World Journal of Nutrition and Food Science

Research Article

Effect of Clove Powder and Garlic Paste on Quality and Safety of Raw Chicken Meat at Refrigerated Storage

Tareq MH, Rahman SME and Hashem MA*

Department of Animal Science, Bangladesh Agricultural University, Bangladesh

Abstract

The present study was conducted to evaluate the shelf life and microbiological quality of raw chicken meat incorporated with clove powder, garlic paste and their combination at refrigerated storage (4°C ± 1°C). Meat samples were divided into four different batches i.e., T_0=Control (without natural preservatives), T_1=0.2% clove powder, T_2=2% garlic paste and T_3=combination of 0.2% clove powder and 2% garlic paste. The samples were evaluated for physicochemical properties (pH, cooking loss), oxidative stability (FFA, POV and TBARS) sensorial properties (color, odor) and microbial counts (TVC, TCC and TYMC) on 0, 3, 6, 9 and 12 days of storage. The obtained results showed that addition of natural preservatives significantly ($p<0.05$) influenced on physicochemical properties, oxidative stability, microbiological and sensory attributes compared to control samples. Throughout the storage, pH value and cooking loss were significantly lower ($P<0.05$) in T_1, T_2 and T_3 batches than control. Among the treated batches, combination of clove powder and garlic paste batch (T_3) showed significantly ($P<0.05$) lower free fatty acid and peroxide values during the storage period. TBARS values were varying significantly among the treated batches but clove powder (T_1) maintained lowest TBARS value till the end of storage. Throughout the storage period, comparatively lower values of both viable count and coli form count were detected in T_3 batch. But yeast mould count was significantly lower in T_2 batch. Color score of T_1 batch and odor score of T_2 batch were significantly higher ($P<0.05$) than other batches. From this comparative study of natural preservatives, it was concluded that 0.2% clove powder and 2% garlic paste are effective in extending shelf life of meat but combination of 0.2% clove powder and 2% garlic paste could be utilized effectively as antioxidant and antimicrobial in preservation of raw chicken meat at refrigerated storage (4°C ± 1°C).

Keywords: Clove powder; Garlic paste; Raw chicken meat; Physicochemical properties; Oxidative quality; Sensory properties

Introduction

Meat is an important protein source throughout the world, especially in developing countries [1]. Fresh meat is also highly perishable product due to its biological composition [2]. The nutritional attributes of meat, which provide a major proportion of consumer requirements for protein, some vitamins and certain minerals, are highlighted in work on the nutritional value of meat in other countries [3-5]. It is liked for its unique taste and is a rich source of nutrients, providing good quality animal proteins, essential amino acids and fatty acids, minerals, trace elements and vitamins particularly B-complex which make them 'natural media' of microorganisms [6]. Microbial contamination can decrease the quality and safety and decrease shelf life of meat. Color, microbial growth and lipid oxidation are important factors for the shelf-life and consumer acceptance of fresh meat (Jakobsen and Bertelsen, 2000). Lipid oxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids [7]. Temperature, light, concentration of oxygen in the surrounding atmosphere, amount and composition of phospholipids, presence of anti-oxidants, pro-oxidants, metal ions, haem pigments, enzymes, mechanical processes etc., are the main factors for lipid oxidation [8]. To prevent or delay the auto-oxidation process, antioxidants have been utilized for many years in meat and meat products (Lahucky et al., 2010). Various synthetic chemicals such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), Tertiary Butyl Hydroquinone (TBHQ) etc., are being used as antioxidant and antimicrobial agents to decrease the above mentioned problems [9]. But those are very harmful for human health. That's why; the growing concern about food safety has led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria. Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavor [10]. Studies done previously confirm that garlic, onion, cinnamon, cloves, thyme, sage, and other spices inhibit the growth of both Gram positive and Gram negative food borne pathogens or spoilage bacteria, yeast, and molds.

Clove a dried floral bud of Syzygium aromaticum, generally used to provide distinctive flavor to the foods, have also been reported to exhibit inhibitory effect on many food borne pathogens [11-13]. The inhibitory effect of spices on microbes is due to the presence of essential oils that contains eugenol [14]. Eugenol has been reported to inhibit the growth of *E. coli* O157: H7 and *Listeria Monocytogenes*. Garlic (*Allium sativum*) is always appreciated for its flavor enhancing and medicinal properties. It has potent antioxidant anti-microbial [15,16], lipid lowering, anti-thrombotic, anti-blood coagulation, anti-hypertension and anti-carcinogenic activity [17]. Though garlic contains various bio-active substances such as allicin, diallyl sulfide, allyl sulfide and propyl sulfide, but allicin is the principal ingredient [18].

Citation: Tareq MH, Rahman SME, Hashem MA. Effect of Clove Powder and Garlic Paste on Quality and Safety of Raw Chicken Meat at Refrigerated Storage. World J Nutr Food Sci. 2018; 1(1): 1002.

Publisher Name: MedText Publications LLC

Manuscript compiled: October 13th, 2018

***Corresponding author:** Rahman SME, Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, E-mail: ehsan_bau@yahoo.com

Various scientific studies have documented the use of these preservatives in meat systems *viz* clove powder in chicken nuggets, clove powder in pork, fresh garlic in raw chicken sausages, garlic in ground beef have been documented individually but their combination role in meat was not practiced [19-22]. Therefore in this present discourse we attempt to compare their efficacy so as to know the best natural preservative for extending the shelf life of chicken meat.

Materials and Methods

Collection and preparation of raw materials

Chicken meat (broiler) was collected from "KR Market", Bangladesh Agricultural University (BAU), Mymensingh. The meat samples were immediately transferred to the "Animal Science Laboratory". Cloves, and garlic were also purchased from the K.R. Market", Bangladesh Agricultural University (BAU), Mymensingh. All necessary instruments and jars were cleaned with hot water and detergent powder, then, dried properly before starting the experimental activities. Cloves were dried properly and the dried cloves were ground using a hand grinder into a fine powder. The fine powder form of CP was stored in a Polyethylene Terephthalate (PET) jar for subsequent use. After cleaning and removing the peel, garlic were taken in a hand grinder to make garlic paste as methods followed by [23]. The garlic paste were packed in low density polyethylene (LDPE) bags and stored in deep freezer at (-18°C ± 1°C) for subsequent use.

Sample preparation

All visible fat and connective tissue of meat were trimmed off as far as possible with the help of knife and the sample was cut into small pieces. Then whole sample was cleaned with distilled water. Four different batches i.e., Control (T_0), T_1, T_2 and T_3 of chicken meat, each contain about 250 gm of meat were prepared. In addition, 0.2% CP, 2% GaP and combination of 0.2% CP and 2% GaP were added to T_1, T_2 and T_3 batches respectively. Control (T_0) was prepared without using any natural preservative. The different groups were packaged in different LDPE bags and stored for 12 days in a refrigerator (4°C ± 1°C). The sample was drawn every alternate day i.e., 0, 3, 6, 9, 12 and analyzed for different physicochemical properties.

Sensory evaluation

Different sensory attributes were examined over the experiment period. A trained 4 member panel was made to evaluate meat the samples. A 5-point balanced semantic scale (weak to strong) was used for sensory evaluation (color, off-odor) where 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor. The judges evaluated the samples based on the above criterions. Panelists were selected among department staff and students and trained according to the American Meat Science Association guidelines [24]. Sensory evaluation was carried out in individual booths under controlled conditions of light, temperature and humidity. All samples were served in the petri dishes. Sensory evaluation was accomplished at 0 day and repeated at 3 day, 6 day, 9 day and 12 day; up to the end of refrigerated storage at 4°C ± 1°C.

Physicochemical properties of meat

The pH was determined with digital pH meter (Seven Easy pH, Mettler-Toledo GmbH, Switzerland) [25]. For this, 10 g of ground sample was homogenized with 50 ml of distilled water using a vortex machine for 1 min and the electrode was dipped into the suspension to note down the PH.

To determine cooking loss, weighed 20 g sample and wrapped in a heat-stable foil paper and kept in water bath at 80°C for 30 minutes. The internal temperature was not measured, but from a previous study it was estimated that the optimum internal meat temperature (75°C to 80°C) would be gained by 30 minutes. Cooking loss was calculated after draining the drip coming from the cooked meat as follows:

$$\text{Cooking loss (\%)} = \frac{W1 - W2}{W1} \text{x } 100$$

Where, W_1=meat weight before cooking and

W_2=meat weight after cooking.

Oxidative stability

Peroxide Value (POV) was determined according to [21]. The sample (3 g) was weighed in a 250 ml glass stopper Erlenmeyer flask and heated in a water bath at 60°C for 3 min to melt the fat, then thoroughly agitated with vortex machine for 3 min with 30 ml acetic acid-chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered with Whatman filter paper number 1 to remove meat particles. Saturated potassium iodide solution (0.5 ml) and starch solution were added to the filtrate. The content was titrated against 0.01 N sodium thiosulphate to get the end point (non-aqueous layer turned to colorless). POV was calculated and expressed as milliequivalent peroxide per kilogram of sample:

$$\text{POV (meq/kg)} = \frac{S \times N}{W} \times 1000$$

Where S is the volume of titration (ml), N is the normality of sodium thiosulfate solution (n=0.01) and W is the sample weight (g).

Free Fatty Acids (FFA) was determined according to Koniecko (1979). 5 g of the sample was weighed in a 250 ml glass stopper Erlenmeyer flask with 30 ml of chloroform, then thoroughly agitated with vortex machine for 1 min. The sample was filtered with Whatman filter paper number 1 to remove meat particles. 5 drops of 1 percent phenolphthalein indicator was added with the filtrate and titrated against 0.1 N alcoholic KOH to get the end point (pink color).

Percent FFA content was calculated as,

$$\text{FFA (\%)} = \frac{0.1 \times \text{mL of 0.1N alc. KOH} \times 0.282}{\text{sample weight (g)}} \times 100$$

TBARS value was determined as per the extraction method described by Witte et al. (1970). For this, 5 g of ground sample was mixed with 25 ml of pre-cooled 20% Trichloroacetic Acid (TCA) solution using vortex machine for 1 min. Then the content was filtered through Whatman filter paper No. 1 to get TCA extract. 2 ml of this TCA extract was mixed with 2 ml of TBA solution (orthophosphoric acid 4.05 ml, TBA 0.09 g, DW 30 ml, for 34.5 ml TBA solution) in small beaker and placed in an oven (100°C) for 30 mins. Then take it in normal temperature. After reducing the temperature TBARS value was measured at fixed wavelength of 532 nm with a scanning range of 531 nm to 533 nm using UV-VIS spectrophotometer (Elico SL-159, Mumbai, India).

Microbial assessment

Total Viable Count (TVC), Total Coliform Count (TCC) and Yeast and Mould Count (TYMC) of the samples were enumerated following the methods as described by American Public Health Association (APHA, 1984). The samples on respective storage days were opened in inoculation laminar flow (Model: RH-58-03.

Rescholar equipment, Ambala, India). About 10 g of sample was blended with 90 ml of sterile 0.1% peptone water in a pestle and mortar and serial dilutions were prepared as per recommendation of International Organization for Standardization (ISO, 1995). Thus 1:10 dilution of the samples was obtained. Later on using whirly mixture machine different serial dilutions ranging from 10^{-2} to 10^{-6} were prepared according to the instruction of the standard method (ISO, 1995). Standard plate counts were determined on Plate Count Agar (PCA), total Coliform count on MacConkey Agar (MA) and plates were incubated at (37°C ± 2°C) for 48 hrs. Yeasts and moulds were determined on Potato Dextrose Agar and plates were incubated at (25°C ± 2°C) for 48 to 72 days. Following incubation, plates exhibiting 30 to 300 colonies were counted. Colonies were counted with the aid of a colony counter. The average number of colonies was multiplied by reciprocal of the dilution and expressed as log 10 cfu/g of sample.

Preparation of media

11.50 g of PCA agar and 15.6 g of MA agar were dissolved in 500 ml and 300 ml of cold distilled water in two separate conical flasks and heated to boiling for dissolving the ingredients completely. In case of PDA, 200 g of previously peeled and sliced potato was taken in 1,000 ml of distilled water and boiled for an hour. After boiling, sieving was done through clean cheesecloth. 20 g of commercial dextrose and 15 g of agar were added to the potato infusion solution and heated up to boiling to dissolve the ingredients completely. Later, the media were sterilized at 121°C (6.795 kg pressure/sq. inch) for 15 minutes in an autoclave. The final reaction was adjusted to pH 7.0 ± 0.1. The agar was then ready for pouring. Before pouring, the medium was kept in a water bath at 45°C.

Statistical model and analysis

The proposed model for the planned experiment was factorial experiment with two factors A (Treatments) and B (Days of Intervals) is:

$$yijk=\mu+Ai+Bj+(AB)ij+\varepsilon ijk \quad i=1,\ldots,a;\ j=1,\ldots,b;\ k=1,\ldots,n$$

Where, yijk=observation k in level i of factor A and level j of factor B

Bμ=the overall mean, Ai=the effect of level i of factor A, Bj=the effect of level j of factor B

Data were statistically analyzed using SAS statistical discovery software, NC, USA. DMRT test was used to determine the significance of differences among treatments means.

Results

Physicochemical quality

The pH values were generally decreased in T_1 and T_2 samples after 3 days of refrigerated storage but these values were significantly ($p<0.05$) increased with the increased storage period (Table 1). The pH values of T_3 and T_4 were gradually increased in compare to 0 day of storage. Throughout the storage period, T_1 maintained lowest pH values than control, T_2 and T_3 samples. Similar findings were observed by Singh et al. [6] in chicken meat products incorporated with clove, garlic and ginger paste during the refrigerated storage. The pH followed an increasing trend throughout the storage period in all the samples. Similar findings were observed in chicken meat products during the refrigerated storage [8,26]. The increase in pH during the storage period might be due to accumulation of metabolites due to growth of Gram-negative bacteria such as *Pseudomonas*, *Moraxella*, *Acinetobacter*, etc., [27,28]. The last pH values increase might have been due to the liberation of ammonia compounds as a result of endoprotease activity or the proteolytic microbial flora present in the raw meat [29].

The cooking loss of different treatments with day's intervals is shown in Table 1. The range of overall observed cooking loss at different treatments was 22.36% to 23.05% and different days of intervals were 14.38% to 29.39%. The different superscripts were observed in all treatment groups that indicates cooking loss content significantly ($P<0.05$) decrease compared to control. Among these treatments, most preferable cooking loss was observed at 2% garlic paste than other groups. The cooking loss was decreased with the increased storage period. The most preferable cooking loss was observed at 12th day and less preferable cooking loss was observed at 0 day observation. Cooking loss refers to the reduction in weight of meat during the cooking process [30]. Major components of cooking losses are thawing, dripping and evaporation. Thawing loss refers to the loss of fluid in meat resulting from the formation of exudates following freezing and thawing [30,31]. Same observation was identified by Kim et al. [32]. Cooking yield is an important data

Table 1: Effect of different natural preservatives on the physico-chemical quality (Mean ± SE) of chicken meat stored at 4°C ± 1°C.

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat	DI	T*DI
pH	0	5.60 ± 0.029	5.59 ± 0.012	5.68 ± 0.018	5.77 ± 0.012	5.66^{d} ± 0.017	P<0.001	P<0.001	P<0.001
	3	5.59 ± 0.012	5.58 ± 0.006	5.73 ± 0.006	5.79 ± 0.012	5.67^{d} ± 0.009			
	6	6.07 ± 0.006	5.87 ± 0.006	5.99 ± 0.012	6.13 ± 0.012	6.02^{c} ± 0.009			
	9	6.69 ± 0.009	6.15 ± 0.006	6.38 ± 0.012	6.41 ± 0.006	6.41^{b} ± 0.008			
	12	6.92 ± 0.012	6.38 ± 0.006	6.53 ± 0.012	6.62 ± 0.006	6.61^{a} ± 0.009			
	Mean	**6.18^{a} ± 0.013**	**5.91^{d} ± 0.007**	**6.06^{c} ± 0.012**	**6.15^{b} ± 0.010**				
Cooking Loss (%)	0	30.77 ± 0.009	28.49 ± 0.006	30.09 ± 0.012	28.23 ± 0.009	29.39^{a} ± 0.009	P<0.001	P<0.001	P<0.001
	3	29.51 ± 0.006	25.50 ± 0.006	28.90 ± 0.012	25.45 ± 0.006	27.34^{b} ± 0.008			
	6	23.35 ± 0.006	23.35 ± 0.012	21.35 ± 0.006	21.10 ± 0.012	22.43^{c} ± 0.009			
	9	20.03 ± 0.009	20.65 ± 0.009	18.95 ± 0.006	20.25 ± 0.009	19.97^{d} ± 0.008			
	12	11.01 ± 0.009	15.99 ± 0.009	12.51 ± 0.006	18.01 ± 0.006	14.38^{e} ± 0.007			
	Mean	**23.05^{a} ± 0.007**	**22.79^{b} ± 0.008**	**22.360^{d} ± 0.008**	**22.61^{c} ± 0.008**				

Mean in each row and column having different superscript varies significantly at values *P<0.05. Again, mean values having same superscript in each row did not differ significantly at P>0.05. T_0=Control group, T_1=0.2% clove treated samples, T_2=2% garlic treated samples, T_3=combined (0.2% clove + 2% garlic) treated samples, DI=Days of Intervals, Treat=Treatment, T*DI=Interaction of Treatment and Days of Intervals

that are used by the meat industry to predict the behavior of their products during processing [33]. The cooking yield of the Kung-Wan significantly decreased with higher natural antioxidant extract levels [34]. The meat also tended to shrink during the cooking process due to the denaturation of meat protein; the loss of water and fat also contributed to the shrinking process [35].

Oxidative stability

Peroxide Value (POV) of control sample remained significantly higher on day 3, 6, 9 and 12 as compared to natural preservative treated samples (Table 2). Within the treated batches PV did not vary significantly. CP batch (T_1) showed non-significantly lower POV than C batch (T_0) and garlic paste batches (T_2) also non-significantly lower PV than CP batch. Among these four treatments most preferable peroxide value was observed from the combine CP and GaP batch (T_3). The lowest amount peroxide value indicates this product is most preferable for consumer's health. Less preferable peroxide value was observed from control group. Peroxide value of control and treatments showed a significant difference (P<0.05) in between the storage period. Similar, results were reported [6], who found non-significantly lower peroxide value with the storage period in chicken meat incorporated with CP, GaP and GinP. Al-Kutby [36] reported that retarded lipid oxidation rate was found when Applications of Cinnamon, clove, and sumac alcoholic extracts to improve microbial safety and shelf-life of cooked, high fat meat products (doner kebab). Longer storage time can increase the peroxide value [37]. Meat with higher lipid oxidation values also showed higher protein oxidation and greater metmyoglobin formation [38]. Higher peroxide values may also be obtained for any extremely rancid products, again because the peroxides initially formed have all undergone further oxidation reactions [39,40].

In the beginning of the storage i.e., day 1, Free Fatty Acid (FFA) content was almost similar in T_1 and T_2 samples whereas the control sample showed a significantly higher (P<0.05) value than T_3 batch (Table 2). On all the storage days FFA was significantly higher (P<0.05) in control as compared to treated batches. Among the treated batches, combination of clove powder and garlic paste batch (T_3) showed significantly (P<0.05) lower FFA during all the storage intervals up to day 12. This may be due to possible low lipolysis and lipolytic enzyme activity, leading to low production of free fatty acids [41]. In general FFA increased as the storage period progressed. Das et al. [42] reported increasing trend of FFA during refrigeration storage of raw ground meat for 9 days. Other workers also suggested similar trends in FFA of chicken meat products and goat meat products during 9 days of refrigeration storage [8,26,43].

TBARS value was significantly lower (P<0.05) in T_1 and T_3 as compared to control and T_2 at 12th days of storage (Table 2). Among these five treatments most preferable TBA value was observed from 0.2% clove powder group. Less preferable TBA value was observed from controlled group. The highest amount of peroxide value indicates that this product is less preferable. Clove powder (T_1) maintained lowest TBARS value in all the storage intervals till the end of the storage among the natural preservatives tried. The finding is very similar in accordance with the study of Vasavada et al. [44] who also documented that antioxidant activity of cloves in cooked ground beef (stored at 2°C for 15 days) was highest in terms of TBARS value than ginger, cinnamon, caraway, fennel, nutmeg and other spices. Same observation also found by Singh et al. [6] in raw chicken meat emulsion and Shan et al. [20] in raw pork. Bali et al. [10] also observed significant increase in TBARS value of chicken sausages (stored at 4°C ± 1°C for 21 days) incorporated with garlic and coriander. Addition of fresh garlic paste to chicken sausage (stored at 3°C for 21 days) significantly delayed lipid oxidation than the control samples [21]. Istrati et al. was found all marinating treatments resulted in significantly lower TBARS. TBA value significantly increased throughout the storage period as concluded by Biswas et al. [8].

Table 2: Effect of different natural preservatives on the oxidative stability (Mean ± SE) of chicken meat stored at 4°C ± 1°C.

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat	DI	T*DI
POV(mg/kg)	0	1.21 ± 0.006	1.31 ± 0.150	1.17 ± 0.150	0.86 ± 0.150	1.14^{b} ± 0.075	P<0.001	P<0.001	P<0.001
	3	1.83 ± 0.009	1.46 ± 0.150	1.29 ± 0.150	0.93 ± 0.150	1.38^{a} ± 0.075			
	6	1.93 ± 0.009	1.52 ± 0.150	1.33 ± 0.150	0.98 ± 0.150	1.44^{a} ± 0.075			
	9	2.00 ± 0.006	1.57 ± 0.150	1.40 ± 0.150	1.11 ± 0.150	1.52^{a} ± 0.075			
	12	2.12 ± 0.063	1.64 ± 0.150	1.45 ± 0.150	1.17 ± 0.150	1.42^{a} ± 0.075			
	Mean	**1.83^{a} ± 0.019**	**1.50^{ab} ± 0.067**	**1.33^{b} ± 0.067**	**1.01^{c} ± 0.067**				
FFA (%)	0	0.17 ± 0.001	0.11 ± 0.001	0.11 ± 0.002	0.06 ± 0.001	0.11^{e} ± 0.001	P<0.001	P<0.001	P<0.001
	3	0.23 ± 0.001	0.17 ± 0.001	0.15 ± 0.001	0.07 ± 0.002	0.16^{d} ± 0.002			
	6	0.28 ± 0.002	0.18 ± 0.001	0.16 ± 0.001	0.11 ± 0.001	0.12^{c} ± 0.002			
	9	0.31 ± 0.001	0.21 ± 0.001	0.19 ± 0.001	0.13 ± 0.001	0.21^{b} ± 0.001			
	12	0.37 ± 0.001	0.29 ± 0.001	0.23 ± 0.001	0.16 ± 0.001	0.26^{a} ± 0.001			
	Mean	**0.27^{a} ± 0.002**	**0.19^{b} ± 0.001**	**0.17^{c} ± 0.001**	**0.11^{d} ± 0.001**				
TBARS (mg MDA/kg)	0	0.186 ± 0.002	0.111 ± 0.001	0.174 ± 0.001	0.163 ± 0.001	0.158^{c} ± 0.002	P<0.001	P<0.001	P<0.001
	3	0.188 ± 0.001	0.111 ± 0.001	0.176 ± 0.001	0.165 ± 0.001	0.160^{d} ± 0.001			
	6	0.188 ± 0.001	0.113 ± 0.001	0.178 ± 0.001	0.167 ± 0.002	0.161^{c} ± 0.002			
	9	0.191 ± 0.001	0.115 ± 0.001	0.181 ± 0.001	0.168 ± 0.001	0.164^{b} ± 0.001			
	12	0.319 ± 0.001	0.212 ± 0.001	0.302 ± 0.001	0.267 ± 0.001	0.275^{a} ± 0.001			
	Mean	**0.214^{a} ± 0.002**	**0.132^{d} ± 0.001**	**0.202^{b} ± 0.001**	**0.186^{c} ± 0.002**				

Mean in each row and column having different superscript varies significantly at values *P<0.05. Again, mean values having same superscript in each row did not differ significantly at P>0.05. T_0=Control group, T_1=0.2% clove treated samples, T_2=2% garlic treated samples, T_3=combined (0.2% clove + 2% garlic) treated samples, DI=Days of Intervals, Treat=Treatment, T*DI=Interaction of Treatment and Days of Intervals, POV=Peroxide Value, FFA=Free Fatty Acid, TBARS=Thiobarbituric acid value

Microbiological assessments

Total viable counts were affected significantly (P<0.05) by mixing natural preservatives compared with Control group (Table 3). The increase in the number of microorganisms in the treated samples with preservatives was significantly (P<0.05) less than control. Among the three different treated batches, there was significant difference on day 0, 3, 6, 9 and 12. However, at the end of the storage T_3 showed the lowest microbial load (Table 3). This indicates that clove and garlic are effective in checking the microbial growth during the storage period but there combination was proved to be a preferred preservative ingredient. The anti-microbial activity of clove and garlic blocked the deteriorating of fat and helped prevent the metabolism of fat by bacteria. As a result, bacterial growth was lower in treated group. Results also showed that combination of CP and GaP had better antimicrobial capacity than control sample, and individual (0.2% CP, 2% GaP) had possible synergistic effect on microbial inhibition. Similar result was found combined with electrolyzed NaCl solution and essential oil compounds on carp fillets [45]. Georgantelis et al. [46] also reported that, in pork sausages, the lowest microbial counts were obtained in samples containing chitosan and rosemary, indicating a possible synergistic effect.

Among the different treated batches, there was significant difference in coliform count on day 0, 3, 6, 9 and 12. It was further noticed that coliform count did not vary significantly (P>0.05) among T_1 and T_2 batches with marginally lower values in T_3 batches. Throughout the storage period, comparatively lower values of coliform counts were detected in combine (0.2% CP, 2% GaP) batch than CP and GaP batches which is in accordance with the findings, who revealed that out of ground clove, fresh garlic and red chilli exhibited strongest antimicrobial systems in broth model systems [47]. The results of the study revealed a significant (P<0.05) difference in TCC among storage period and among treatments. A number of studies have demonstrated that compounds existing in many spices also possess antimicrobial activity. Smith-Palmer et al. [13] showed that the oils of bay, cinnamon, clove and thyme were the most inhibitory against five important food-borne pathogens, *Campylobacter jejuni*, Salmonella enteritidis, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*. In general, Gram-positive bacteria were more sensitive to inhibition by plant essential oils than the Gram-negative bacteria. *Campylobacter jejuni* was the most resistant of the bacteria investigated to plant essential oils, with only the oils of bay and thyme having a bactericidal concentration of less than 1%.

Among four treatments, the yeast and mold counts in the control sample (2.06 log 10 CFU/g) was significantly higher than in the samples treated with 0.2% CP, 2% GaP and combination of 0.2% CP and 2% GaP respectively. During storage TYMC was increased gradually in different treatments at increasing storage days. But the same superscript in T_1 and T_3 batches indicates that there was no significant difference among these group where in T_3 batch shows significantly (P<0.05) lower TYMC than other treatment batches (Table 3). The antimicrobial activity of CP and GaP blocked the deteriorating of fat and helped prevent the metabolism of fat by bacteria. The results of the study revealed a significant (P<0.05) difference in yeast and mold counts among storage period and among treatments and Yeast and mold count increased significantly (P<0.05) with storage period. Bali et al. [10] also observed that in chicken sausages (incorporated with garlic and coriander and stored at (4°C ± 1°C) for 21 days) yeast and mould were not detected initially but after 7 days onwards there was significant increase in all the groups throughout the storage period. Asha et al. [48] discovered that spice essential oil was also effective in increasing the shelf life of minced meat to six days when stored at (4°C ± 1°C). Sunil [11] had reported an extended shelf life in buffalo meat mince treated with clove essential oil stored at refrigeration temperature as compared to control samples. Kumar and Berwal [16] also found the Minimum inhibitory concentration (MIC) of garlic at 80% inhibition level was calculated for yeast and mold and other pathogenic bacteria.

Table 3: Effect of different natural preservatives on different microbial population (Mean ± SE) of chicken meat stored at 4°C ± 1°C.

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat	DI	T*DI
TVC (log CFU/g)	0	5.44 ± 0.009	5.32 ± 0.009	5.24 ± 0.006	4.90 ± 0.009	5.23^{e} ± 0.008	P<0.001	P<0.001	P<0.001
	3	5.48 ± 0.012	5.36 ± 0.006	5.31 ± 0.012	5.00 ± 0.006	5.29^{d} ± 0.009			
	6	5.52 ± 0.006	5.41 ± 0.009	5.41 ± 0.009	5.17 ± 0.006	5.38^{c} ± 0.008			
	9	5.78 ± 0.009	5.55 ± 0.012	5.58 ± 0.006	5.49 ± 0.012	5.59^{b} ± 0.010			
	12	5.96 ± 0.006	5.69 ± 0.009	5.72 ± 0.006	5.65 ± 0.006	5.76^{a} ± 0.007			
	Mean	**5.64^{a} ± 0.008**	**5.47^{b} ± 0.009**	**5.45^{c} ± 0.008**	**5.24^{d} ± 0.004**				
TCC (log CFU/g)	0	2.48 ± 0.006	2.25 ± 0.006	2.34 ± 0.006	2.35 ± 0.018	2.36^{e} ± 0.009	P<0.001	P<0.001	P<0.001
	3	2.44 ± 0.012	2.31 ± 0.012	2.38 ± 0.006	2.40 ± 0.009	2.38^{d} ± 0.010			
	6	2.51 ± 0.009	2.34 ± 0.012	2.45 ± 0.009	2.42 ± 0.009	2.43^{c} ± 0.010			
	9	2.75 ± 0.009	2.90 ± 0.009	2.73 ± 0.006	2.68 ± 0.006	2.77^{b} ± 0.008			
	12	2.93 ± 0.006	3.03 ± 0.009	2.92 ± 0.006	2.86 ± 0.006	2.93^{a} ± 0.007			
	Mean	**2.62^{a} ± 0.008**	**2.57^{b} ± 0.010**	**2.57^{b} ± 0.007**	**2.54^{c} ± 0.010**				
TYMC (log CFU/g)	0	2.70 ± 0.006	2.50 ± 0.012	2.45 ± 0.006	2.45 ± 0.006	2.53^{e} ± 0.008	P<0.001	P<0.001	P<0.001
	3	3.05 ± 0.012	2.76 ± 0.006	2.59 ± 0.006	2.69 ± 0.006	2.77^{d} ± 0.008			
	6	3.28 ± 0.009	2.91 ± 0.006	2.65 ± 0.012	2.86 ± 0.009	2.93^{c} ± 0.009			
	9	3.38 ± 0.006	2.93 ± 0.009	2.78 ± 0.006	3.00 ± 0.006	3.02^{b} ± 0.007			
	12	3.51 ± 0.006	3.11 ± 0.012	2.91 ± 0.006	3.18 ± 0.012	3.18^{a} ± 0.009			
	Mean	**3.18^{a} ± 0.008**	**2.84^{b} ± 0.009**	**2.68^{c} ± 0.007**	**2.84^{b} ± 0.008**				

Mean in each row and column having different superscript varies significantly at values *P<0.05. Again, mean values having same superscript in each column did not differ significantly at P>0.05. T_0=Control group, T_1=0.2% clove treated samples, T_2=2% garlic treated samples, T_3=combined (0.2% clove + 2% garlic) treated samples, DI=Days of Intervals, Treat=Treatment, T*DI=Interaction of Treatment and Days of Intervals

Sensory evaluation

The color was affected significantly (P<0.05) by treated groups compared with control group (Table 4). The different superscripts were observed in all treatments groups which indicate there were significant (P<0.05) difference of color content. In different treatment groups color content significantly (P<0.05) decreased but in the garlic treated group color content decreased rapidly. In 0.2% CP treated sample was maintain desirable color than other treatment groups. A decrease in appearance and color scores of meat products with increase in storage period was also reported [49-51]. It was obvious that lightness values increased significantly (P<0.05) in all meat samples. Some authors reported that lightness values in meat and meat products are related to surface water, water vapor exchanges between the products and the environment and modifications of the different states of the heme pigments [52]. The decreased color test scores during storage resulted from the denaturation of proteins, particularly the myofibrillar protein (actin and myosin) that affects gel formation [53].

The range of odor score among four treatments was 3.51 to 4.19. There were significant (P<0.05) difference in odor scores of all treatment. The range of odor among different days of intervals was 3.09 to 4.76. It showed that the quality was deteriorated with increased storage period. The most preferable odor was observed from 2% garlic paste treatment and the undesirable off odor from control group. The observed odor scores were in agreement with the indicators for lipid oxidation (TBARS, PV, and FFA) in raw chicken meat. Throughout the storage period, both color and odor scores declined linearly. Similar trends were observed, in tocopherol preblended ground chevon (stored at 4°C ± 1°C for 9 days) [26]. The odor score of different treatments was decreased with increased storage period. Similar value was observed by Raghavan and Richards (2007). The progressive decrease in odor could be correlated to increase in TBARS values of meat products stored under aerobic conditions. Flavor is one of the major causes of quality deterioration because it can negatively affect sensory attributes such as color, texture, and odor as well as the nutritional quality of the product [54-58].

Conclusion

From the findings results of the present study, it may be concluded that clove and garlic will be used in future for manufacturing meat with providing antioxidant and antimicrobial agents as value addition through inhibiting lipid oxidation and prolonged the shelf- life of stored meat and meat products instead of synthetic antioxidant. But there combination (0.2% clove powder and 2% garlic paste) is more effective and produce better results in terms of physico-chemical characteristics, oxidative stability and microbiological parameters during raw meat preservation. Therefore, meat industry can effectively combination of 0.2% clove powder and 2% garlic paste to improve color and minimize oxidation-induced deteriorative changes in raw chicken meat emulsion without compromising the sensory attributes.

Table 4: Effect different natural preservatives on sensory parameters (Mean ± SE) of chicken meat stored at 4°C ± 1°C.

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat	DI	T*DI
Color	0	4.91 ± 0.009	4.76 ± 0.006	4.71 ± 0.006	4.76 ± 0.006	4.78^{a} ± 0.007	P<0.001	P<0.001	P<0.001
	3	4.51 ± 0.012	4.65 ± 0.009	4.57 ± 0.009	4.64 ± 0.009	4.59^{b} ± 0.010			
	6	4.11 ± 0.009	4.20 ± 0.009	4.16 ± 0.006	4.15 ± 0.009	4.16^{c} ± 0.008			
	9	3.56 ± 0.009	3.76 ± 0.006	3.31 ± 0.006	3.60 ± 0.012	3.56^{d} ± 0.008			
	12	3.43 ± 0.008	3.65 ± 0.009	2.56 ± 0.008	3.23 ± 0.012	3.22^{e} ± 0.009			
	Mean	**4.11^{b} ± 0.009**	**4.20^{a} ± 0.008**	**3.86^{d} ± 0.007**	**4.08^{c} ± 0.010**				
Odor	0	4.82 ± 0.012	4.71 ± 0.009	4.79 ± 0.006	4.72 ± 0.006	4.76^{a} ± 0.008	P<0.001	P<0.001	P<0.001
	3	4.39 ± 0.006	4.43 ± 0.010	4.60 ± 0.006	4.52 ± 0.009	4.49^{b} ± 0.008			
	6	3.81 ± 0.006	4.11 ± 0.012	4.23 ± 0.012	4.13 ± 0.012	4.07^{c} ± 0.010			
	9	2.53 ± 0.017	3.50 ± 0.006	3.75 ± 0.006	3.71 ± 0.006	3.37^{d} ± 0.009			
	12	1.98 ± 0.009	3.33 ± 0.009	3.58 ± 0.009	3.48 ± 0.006	3.09^{e} ± 0.008			
	Mean	**3.51^{d} ± 0.010**	**4.02^{c} ± 0.009**	**4.19^{a} ± 0.008**	**4.11^{b} ± 0.008**				

Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair, and 1 for poor. Mean in each row and column having different superscript varies significantly at values *P<0.05. T_0=Control group, T_1=0.2% clove treated samples, T_2=2% garlic treated samples, T_3=combined (0.2% clove + 2% garlic) treated samples, DI=Days of Intervals, Treat=Treatment, T*DI=Interaction of Treatment and Days of Intervals

References

1. Biswas S, Das AK, Banerjee R, Sharma N. Effect of electrical stimulation on quality of tender stretched chevon sides. Meat Sci. 2007;75(2);332-6.
2. Zhou GH, Xu XL, Liu Y. Preservation technologies for fresh meat - A review. Meat Sci. 2010;86:119-28.
3. Breidenstein BC. Nutrient composition: Nutrient value of meat. Food Nutr News. 1987;59:43-58.
4. Johnson AR. The nutrient composition of Australian meats and poultry: A preface. Food Technol Aust. 1987;39:183-4.
5. Robinson F. The nutritional contribution of meat to the British diet: Recent trends and analyses. Nutr Bull. 2002;26(4):283-93.
6. Singh P, Sahoo J, Chatli MK, Biswas AK. Shelf life evaluation of raw chicken meat emulsion incorporated with clove powder, ginger and garlic paste as natural preservatives at refrigerated storage (4 ± 1°C). Int Food Res J. 2014;21(4):1363-73.
7. Verma AR, Vijayakumar M, Mathela CS, Rao CV. In vritro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem Toxicol. 2009;47(9):2196-201.
8. Biswas AK, Chatli MK, Sahoo J, Singh J. Storage stability of chicken meat patties, balls and nuggets incorporated with eugenol and chitosan at refrigeration temperature (4 ± 1°C) under aerobic packaging condition. Ind J Poult Sci. 2012;47(3):348-56.
9. Valencia I, Ansonera D, Astiasaran I. Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae Schizochytrium sp: Influence on nutritional properties, sensorial quality and oxidation stability. Food Chem. 2007;104(3):1087-96.
10. Bali A, Das SK, Khan A, Patra D, Biswas S, Bhattacharyya D. A comparative

study on the antioxidant and antimicrobial properties of garlic and coriander on chicken sausage. Int J Meat Sci. 2011;1(2):108-16.

11. Sunil B. Antimicrobial efficacy of bio preservatives in buffalo meat mince. PhD thesis. Izatnagar, India: Deemed University, IVRI; 2006. p. 178.
12. Kumudavally KV, Padmini S, Radhakrishna K, Bawa AS. Effect of surface treatment of fresh mutton with spices on lipolytic bacteria, Enterobacteriaceae and their degradation products during storage. J Food Sci Technol. 2005;42(3):249-53.
13. Smith-Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett appl microbiol. 1998;26(2):118-22.
14. Sharma K, Verma BB. Herbs and spices, apotential tool for preservation of dairy products. Indian Food Industry. 2006;25:36-8.
15. Jackson R, McNeil B, Taylor C, Holl G, Ruff D, Gwebu ET. Effect of aged garlic extract on casepase-3 activity, in vitro. Nutr Neurosci. 2002;5(4):287-90.
16. Kumar M, Berwal JS. Sensitivity of food pathogens to garlic(Allium sativum). J Appl Microbiol. 1998;84(2):213-5.
17. Rahman MM, Fazlic V, Saad NW. Antioxidant properties of raw garlic (Allium sativum) extract. Int Food Res J. 2012;19(2):589-91.
18. Aguirrezabal MM, Mateo J, Domiguez MC, Zumalacarregui JM. Compounds of technological interest in the dry sausage manufacture. Sci Aliments. 1998;18:409-14.
19. Kumar D, Tanwar VK. Utilization of clove powder as phytopreservative for chicken nuggets preparation. J Stored Postharvest Res. 2011;2:11-4.
20. Shan B, Cai YZ, Brooks JD, Corke H. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. J Sci Food Agric. 2009;89(11):1879-85.
21. Sallam KI, Ishioroshi M, Samejima K. Antioxidants and antimicrobial effects of garlic in chicken sausage. Lebenson Wiss Technol. 2004;37(8):849-55.
22. Yin MC, Cheng WS. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Sci. 2003;63:23-8.
23. Akhtar J, Omre PK, Alam MA. Effect on rquality of developed ginger-garlic paste during storage. Int J Appl Pure Sci Agric. 2015;1:32-41.
24. AMSA. Research guidelines for cookery, sensory evaluation, and tenderness measurements of fresh meat. US: Chicago III: American Meat Science Association and Nutritional Livestock and Meat Board. 1995.
25. Trout ES, Hunt MC, Johnson DE, Claus JR, Kastner CL, Kropf DH. Characteristics of low fat ground beef containing texture-modifying ingredients. J Food Sci. 1992;57:19-24.
26. Verma SP, Sahoo J. Improvement in the quality of ground chevon during refrigerated storage by tocopherol acetate preblending. Meat Sci. 2000;56(4):403-13.
27. Kirsch RH, Berry FE, Baldwin CL, Foster EM. The bacteriology of refrigerated ground beef. Food Res. 1952;17(1-6):495-503.
28. McDowell DA, Hobson I, Strain JJ, Owens JJ. Bacterial microflora of chill stored beef carcasses. J Environ Health. 1986;95:65-8.
29. Mokhtar S, Mostafa G, Taha R, Eldeep GSS. Effect of different starter cultures on the biogenic amines production as a critical control point in fresh fermented sausages. Eur Food Res Technol. 2012;235(3):527-35.
30. Jama N, Muchenje V, Chimonyo M, Strydom PE, Dzama K, Raats JG. Cooking loss components of beef from Nguni, Bonsmara and Angus steers. Afr J Agr Res. 2008;3(6):416-20.
31. Muchenje V, Dzama K, Chimonyo M, Strydom PE, Hugo A, Raats JG. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chem. 2009;112(2):279-89.
32. Kim GD, Jung EY, Lim HJ, Yang HS, Joo ST, Jeong JY. Influence of meat exudates on the quality characteristics of fresh and freeze-thawed pork. Meat Sci. 2013;95(2):323-9.
33. Ulu H. Effects of carrageenam and guar gum on the cooking and textural properties of low fat meatballs. Food Chem. 2006;95(4):600-5.
34. Hsu SY, Sun LY. Comparison on 10 non- meat protein fat substitutes for low-fat Kung-Wans. J Food Eng. 2006;74:47-53.
35. Serdaroglu M, Yildiz-Turp G, Abrodimov K. Quality of low-fat meatballs containing Legume flours as extenders. Meat Sci. 2004;70:99-105.
36. Al-Kutby S. Applications of spice extracts and other hurdles to improve microbial safety and shelf-life of cooked, high fat meat products (doner kebab). Plymouth: University of Plymouth; 2012.
37. Novelli E, Zanardi E, Ghiretti GP, Campanini G, Dazzi G, Madarena G, et al. Lipid and cholesterol oxidation in frozen stored pork, salame Milano and mortadella. Meat Sci. 1998;48(1-2):29-40.
38. Insani EM, Eyherabide A, Grigioni G, Sancho AM, Pensel NA, Descalzo AM. Oxidative stability and its relationship with natural antioxidants during refrigerated retail display of beef produced in argentina. Meat Sci. 2008;79(3):444-52.
39. Levermore R. Rancidity in fresh and stored pork products. Meat International. 2004;14:16-8.
40. Gotoh N, Wada S. The importance of peroxide value in assessing food quality and food safety. J Am Oil Chem Soc. 2006;83(5):473-4.
41. Aguirrezabal MM, Mateo J, Dominguez MC, Zumalacarregui JM. The effect of paprika, garlic and salt on rancidity in dry sausages. Meat Sci. 2000;54:77-81.
42. Das AK, Rajkumar V, Dwivedi DK. Antioxidant effect of curry leaf (Murraya koenigii) powder on quality of ground and cooked goat meat. Int Food Res J. 2011;18:563-9.
43. Das AK, Anjaneyulu ASR, Verma AK, Kondaiah N. Physicochemical, textural, sensory characteristics and storage stability of goat meat patties extended with full-fat soy paste and soy granules. Int J Food Sci Technol. 2008;43:382-92.
44. Vasavada MN, Dwivedi S, Cornforth D. Evaluation of garam masala spices and phosphates as antioxidants in cooked ground beef. J Food Sci. 2006;71(5):292-7.
45. Mahmoud BS, Yamazaki K, Miyashita K, Kawai Y, Shin IS, Suzuki T. Preservative effect of combined treatment with electrolyzed NaCl solutions and essential oil compounds on carp fillets during convectional air-drying. Int J Food Microbiol. 2005;106(3):331-7.
46. Georgantelis D, Blakes G, Katikou P, Ambrosiadis I, Fletouris DJ. Effect of rosemary extract, chitosan and a-tocopherol on lipid oxidation and colour stability during frozen storage of beef burgers. Meat Sci. 2007;75(2):256-64.
47. Leuschner RG, Lelsch V. Antimicrobial effects of garlic, clove and red hot chilli on Listeria monocytogenes in broth model systems and soft cheese. Int J Food Sci Nutr. 2003;54(2):127-33.
48. Asha K, Sunil B, George G, Prejit. Effect of clove on the bacterial quality and shelf life of chicken meat. J Meat Sci Technol. 2014;2(2):37-9.
49. Kandeepan G, Anjaneyulu ASR, Kondaiah N, Mendiratta SK. Quality of buffalo meat keema at different storage temperature. Afr J Food Sci. 2010;4(6):410-7.
50. Chidanandaiah KRC, Sanyal MK. Effect of sodium alginate coating with preservatives on the quality of meat patties during refrigerated (4 ± 1°C) storage. J Muscle Foods. 2009;20(3):275-92.
51. Kilinc B. Microbiological, sensory and color changes of anchovy (Engraulis encrasicholus) patties during refrigerated storage. J Muscle Foods. 2009;20(2):129-37.

52. Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Perez-Alvarez JA, Kuri V. Antioxidant and antibacterial activities of natural extracts: Application in beef meatballs. Meat Sci. 2011;69(3):371-80.

53. Agustini TW, Darmanto YS, Putri DPK. Evaluation on utilization of small marine fish to produce surimi using different cryoprotective agents to increase the quality of surimi. J Coast Develop. 2008;11:131-40.

54. Naidu AS. Natural food antimicrobial systems. 1st ed. USA: CRC Press, Boca Raton FL; 2000.

55. Norrung B, Buncic S. Microbial safety of meat in the European Union. Meat Sci. 2008;78(1-2):14-8.

56. Park SY, Yoo SS, Eun JB, Lee HC, Kim YJ, Chin KB. Evaluation of lipid oxidation and oxidative products as affected by pork meat cut, packaging, method and storage time during frozen storage (-10°C). J Food Sci. 2007;72(2):114-9.

57. Singh P, Sahoo J, Talwar G, Chatli MK, Biswas AK. Development of chicken meat caruncles on the basis of sensory attributes: Process optimization using response surface methodology. J Food Sci Technol. 2015;52(3):1290-303.

58. Whitney EN, Rolfes SR. Understanding nutrition. 9th ed. Australia: Wadsworth; 2002.