expressed as mg of malondialdehyde (MDA)/kg sample =7.8 D.

Antimicrobial activity testing

Collection of samples

Nugget samples from each treatment were collected into sterilized plastic containers and transferred to the laboratory with the aid of ice bags, and analyzed within an hour after collection as recommended by Nwachukwu *et al*. (2008).

Media preparation

The media included plate count agar, McConkey agar, potato dextrose agar, peptone water and nutrient agar which were prepared according to manufacturer's instructions (Oxoid, U.K.). The media were sterilized by autoclaving at 121°^{C} for 15 minutes.

Enumeration of bacteria and fungi

A sterile knife was used to cut 25 g of each nugget sample. The sample was homogenized in 225 ml peptone water (0.1 peptone water) to form the stock solution. One milliliter from each stock solution was transferred aseptically into 9 ml of peptone water and this was serially diluted to 10^3 dilutions. Thereafter, 0.1 ml of 10^3 dilutions was transferred aseptically into plates containing plate count agar, McConkey agar and potato dextrose agar. A sterile bent glass rod was used to spread the inocula on the surface of the culture media. The inoculated media were incubated inversely at 37°C for 24 hours (Cheesebrough, 2004) for total plate count and total coliform count while the inoculated media for total fungal count was incubated at room temperature (28-32°C) for 7 days. The number of bacterial and fungal colonies were counted, recorded and expressed as colony-forming unit per gram (cfu/g).

$$cfu/g = \frac{Total\ Number\ of\ Colonies\ Counted \times Dilution\ factor}{Volume\ of\ Inocula}$$

Statistical Analysis

The experiment was replicated six times and data obtained were subjected to Analysis of Variance Procedure of SAS (SAS, 2002) and significant means were separated using Duncan Multiple Range Test (Duncan D. B., 1955). The following model was used:

$$Y_{ij} = \mu + T_i + e_{ijk}$$

Where;

Y_{ij} = dependent variable; μ = overall mean; T_i = effect of the ith treatment; e_{ijk} = random error.

RESULTS AND DISCUSSION

Effect of spice extracts and storage time on pH and thiobarbituric acid reactive substances

Spice extracts added in chicken nugget controlled the pH changes throughout the period of storage (Fig. 1). As storage days increased, there was gradual increase in pH values among optimized products which significantly differed ($P<0.05$) from the control on day 9. On the 12th day of storage, the combination of clove and ginger (CG), and that of ginger and pepper (GP) were not statistically different ($P>0.05$) but significantly differed from the control. The combination of ginger and pepper (GP) extracts and cloves and pepper (CP) extracts were able to stabilize the product better than the other treatments till the last day of storage. Generally, the break-down of meat protein results in accumulation of ammonia and amino acid, with consumption of the latter by microbes due to

glucose depletion during storage. Therefore, the lower pH values observed in the treated products suggests inhibition of microbial growth and propagation by compounds present in spice extracts (Kumar and Tanwar, 2011; Zhang *et al.,* 2016).

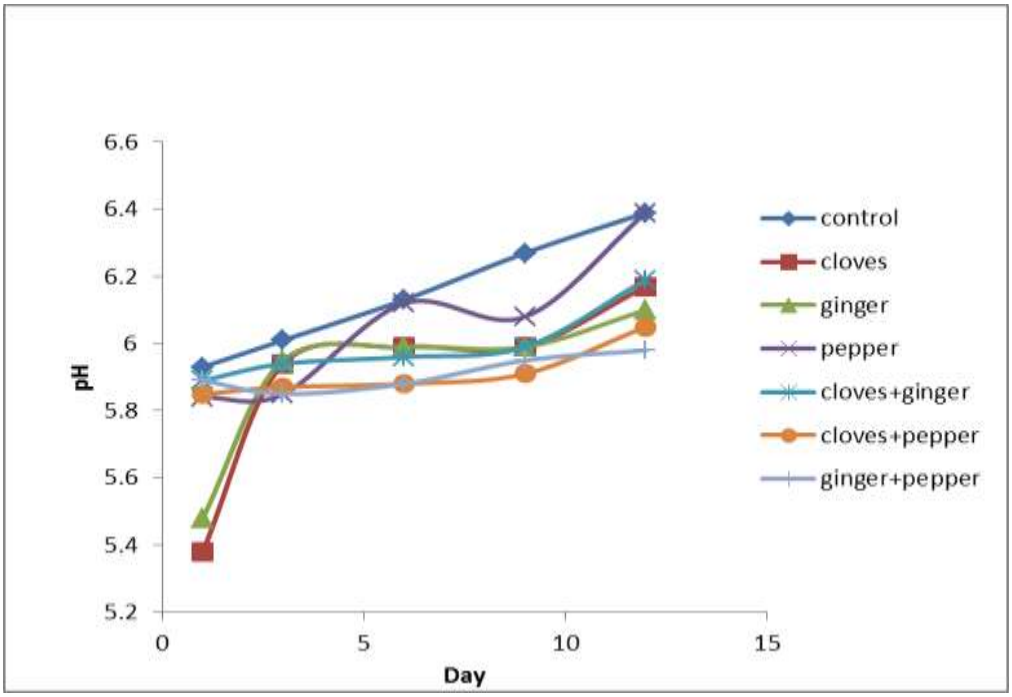

Figure 1: **Effect of spice extracts on pH changes of chicken nugget.**

Thiobarbituric acid of reactive substances (TBARS) analysis determines the formation of secondary products of lipid oxidation, mainly malondialdehyde (MDA), which may contribute to the off-flavor of oxidized fat (Fig. 2). On the 9th day of storage, lower TBARS value was observed in pepper (0.38 mg of MDA/kg) compared to ginger (0.43 mg of MDA/kg) and cloves (0.46 mg of MDA/kg). However, the GP treatment significantly ($P<0.05$) decreased TBARS levels throughout the storage period followed by the CG and CP treatments. In contrast to the control, where the values increased astronomically, all optimized products were associated with lower TBARS, suggesting their antioxidant properties. Similarly, previous studies reported that clove, ginger and pepper have high antioxidant properties (Ibrahim *et al.*,2001; Tanabe *et al.*,2002) due to their phenolic content, which exert positive effects on lipid oxidation by reducing 2-thiobarbituric acid (TBA) or malondialdehyde (MDA) formation on different types of meat products during storage (Gurnani *et al.,* 2015; Shan *et al.,* 2005; Su *et al.,* 2007). It is believed that natural antioxidants interfere with free radical chains by offering hydrogen from the phenolic groups, resulting in the formation of a stable end product (Negi *et al.,* 2003; Zhang *et al.,* 2016). Synergism between compounds in pepper and ginger extracts could be responsible for its better keeping quality.

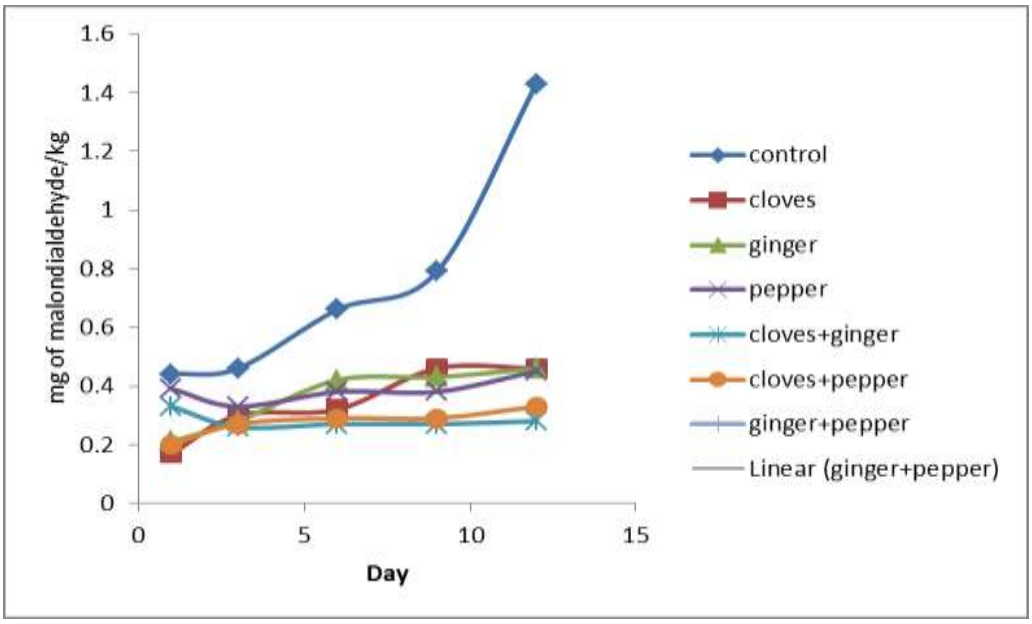

Figure 2: **Effect of spice extracts on thiobarbituric acid reactive substances**

Effect of spice extracts and storage time on microbial count of chicken nuggets

The effect of spice extracts on the total plate counts (TPC), total coliform counts (TCC) and total fungal counts (TFC) of chicken nuggets are presented in Figure 3, 4 and 5, respectively. The CG treatment remarkably lowered TPC compared to other optimized products but did not differ significantly ($P>0.05$), except on the 12^{th} day ($P<0.05$; Fig. 3). During the first 6 days, the TCC from the optimized products were not significantly different ($P>0.05$). On day 9, significantly lower TCC ($P<0.05$) were recorded in CG and clove extract treatments than other optimized treatments. On the last storage day, the TCC were significantly ($P<0.05$) retarded by CG treatment relative to other optimized products (Fig. 4). No significant difference ($P>0.05$) were found in TFC between optimized products for the first 6 days. On the 9^{th} day, the TFC did not differ among CG and clove treatments ($P>0.05$), but were considerably lower ($P<0.05$) than other optimized products. CG completely inhibited fungal growth from the 1^{st} to the 9^{th} day of storage (Fig. 5). On the overall, the CG treatment performed better compared to other optimized products. The CG treatment remarkably reduced TPC, TCC and TFC in chicken nugget, highlighting the potential of this combination in stabilizing the quality of the studied meat product, especially when stored at 4ºC. Based on their inhibitory effect against a wide range of microorganisms, spice extracts have been widely used to increase refrigerated meat shelf-life (Chao *et al*., 2017; Djenane *et al*., 2002; Zhang *et al*., 2009). Moreover, extracts of ginger, cloves and pepper have been identified as good antimicrobial agents (Ibrahim *et al*., 2011; Zhang *et al*., 2009; Zia-Ur-Rehman *et al*., 2003), for their inhibitory effects against spoilage microbes: coliform bacteria and certain fungi (Kenar *et al*., 2010; Kong *et al*., 2007; Kumudavally *et al*., 2011). The mechanism of action of spice extracts on inhibition of microbial growth is not well understood. However, studies have shown that phenol-rich essential oils from spice extracts are hydrophobic in nature. Their hydrophobicity is shown to be responsible for altering lipids of the cell membrane and mitochondria of microbes. As a result, these structures leak their contents due to permeability, and finally, cell death (Burt, 2004; Mau *et al*., 2001; Shan *et al*., 2007; Taylor *et al*., 2011). On the other hand, the mode of action of spice extracts that contains

flavonoids involves metal chelation (Zhang *et al.*, 2016). The observed strong antimicrobial effect of combined spice extracts when compared to the spices used singly may be due to the synergic effect of compound present in mixed spices (Dufour *et al.*, 2002). A similar trend was reported by Mau *et al*. (2001) and Zhang *et al*. (2009) who used mixed spice extracts in fresh pork and ham products.

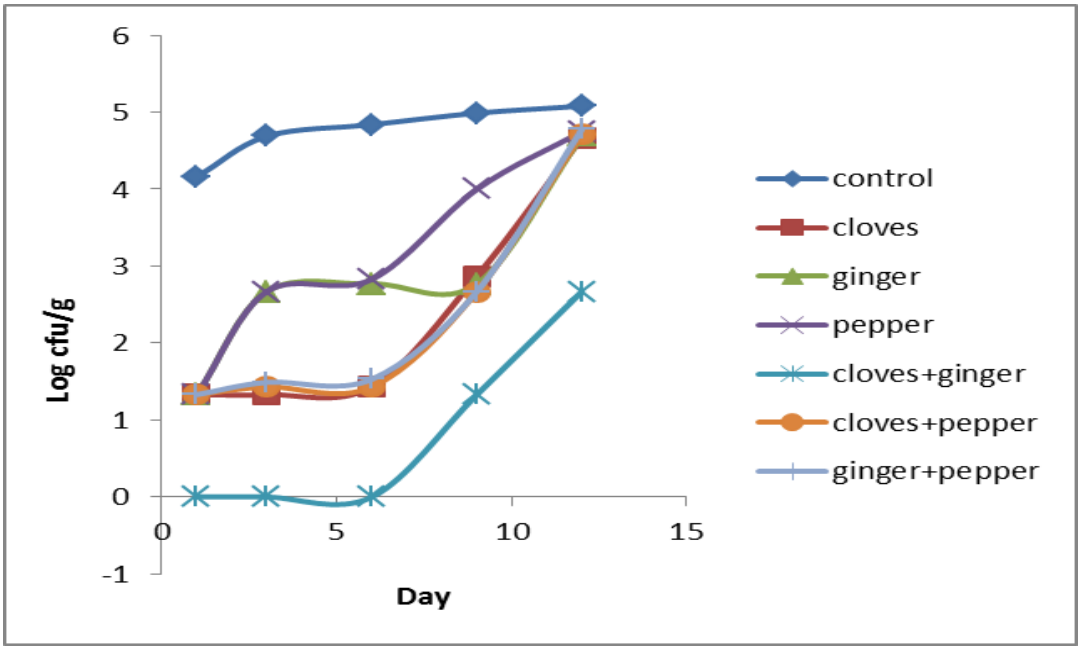

Figure 3: **Effect of spice extracts on Total Plate Count (TPC)**

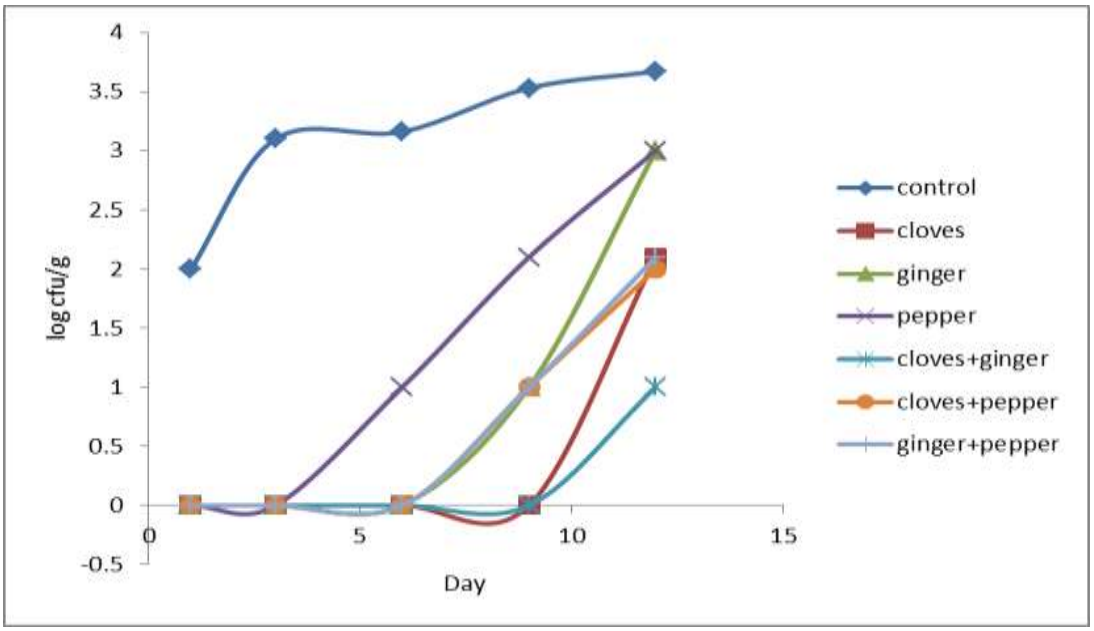

Figure 4: **Effect of spice extracts on Total Coliform Count (TCC)**

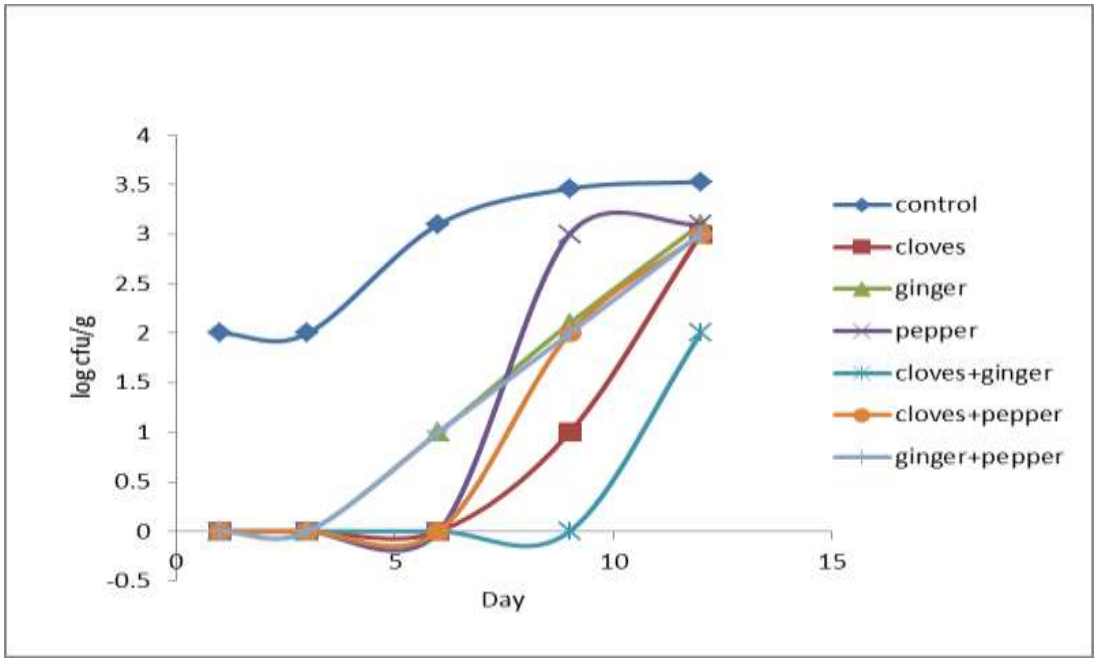

Figure 5: **Effect of spice extracts on Total Fungal Count (TFC)**

CONCLUSIONS

Generally, the findings demonstrated the effectiveness of clove, ginger, and pepper extracts and their combination in inhibiting microbial growth, reducing lipid oxidation and stabilizing pH thus extending the shelf-life of chicken nugget stored at 4^{oC} over a 12-day period. However, combination of the CG and GP gave a better result. The CG treatment showed good stability against lipid oxidation and had the strongest preservative effect on microbial growth in chicken nugget whereas the GP treatment had the strongest anti-oxidant effect. The result of this study shows the additive effects of combining spice extracts and therefore, their application in enhancing the quality of chicken nugget is highly desirable.

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