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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)

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Abstract

The present study was conducted to study the shelf life evaluation of raw chicken meat emulsion incorporated with clove powder, ginger and garlic paste at refrigerated storage (4±1ºC). Four different batches of chicken meat emulsion i.e. C = Control (without natural preservatives), T_1 = 0.2% clove powder, T_2 = 3% ginger paste and T_3 = 2% garlic paste, were analyzed during refrigerated storage (4±1ºC) of 9 days under aerobic packaging. Throughout the storage, free fatty acids and peroxide value was significantly higher ($P < 0.05$) in control than T_1, T_2 and T_3 batches. Among the treated batches, garlic paste batch (T_3) showed significantly ($P < 0.05$) lower free fatty acid values during the storage period. Thiobarbituric acid value (TBA) did not vary significantly among the treated batches but clove powder (T_1) maintained lowest TBA value till the end of storage. 1, 1-diphenyl-2-picrylhydrazyl (DPPH % inhibition) radical scavenging activity and 2-2-azinobis-3ethylbenthiazoline-6-sulphonic acid (ABTS % inhibition) radical scavenging activity was significantly higher ($P < 0.05$) in T_1 batch as compared to control, T_2 and T_3 batches throughout the storage interval. L^* value did not vary significantly among different batches, while a^* value was found to be significantly higher ($P < 0.05$) in T_1 batch. Percentage metmyoglobin was significantly lower for T_1 batch than control, T_2 and T_3 batches during storage. Colour and odour scores of T_1 batch were significantly higher ($P < 0.05$) than control, T_2 and T_3 batches. Standard plate count was significantly higher ($P < 0.05$) in control than T_1, T_2 and T_3 batches. Throughout the storage period, comparatively lower values of both standard plate count and coliform counts were detected in T_1 batch than control, T_2 and T_3 batches. From this comparative study of natural preservatives, it was concluded that 0.2% clove powder could be utilized effectively as antioxidant and antimicrobial in raw chicken meat emulsion.

Introduction

Meat forms an essential part of non-vegetarian diet. 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 (Singh *et al*., 2013). Meat purchasing decisions are influenced by colour more than any other quality factor because it is an indicator of freshness and wholesomeness (Mancini and Hunt, 2005). Colour, microbial growth and lipid oxidation are important factors for the shelf-life and consumer acceptance of fresh meat (Jakobsen and Bertelsen, 2000). Lipid oxidation causes deterioration of meat by adversely affecting its colour, flavour and nutritional value. During lipid oxidation, the decrease in nutritional value occurs due to loss of essential fatty acids and vitamins and generation of toxic products such as malonaldehyde and cholesterol oxidation products (Tang *et al.*, 2001). Numerous factors affect lipid oxidation such as 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 (Biswas *et al.*, 2012). Various synthetic chemicals such as butylated hydroxyanisole (BHA), butylated hydroxytoulene (BHT), tertiary butyl hydroquinone (TBHQ) etc. are being used as antioxidant and antimicrobial agents to combat the above mentioned problems (Valencia *et al*., 2007). However, the use of synthetic compounds is quite debatable because of their ill effects on human health. This has revived the search for natural preservatives having both antioxidant and antimicrobial activities such as clove powder, ginger, garlic, chitosan, oregano oil, green tea, cloud berry, beetroot, willow herb, rosemary, clove and red chilli etc. for maintaining meat quality, extending shelf-life and preventing economic loss.

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Clove is a dried floral bud of *Syzygium aromaticum* and is known to have antioxidant and antimicrobial activity for long time due to its active ingredient - eugenol (Sofia *et al.*, 2007; Cort, 1974). Ginger (*Zingiber officinale*) being rich in polyphenolic compounds, has high antioxidant (Stoilova *et al*., 2007) and antimicrobial activity (Gupta and Ravishankar, 2005). Besides it is also used for the treatment of a wide spectrum of affections including atherosclerosis, rheumatoid arthritis, high cholesterol, ulcers and impotence (Liang, 1992). Garlic (*Allium sativum*) is always appreciated for its flavour enhancing and medicinal properties. It has potent antioxidant (Jackson *et al.*, 2002), antimicrobial (Kumar and Berwal, 1998 and Naidu, 2000), lipid lowering, antithrombotic, anti-blood coagulation, anti-hypertension and anti-carcinogenic activity (Rahman *et al*., 2012). Though garlic contains various bio-active substances such as allicin, diallyl sulfide, allyl sulfide and propyl sulfide, but allicin is the principal ingredient. In addition, it also contains ascorbic acid, nitrates and nitrites (Aguirrezabal *et al*., 1998). Various scientific studies have documented the use of these preservatives in meat systems viz. clove powder in chicken nuggets (Kumar and Tanwar, 2011), 2% ginger extract and 0.2% clove powder in chevon patties (Raj *et al.*, 2005), clove powder in pork (Shan *et al.*, 2009), 2.5% v/v ginger extract in smoked spent hen meat (Naveena *et al*., 2001), 6% ginger paste in chicken meat balls (Rongsensusang *et al.*, 2005), 3% garlic juice in emulsified sausage (Park and Kim, 2009), fresh garlic in raw chicken sausages (Sallam *et al*., 2004), garlic in ground beef (Yin and Cheng, 2003) have been documented individually but their comparative role in meat products is yet to be ascertained fully. 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

Raw materials

The white Leghorn layer spent hens (80-100 weeks old) were obtained from poultry farm of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana and slaughtered as per standard procedure in the experimental slaughterhouse of Department of Livestock Products Technology, GADVASU, Ludhiana, Punjab. The dressed layer carcasses were chilled at 4±1ºC for 18 hrs. and then after deboning manually, these were packed in low density polyethylene (LDPE) bags and stored in deep freezer at -18±1°C for subsequent use. Frozen meat samples were taken out and after partial thawing in a refrigerator (4±1°C), these meat cubes were tenderized by dipping in a solution containing 0.25% papain (w/w) and 0.15 M calcium chloride (w/v) for about 36-40 hrs at refrigeration temperature (4±1°C) (Biswas *et al*., 2009). Thereafter the meat was taken out from the solution, washed thoroughly 2-3 times with running water; extra moisture was drained out, then packed in LDPE bags and kept at -18±1°C for subsequent use. Frozen tenderized meat sample was taken out and after partial thawing in a refrigerator (4±1°C), the meat chunks were double minced using 6 mm and 4 mm grinder plates (KL-32, Kalsi, Ludhiana, India) to get fine tenderized minced chicken meat (TMCM). Refined wheat flour, salt, sugar, refined oil, baking powder, cloves, ginger and garlic were purchased from the local market of Ludhiana, India. Clove powder (CP) was obtained by grinding the good quality cloves in a grinder (Inalsa Maxie plus, 07120219, Inalsa Technologies, New Delhi, India) and sieved through a fine mesh. The fine powder form of CP was stored in a Polyethylene Terephthelate (PET) jar for subsequent use. Ginger paste (GiP) and Garlic paste (GaP) were prepared separately after washing, peeling and mincing the ginger and garlic respectively in a grinder. Tapioca starch (Appearance = white, moisture = 13.5%, protein = 0.3%, fibre = 0.05%, ash = 0.35%, Iron = 35 ppm) was purchased from Shubham Starch Chemical Pvt. Limited, Faridabad, Haryana, India.

Preparation and packaging of chicken meat emulsion

Four different batches i.e Control (C), T_1, T_2 and T_3 of chicken meat emulsion (CME) having 65% TMCM, 14% refined wheat flour, 21% tapioca starch, 5% refined oil (FORTUNE Soyabean oil), 1% salt (TATA salt, Tata chemicals Ltd. Mumbai), 1% sugar, 0.7% carboxymethyl cellulose (Sodium salt High Viscosity carboxymethyl, S d fine-CHEM Ltd., Mumbai, India; Code No. 56095) and 0.5% baking powder (Ajanta Baking powder, Ajanta Food Products Co., Solan, India; Code No. 288668) were prepared. In addition, 0.2% CP, 3% GiP and 2% GaP were added to T_1, T_2 and T_3 batches respectively. Control (C) was prepared without using any natural preservative. TMCM was blended with common salt and sugar and mixed in Inalsa mixer for 1 min, followed by mixing of baking powder, carboxymethyl cellulose, refined wheat flour, tapioca starch and refined oil for 1-2 min. The different groups were packaged in LDPE bags and stored for 9 days in a refrigerator (4±1°C). The sample was drawn every alternate day i.e. 1, 3, 5, 7, 9 and analyzed for different physico-chemical

quality attributes.

Chemical analysis

The pH was determined (Trout *et al*., 1992) with digital pH meter (SAB 5000, LABINDIA, New Delhi, India). For this, 10 g of CME was homogenized with 50 ml of distilled water and the electrode was dipped into the suspension to note down the pH.

Titrable acidity (Shelf and Jay, 1970) was determined by blending 10 g of CME with 200 ml of distilled water and made the volume to 250 ml in a volumetric flask. The slurry was filtered through Whatman filter paper No.1 and 25 ml of this filtrate was added with 75 ml distilled water with three drops of 1% phenolphathalein indicator solution and titrated against 0.1 N NaOH to get the end point (pink colour). Titrable acidity was calculated as,

$$\text{Titrable acidity} = [(\text{ml of } 0.1\text{N NaOH} \times 0.1 \times \text{meq wt. of lactic acid}) / \text{weight of sample (g)}] \times 100$$

Extract release volume (ERV) (Jay, 1964) was determined by blending 15 g of CME with 60 ml of 0.05 M phosphate buffer solution (pH 5.8). The filtration was carried out using Whatman filter paper No. 1 for 15 min. The filtrate collected was measured and expressed as ERV (ml).

For Free fatty acids (FFA) (Koniecko, 1979) estimation, 5 g of the CME was blended with 30 ml of chloroform in the presence of anhydrous sodium sulphate. The filtrate (Whatman filter paper No. 1) was added with 2 drops of 0.2 percent phenolphthalein indicator and titrated against 0.1N alcoholic KOH to get the end point (pink colour). Percent FFA content was calculated as,

$$\text{FFA (\%)} = [(0.1 \times \text{ml of } 0.1\text{N alc. KOH} \times 0.282) / \text{sample weight (g)}] \times 100$$

Peroxide value (PV) (Koniecko, 1979) was determined by blending 5 g of CME with 30 ml chloroform in the presence of anhydrous sodium sulphate. The filtrate (Whatman filter paper No. 1) was added with 30 ml of glacial acetic acid and 2 ml of saturated KI solution and left for 2 min with occasional shaking after which 100 ml of distilled water and 2 ml of fresh 1 percent starch solution were added. The content was titrated against 0.1N sodium thiosulphate to get the end point (non-aqueous layer turned to colourless). The peroxide value was calculated as,

$$\text{PV (meq/kg sample)} = [(0.1 \times \text{ml of } 0.1\text{N sodium thiosulphate}) / \text{sample weight (g)}] \times 1000$$

Thiobarbituric acid value (TBA value) was determined as per the extraction method described by Witte *et al*. (1970). Briefly, 10 g of sample was triturated with 25 ml of pre-cooled 20% trichloroacetic acid (TCA) in 2 M orthophosphoric acid solution for 2 min. The content was filtered through Whatman filter paper No. 1 to get TCA extract. 3 ml of this TCA extract was mixed with 3 ml of TBA reagent (0.005 M) in test tubes and placed in a dark room for 16 hrs. A blank sample was prepared by mixing 1.5 ml of 20% TCA, 1.5 ml distilled water and 3 ml of 0.005 M TBA reagent. Absorbance (O.D.) 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). TBA value was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with a factor 5.2.

The metmyoglobin percentage (MMb%) was measured as per the method described by Trout (1989). 3 g of CME was blended with 30 ml of cold phosphate buffer 0.04 M (pH 6.8). The meat sample was homogenized with the help of pestle and mortar for 20 sec and kept at refrigerated temperature (4°C) for 1 hour. Then, it was centrifuged at 10,000 rpm for 5 minutes in a refrigerated centrifuge (Eltek MP-400-R Eltek India, Delhi) at 4°C. The supernatant was collected and filtered through a Whatman filter paper No. 42. The optical density was measured in a UV-VIS spectrophotometer (Elico India Limited, Mumbai) at 525, 572 and 700 nm and percentage metmyoglobin was calculated using the formula of Krzywicki (1979).

$$\text{MMb\%} = [1.395-(OD_{572} - OD_{700}) / (OD_{525} - OD_{700})] \times 100$$

The ability to scavenge 1, 1 diphenyl-2picrylhydrazyl (DPPH) radical by added antioxidants in CME was estimated following the method of Kato *et al.* (1988) with slight modifications. DPPH can make stable free radicals in aqueous or ethanol solution, however, fresh DPPH solution was prepared before every measurement. Sample extract was prepared by blending 10 g of CME with 20 ml of ethanol for 2 min followed by filtering through Whatman filter paper No 42. Prior to use about 1 ml of DPPH stock solution was diluted with 9 ml of ethanol to make working solution. Then, 200 µl of the sample extract was mixed with 1300 µl of 0.1M Tris-HCl buffer previously adjusted to a pH of 7.4 and 1 ml of DPPH working solution (250 µM) in test tubes. Ethanol was used as blank sample. After properly mixing the samples, the absorbance (At_0) at time t=0

min, was measured at 517-518 nm using a UV-VIS Spectrophotometer (Elico India Limited, Mumbai) and then incubated at room temperature in dark for 20 mins. After 20 mins, the absorbance (At_{20}) at time t=20 min was measured at the same wavelength. The free radical scavenging activity was calculated as a decrease of absorbance from the equation:

$$\text{Scavenging activity (\% inhibition)} = 100 - [(At_{20}/At_0) \times 100].$$

The spectrophotometric analysis of $ABTS^+$ radical scavenging activity was determined as per the method given by Shirwaikar *et al.* (2006). This method was based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of standard antioxidants. $ABTS^+$ was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^+$) was produced by reacting $ABTS^+$ stock solution with 2.45 mM potassium persulphate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 16 hrs before use. Because $ABTS^+$ and potassium persulphate react stoichiometrically at a ratio of 1:0.5 (mol/mol), this will result in complete oxidation of $ABTS^+$. Oxidation of $ABTS^+$ commenced immediately, but the absorbance was not maximal and stable until 6 hrs had elapsed. The radical was stable in this form more than two days, when stored in dark at room temperature. Prior to use, the stock solution was diluted with ethanol to an absorbance of 0.70 at t_0 (t=0 min) and equilibrated at 30°C exactly 6 min after initial mixing. About 2 ml of $ABTS^+$ working standard solution was mixed with 1ml of sample extract (sample extract was prepared similar to the procedure as mentioned for DPPH) and absorbance was measured after 20 min (t_{20}) at 734 nm. The $ABTS^+$ activity was calculated by using formula:

$$ABTS^+ \text{ activity (\% inhibition)} = [(0.7 - At_{20}) / 0.7] \times 100$$

Colour profile analysis was performed using Lovibond Tintometer (Lovibond RT-300, Reflactance Tintometer, United Kingdom) set at 2° of cool white light (D65) and known as L^*, a^* and b^* values. L^* value denotes (brightness 100) or lightness (0), a^* (+ redness/- greenness), b^* (+ yellowness/-blueness) values were recorded from the surface of petriplates uniformly filled with CME variants. The Hue and chroma were determined by using the equation (Little, 1975).

$$\text{Hue} = (\tan^{-1}) \, b/a; \quad \text{Chroma} = [a^2 + b^2]^{0.5}$$

Colour and odour scores

Colour and odour scores evaluation was performed by a panel of seven judges consisting of faculty and postgraduate students of College of Veterinary Science, GADVASU. A 5-point descriptive scale (Sahoo and Anjaneyulu, 1997a) was used where 1- Very undesirable, 2- Moderately undesirable, 3- Moderately desirable, 4- Desirable and 5- Very desirable, for colour and 1- Very unpleasant, 2-Moderately unpleasant, 3-Moderately pleasant, 4-Pleasant and 5- Very pleasant, for odour.

Microbiological analysis

Standard plate count (SPC), *Staphylococcus* spp., Total Coliform count and Yeast and mould count 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 equipments, Ambala, India) pre-sterilized by ultra-violet (UV) radiation, under aseptic conditions. 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 requirement. Standard plate counts were determined on Plate Count Agar (PCA), total Coliform count on Violet Red Bile Glucose Agar and *Staphylococcus* spp. were counted on Baird Parker Agar. In all cases, plates were incubated at 37±2°C for 48 hrs. Yeasts and moulds were determined on Potato Dextrose Agar and plates were incubated at 25±2°C for 7 days. Pour plate technique was used to analyze the samples in duplicates (n=6). Cultural media and standard chemicals were procured from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. The average number of colonies was multiplied by reciprocal of the dilution and expressed as $\log_{10}$ cfu/g of sample.

Statistical analysis

Data were analyzed statistically on SPSS-16.0 software packages (SPSS Inc. Chicago, IL, USA) as per standard methods (Snedecor and Cochran, 1994). Duplicate samples were drawn for each parameter, and the experiment was repeated thrice (n=6). Visual colour and odour evaluation was performed by a seven-member panel in each trial (n=21). Means between the periods of storage, between treatments and within treatments were compared by two-way analysis of variance using Duncan's Multiple Range Tests and Homogeneity tests to test the significance of difference between means at 5% level ($P < 0.05$).

Results and Ddiscussions

Physico-chemical quality attributes

The pH (Table 1) of control and T_1 batches did not show any significant change up to day 7, but it was significantly higher ($P < 0.05$) in control than T_1 on day 9. On day 1, T_2 batch showed significantly lower ($P < 0.05$) pH out of all the samples. Throughout the storage period, T_1 and T_2 maintained lowest pH values than control and T_3 samples. The pH followed an increasing trend throughout the storage period in all the samples. These results are in agreement with the study of Kumar and Tanwar (2011) who also observed a non-significant effect on the pH value after incorporation of clove powder in chicken nuggets and simultaneously also reported a significant increase in the pH of both control and treated batches with the advancement of the storage period. Similar findings were observed by Verma and Sahoo (2000) in chevon and Biswas *et al*. (2012) in chicken meat products during the refrigerated storage. 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. (Kirsch *et al.*, 1952; McDowell *et al*., 1986).

At the beginning of storage, titrable acidity (Table 1) of T_2 batch was significantly higher ($P < 0.05$) than control, T_1 and T_3 batches. At the end of storage period, there was no significant change in titrable acidity among control and three treated CME samples. This indicates that natural preservatives did not affect the titrable acidity of CME. As the storage period advanced, titrable acidity decreased significantly ($P < 0.05$) in control, T_1, T_2 and T_3 samples which correspond to increase in their respective pH values. On day 1, values of titrable acidity of raw CME ranged from 0.068-0.088%. Stoltenberg *et al.* (2006) also reported that titrable acidity of raw batter prepared from beef (with each 10% and 25% citric acid and lactic acid) was ranged from 0.85% to 0.94%.

Extract release volume (ERV) (Table 1) did not vary significantly within control and treated batches of CME throughout the storage period. However, all the treated samples showed a marginal increase in ERV in all the storage intervals. In general the ERV decreased as the storage period advanced in all CME samples. This might be due to variation in composition and changes occurred in protein structure and composition due to bacterial action during refrigerated storage under aerobic packaging (Shelef and Jay, 1970). These results are in accordance with the study of Kumar *et al.* (2007) who also observed significant decrease in ERV of chicken meat patties stored at 4±1°C.

Table 1. Effect of different natural preservatives on the physico-chemical quality of chicken meat emulsion stored at 4±1°C

Treatments	Refrigerated storage (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
	pH				
C	5.50 ± 0.12^{Aa}	5.61 ± 0.11^{Aa}	5.70 ± 0.10^{Aa}	5.74 ± 0.07^{Aa}	5.82 ± 0.10^{Ab}
T_1	5.59 ± 0.13^{Aa}	5.62 ± 0.11^{Aa}	5.63 ± 0.13^{Aa}	5.76 ± 0.09^{Aa}	5.77 ± 0.02^{Aa}
T_2	5.38 ± 0.11^{Ab}	5.54 ± 0.09^{ABa}	5.64 ± 0.05^{Ba}	5.65 ± 0.10^{Ba}	5.69 ± 0.06^{Ba}
T_3	5.53 ± 0.12^{Aa}	5.67 ± 0.08^{ABa}	5.75 ± 0.03^{ABa}	5.76 ± 0.07^{ABa}	5.85 ± 0.03^{Bb}
	Titrable acidity (% lactic acid)				
C	0.068 ± 0.013^{Ba}	0.067 ± 0.013^{Ba}	0.045 ± 0.005^{ABa}	0.038 ± 0.003^{Aa}	0.034 ± 0.002^{Aa}
T_1	0.076 ± 0.014^{Cab}	0.071 ± 0.014^{BCab}	0.048 ± 0.002^{ABa}	0.037 ± 0.002^{Aa}	0.035 ± 0.001^{Aa}
T_2	0.088 ± 0.018^{Bc}	0.087 ± 0.019^{Bc}	0.048 ± 0.004^{Aa}	0.042 ± 0.004^{Ab}	0.037 ± 0.002^{Aa}
T_3	0.073 ± 0.014^{Bab}	0.072 ± 0.015^{Bab}	0.044 ± 0.005^{Aa}	0.038 ± 0.005^{Aa}	0.035 ± 0.003^{Aa}
	Extract Release Volume (ml)				
C	22.33 ± 1.65^{Ca}	20.33 ± 1.17^{BCa}	19.33 ± 0.84^{ABCa}	18.33 ± 0.99^{ABa}	16.33 ± 0.80^{Aa}
T_1	24.17 ± 1.99^{Ba}	22.83 ± 1.70^{ABa}	21.83 ± 1.56^{ABa}	20.33 ± 1.56^{ABa}	18.50 ± 1.67^{Aa}
T_2	23.00 ± 1.79^{Aa}	21.50 ± 1.73^{Aa}	20.67 ± 1.67^{Aa}	19.67 ± 1.61^{Aa}	18.50 ± 1.34^{Aa}
T_3	23.33 ± 1.54^{Ba}	22.17 ± 1.45^{ABa}	21.17 ± 1.45^{ABa}	20.33 ± 1.20^{ABa}	18.83 ± 1.20^{Aa}

Mean ± S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P < 0.05$). C = Control (without natural preservatives), T_1 = 0.2% CP, T_2 = 3% GiP and T_3 = 2% GaP.

Table 2. Effect of different natural preservatives on the oxidative stability of chicken meat emulsion stored at 4±1°C

Treatments	Refrigerated storage (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
	Free Fatty acids (%)				
C	0.11 ± 0.009^{Ab}	0.16 ± 0.004^{Bc}	0.17 ± 0.004^{Bc}	0.19 ± 0.004^{Cc}	0.20 ± 0.003^{Cc}
T_1	0.10 ± 0.006^{Aab}	0.14 ± 0.006^{Bb}	0.15 ± 0.005^{BCb}	0.16 ± 0.002^{Cb}	0.18 ± 0.005^{Db}
T_2	0.10 ± 0.008^{Aab}	0.15 ± 0.005^{Bbc}	0.15 ± 0.004^{Bb}	0.17 ± 0.008^{BCb}	0.17 ± 0.008^{Cb}
T_3	0.08 ± 0.003^{Aa}	0.10 ± 0.005^{Ba}	0.12 ± 0.004^{Ca}	0.14 ± 0.005^{Da}	0.15 ± 0.005^{Da}
	Peroxide value (meq/kg)				
C	1.03 ± 0.24^{Aa}	1.13 ± 0.21^{ABa}	1.30 ± 0.13^{ABCb}	1.60 ± 0.05^{BCb}	1.73 ± 0.04^{Cb}
T_1	0.50 ± 0.11^{Aa}	0.63 ± 0.17^{ABa}	0.77 ± 0.10^{ABa}	1.00 ± 0.18^{BCa}	1.27 ± 0.10^{Ca}
T_2	0.73 ± 0.15^{Aa}	0.73 ± 0.15^{Aa}	0.73 ± 0.15^{Aa}	0.97 ± 0.12^{Aa}	1.13 ± 0.18^{Aa}
T_3	0.87 ± 0.31^{Aa}	0.93 ± 0.38^{Aa}	1.03 ± 0.57^{Aab}	1.33 ± 1.08^{Aab}	1.40 ± 1.02^{Aab}
	TBA value (mg MDA/kg)				
C	1.98 ± 0.13^{Ac}	2.10 ± 0.14^{Ab}	2.33 ± 0.07^{Ab}	2.36 ± 0.07^{Ac}	2.77 ± 0.20^{Bb}
T_1	1.30 ± 0.03^{Aa}	1.47 ± 0.12^{ABa}	1.62 ± 0.12^{ABCa}	1.66 ± 0.07^{BCa}	1.86 ± 0.17^{Ca}
T_2	1.49 ± 0.14^{Aab}	1.61 ± 0.23^{Aa}	1.75 ± 0.11^{ABa}	1.66 ± 0.05^{Aa}	2.16 ± 0.18^{Ba}
T_3	1.75 ± 0.08^{Abc}	1.91 ± 0.08^{Aab}	2.18 ± 0.05^{Bb}	1.98 ± 0.14^{ABb}	2.23 ± 0.01^{Ba}
	DPPH (% inhibition)				
C	27.02 ± 4.60^{BCa}	29.83 ± 2.96^{Ca}	19.98 ± 1.53^{ABa}	16.60 ± 0.87^{Aa}	12.88 ± 2.60^{Aa}
T_1	29.39 ± 4.97^{Aa}	28.61 ± 6.61^{Aa}	43.93 ± 4.74^{Ab}	39.00 ± 5.67^{Ab}	35.17 ± 3.80^{Ab}
T_2	26.16 ± 6.54^{Aa}	28.75 ± 5.07^{Aa}	24.76 ± 7.40^{Aa}	17.00 ± 3.03^{Aa}	18.13 ± 1.41^{Aa}
T_3	29.43 ± 5.28^{Ba}	28.28 ± 3.26^{Ba}	21.08 ± 3.44^{ABa}	13.58 ± 2.57^{Aa}	11.86 ± 2.26^{Aa}
	ABTS (% inhibition)				
C	41.93 ± 3.08^{Da}	35.98 ± 3.55^{CDa}	29.81 ± 1.32^{BCa}	26.21 ± 1.06^{ABa}	19.10 ± 2.90^{Aa}
T_1	63.36 ± 1.73^{Dc}	60.74 ± 1.62^{CDc}	56.24 ± 0.96^{Cc}	47.62 ± 2.47^{Bc}	37.55 ± 2.73^{Ac}
T_2	48.69 ± 3.33^{Dab}	43.74 ± 2.71^{CDab}	38.93 ± 2.77^{BCb}	33.31 ± 2.41^{ABb}	27.88 ± 1.41^{Ab}
T_3	51.41 ± 3.33^{Cb}	48.33 ± 2.64^{Cb}	43.79 ± 3.13^{BCb}	37.69 ± 3.09^{ABb}	32.36 ± 2.67^{Abc}

Mean ± S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P < 0.05$). C = Control (without natural preservatives), T_1 = 0.2% CP, T_2 = 3% GiP and T_3 = 2% GaP.

Storage stability indicators

In the beginning of the storage i.e. day 1, free fatty acid (FFA) content (Table 2) was almost similar in the natural preservative treated samples whereas the control sample showed a significantly higher ($P < 0.05$) value than T_3 batch. On all the storage days FFA was significantly higher ($P < 0.05$) in control as compared to treated CME batches. Among the treated batches, garlic paste batch (T_3) showed significantly ($P < 0.05$) lower FFA during all the storage intervals up to day 9. This may be due to possible low lipolysis and lipolytic enzyme activity in garlic treated batch, leading to low production of free fatty acids (Aguirrezabal *et al.*, 2000). In general FFA increased as the storage period progressed. Das *et al.* (2011) 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 (Biswas *et al*., 2012) and goat meat products

(Verma and Sahoo, 2000; Das *et al*., 2008) during 9 days of refrigeration storage.

Peroxide value (PV) (Table 2) of control sample remained significantly higher on day 5, 7 and 9 as compared to natural preservative treated samples. PV did not show significant variation on day 1 and 3 among control and treated CME batches. Within the treated batches PV did not vary significantly. Ginger paste batch (T_2) showed non-significantly lower PV than CP and garlic paste batches (T_3). Among the treated batches, the greater PV of T_3 may be explained on the basis of its greater pH and lower level of active components than T_1 and T_2. As the storage period advanced, there was increase in PV in control and T_1 batch but T_2 and T_3 batch did not show any significant difference. The reasons for increase in FFA values hold good here too.

TBA value (Table 2) was significantly lower ($P < 0.05$) in T_1 and T_2 as compared to control and T_3 at the beginning of the storage. Clove powder (T_1) maintained lowest TBA value in all the storage intervals till the end of the storage among the natural preservatives tried. The finding is very well in accordance with the study of Vasavada *et al.* (2006) who also documented that antioxidant activity of cloves in cooked ground beef (stored at 2°C for 15 days) was highest in terms of TBA value than ginger, cinnamon, caraway, fennel, nutmeg and other spices. In all the CME batches, TBA value significantly increased throughout the storage period as concluded by Biswas *et al.* (2012). Also at the end of storage period, highest TBA value was found in T_3 and lowest in T_1 batch among the treated samples but they did not significantly vary among themselves showing that all the three preservatives are potential antioxidants. At day 9 of storage, comparatively lower TBA values shown by ginger than control and garlic is in accordance with the study of Stoilova *et al.* (2007) who documented that samples with 0.05% ginger extract showed lower TBA values as compared to control and BHT samples. Shan *et al.* (2009) also revealed that out of clove, cinnamon, oregano, pomegranate peel and grape seed, clove exhibited strongest antioxidant activity in terms of TBA value in raw pork at room temperature. Bali *et al.* (2011) also observed significant increase in TBA value of chicken sausages (stored at 4±1°C for 21 days) incorporated with garlic and coriander. Sallam *et al*. (2004) also revealed that addition of fresh garlic paste to chicken sausage (stored at 3°C for 21 days) significantly delayed lipid oxidation (both in terms of PV and TBA value) than the control samples.

DPPH % inhibition did not vary significantly among control and treated CME batches up to day 3 thereafter it was found to be significantly higher ($P < 0.05$) in T_1 batch showing that clove powder is better inhibitor of free radicals formation. The maximum % inhibition in terms of DPPH was shown by CP batch on day 5, 7 and 9 indicating that it is potentially superior to ginger and garlic paste in scavenging the free radicals. As the storage period progressed, DPPH % inhibition significantly decreased in control and T_3 samples whereas the natural preservative groups (T_1 and T_2) exhibited no significant change in DPPH till the end of storage period. Both CP and ginger showed better results in terms of DPPH % inhibition but the effect of CP was double than that of ginger. This finding is in accordance with the results of Gulcin *et al.* (2012) who reported a significant decrease ($P < 0.01$) in the concentration of DPPH radical due to its scavenging ability and the scavenging effect of clove oil and standards on the DPPH radical decreased in the order of clove oil > BHT > α-tocopherol > BHA > trolox. In an another study, Gulcin *et al*. (2004) also reported that DPPH radical decreased in the order of ethanol extract of clove buds > water extract of clove buds = BHA > BHT > ethanol extract of lavender > water extract of lavender > α-tocopherol and were 74%, 62%, 62%, 60%, 50%, 45% and 31% at the concentration of 60 µg/ml, respectively. At day 9 of storage, comparatively higher DPPH (% inhibition) shown by ginger as compared to control and garlic is exactly in accordance with the study of Stoilova *et al.* (2007) who documented that ginger extract showed significant effect in inhibition of DPPH as compared to control and BHT samples.

On day 1, ABTS % inhibition was significantly higher ($P < 0.05$) in T_1 batch as compared to other treated and control batches and same trend was continued in all other storage intervals till the end of storage period showing that CP is potentially much better than ginger and garlic in scavenging the free radicals. At the end of storage maximum ABTS % inhibition was found in T_1 batch as compared to T_2, T_3 and control samples. In general ABTS % inhibition significantly decreased as the storage period increased in all the CME batches. These findings are in accordance with Gulcin *et al.* (2012) who reported a significant decrease ($P < 0.01$) in the concentration of $ABTS^{\cdot+}$ due to potent radical scavenging action of clove oil than BHT, α-tocopherol and trolox, and they documented that scavenging effect of clove oil and standards on the $ABTS^{\cdot+}$ decreased in the order of BHA = clove oil ≈ BHT > α-tocopherol > trolox.

Colour quality parameters

L^* value (Table 3) was lowest in T_2 batch on day 1. There was no significant difference in L^* value

Table 3. Effect of different natural preservatives on the colour profile of chicken meat emulsion stored at 4±1°C

Treatments	Refrigerated storage (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
			L* value		
C	34.32±0.13^{Aab}	35.29±1.60^{Aa}	37.41±1.60^{Ab}	38.62±2.41^{Aa}	36.84±2.18^{Aa}
T_1	37.63±0.86^{Bb}	36.93±1.38^{Ba}	31.27±1.83^{Aa}	35.60±2.43^{ABa}	35.85±1.96^{ABa}
T_2	33.29±1.16^{Aa}	38.64±1.99^{Aa}	33.54±1.53^{Aab}	38.56±2.10^{Aa}	38.43±2.30^{Aa}
T_3	37.73±2.04^{ABb}	39.93±1.53^{Ba}	34.99±0.32^{Aab}	40.04±1.60^{Ba}	35.71±1.66^{ABa}
			a* value		
C	0.90±0.14^{Aa}	0.60±0.14^{Aa}	0.79±0.18^{Aa}	0.59±0.13^{Aa}	0.46±0.13^{Aa}
T_1	1.29±0.05^{Ab}	1.13±0.08^{Ab}	0.97±0.10^{Aa}	1.25±0.17^{Ab}	1.11±0.12^{Ab}
T_2	0.65±0.18^{Aa}	0.64±0.16^{Aa}	0.66±0.11^{Aa}	0.76±0.20^{Aa}	0.53±0.13^{Aa}
T_3	0.75±0.07^{Aa}	0.69±0.18^{Aa}	0.60±0.17^{Aa}	0.67±0.14^{Aa}	0.40±0.04^{Aa}
			b* value		
C	8.61±0.31^{Aa}	8.83±0.41^{Aa}	9.42±0.59^{Ab}	9.09±0.09^{Ab}	8.99±0.22^{Aa}
T_1	8.73±0.22^{Ba}	8.48±0.33^{ABa}	7.71±0.41^{Aa}	8.34±0.10^{ABa}	8.55±0.35^{ABa}
T_2	8.99±0.25^{Aab}	9.78±0.53^{Aa}	8.86±0.49^{Aab}	9.40±0.29^{Ab}	9.43±0.14^{Aa}
T_3	9.70±0.24^{Ab}	9.78±0.47^{Aa}	8.98±0.30^{Aab}	9.68±0.35^{Ab}	9.22±0.45^{Aa}
			Hue angle (°)		
C	84.17±0.72^{Ab}	86.27±0.76^{ABb}	85.39±0.76^{ABb}	86.29±0.84^{ABb}	87.16±0.74^{Bb}
T_1	81.57±0.54^{Aa}	82.44±0.46^{Aa}	82.88±0.57^{Aa}	81.43±1.25^{Aa}	82.67±0.63^{Aa}
T_2	86.01±1.02^{Ab}	86.44±0.71^{Ab}	85.68±0.70^{Ab}	85.32±1.28^{Ab}	86.80±0.77^{Ab}
T_3	85.53±0.47^{Ab}	86.12±0.88^{Ab}	86.33±0.97^{Ab}	86.14±0.66^{Ab}	87.59±0.23^{Ab}
			Chroma		
C	8.66±0.32^{Aa}	8.85±0.42^{Aa}	9.46±0.60^{Ab}	9.12±0.09^{Aab}	9.00±0.23^{Aa}
T_1	8.83±0.22^{Ba}	8.55±0.33^{ABa}	7.77±0.41^{Aa}	8.44±0.08^{ABa}	8.63±0.36^{ABa}
T_2	9.02±0.26^{Aab}	9.81±0.54^{Aa}	8.89±0.49^{Aab}	9.44±0.28^{Ab}	9.45±0.14^{Aa}
T_3	9.73±0.24^{Ab}	9.81±0.48^{Aa}	9.01±0.31^{Aab}	9.71±0.35^{Ab}	9.23±0.45^{Aa}
			Metmyoglobin (%)		
C	79.58±1.68^{Aa}	80.13±1.59^{Ab}	82.13±1.97^{Aa}	82.93±1.55^{Ab}	84.09±2.00^{Aa}
T_1	71.03±1.92^{Aa}	72.55±1.66^{Aa}	74.03±1.04^{Aa}	74.94±0.88^{Aa}	79.05±1.67^{Aa}
T_2	73.32±1.63^{Ba}	74.92±1.07^{Bab}	76.31±1.17^{Ba}	78.01±1.74^{Ab}	81.12±1.12^{Ba}
T_3	75.76±1.19^{Aa}	76.48±1.65^{Ab}	78.38±1.43^{Aa}	79.44±1.56^{Ab}	81.45±1.14^{Aa}

Mean ± S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P < 0.05$). C = Control (without natural preservatives), T_1 = 0.2% CP, T_2 = 3% GiP and T_3 = 2% GaP.

between control, T_1 and T_3 batches at the beginning of the storage. L^* value did not change significantly on day 3, 7 and 9 among control and three different CME batches. While on day 5, the lowest L^* value was observed in T_1 batch but it was not significantly different from T_2 and T_3 batches. On day 1, the highest a^* value was found in T_1 batch (treated with CP) which was also significantly higher ($P < 0.05$) than control, T_2 and T_3 batches showing that CP induced better appeal. The same trend was observed on day 3, 7 and 9 showing superiority of CP over ginger and garlic in maintaining the colour of the product. On day 9, the effect of CP was double than ginger paste and about triple as compared to garlic paste in maintaining the good colour of the product. There was no significant difference of b^* value between control, T_1, T_2 and T_3 on day 1 whereas b^* value of T_3 was significantly higher than control and T_1. On day 3, b^* value did not show any significant results between different batches and the similar trend was observed at the end of storage. On day 5, highest b^* value was obtained in control sample and lowest value was in T_1 batch. It was significantly lower ($P < 0.05$) than control, T_2 and T_3 batches. This indicates that CP is preferred preservative among the natural preservatives used. Hue angle, which indicates visual assessment of meat discolouration (Giroux *et al.*, 2001), was significantly lower ($P < 0.05$) for T_1 than control, T_2 and T_3 on day 1 at the beginning of the storage. Same trend was continued on all other storage intervals i.e. on day 3, 5, 7 and 9. This speaks of CP as a preservative of ingredient as compared to ginger and garlic. There was no significant difference of chroma between control, T_1 and T_2 CME whereas it was significantly higher ($P < 0.05$) in T_3 as compared to control and T_1 batches on day 1. On day 3, no significant variation was observed among four different CME batches. Similar trend was also observed on day 9 whereas on day 5, chroma of control was significantly higher ($P < 0.05$) than T_1 but without any variation from T_2 and T_3 samples. Chroma also remained lowest on T_1 batch which was significantly lower than T_2 and T_3 batches. Similar trends were observed by Naveena *et al.* (2006) in buffalo meat steaks (incorporated with lactic acid, clove and vitamin C and stored at 4±1°C for 12 days). Sahoo and Anjaneyulu (1997b) also reported a significant increase in chroma values in ground buffalo meat preblended with 500 ppm of sodium ascorbate during 10 days of refrigerated storage.

Formation of metmyoglobin in stored meat indicates lipid oxidation and discolouration of meat. There was no significant change in metmyoglobin % among control, T_1, T_2 and T_3 at the beginning of the storage on day 1. On day 3, metmyoglobin % was significantly lower ($P < 0.05$) in T_1 than control and T_3 but it was not significantly different from ginger paste batch. On day 5, no significant change in metmyoglobin % between all the variants was observed and same trend was observed on day 9. Whereas on day 7, T_1 batch showed lowest metmyoglobin % which was also significantly lower ($P < 0.05$) than control, T_2 and T_3. Non-significantly lower values were obtained in T_1 batch. This might be due to the potent antioxidant action of clove powder than ginger and garlic. There was non-significant increase in metmyoglobin % values throughout the storage period which might be due to decreasing oxidative stability of all the batches. Here overall the values of metmyoglobin % were on higher side than the normal values. The probable reason may be due to the effect of tenderizing agents (papain and $CaCl_2$) on the intact structure of proteins or there may be denaturation of myoglobin. Marginally lower values of metmyoglobin % were observed in antioxidant-treated samples than controls and this might be due to decrease in lipid oxidation, which is a major factor promoting myoglobin oxidation (Faustman *et al*., 2010). Kumudavally *et al.* (2011) also reported that application of 95% clove extract on fresh mutton (stored at 25±2°C for 4 days) lead to significantly ($P < 0.05$) lower increase in metmyoglobin % as compared to the control without clove treatment as the storage period progressed.

Colour and odour scores

Colour scores (Table 4) of CP batch (T_1) remained highest on all the storage intervals till the end, indicating that CP is a potent preservative

Table 4. Effect of different natural preservatives on the colour and odour scores of chicken meat emulsion stored at 4±1°C

Treatments	Refrigerated storage (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
	Colour scores (5 Point scale)				
C	4.00 ± 0.13^{Da}	3.42 ± 0.20^{Ca}	2.92 ± 0.15^{Ba}	2.75 ± 0.21^{Ba}	2.25 ± 0.11^{Aa}
T_1	4.25 ± 0.11^{Da}	4.08 ± 0.08^{Db}	3.67 ± 0.11^{Cc}	3.08 ± 0.08^{Ba}	2.75 ± 0.11^{Ab}
T_2	4.17 ± 0.11^{Da}	4.00 ± 0.00^{Db}	3.50 ± 0.13^{Cbc}	3.00 ± 0.13^{Ba}	2.42 ± 0.15^{Aab}
T_3	4.00 ± 0.13^{Da}	3.50 ± 0.18^{Ca}	3.17 ± 0.11^{BCab}	2.92 ± 0.15^{Ba}	2.42 ± 0.20^{Aab}
	Odour scores (5 Point scale)				
C	4.00 ± 0.00^{Da}	3.58 ± 0.15^{Ca}	3.08 ± 0.08^{Ba}	2.83 ± 0.11^{Ba}	2.25 ± 0.11^{Aa}
T_1	4.42 ± 0.08^{Cb}	3.92 ± 0.20^{BCa}	3.58 ± 0.30^{Ba}	3.33 ± 0.17^{ABb}	2.92 ± 0.15^{Ab}
T_2	4.08 ± 0.20^{Ca}	3.83 ± 0.11^{Ca}	3.33 ± 0.21^{Ba}	2.92 ± 0.15^{ABab}	2.58 ± 0.08^{Aab}
T_3	4.00 ± 0.00^{Da}	3.58 ± 0.15^{Ca}	3.17 ± 0.17^{Ba}	2.92 ± 0.15^{Bab}	2.25 ± 0.11^{Aa}

Mean ± S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P < 0.05$). C = Control (without natural preservatives), T_1 = 0.2% CP, T_2 = 3% GiP and T_3 = 2% GaP.

Table 5. Effect of different natural preservatives on the microbiological quality of chicken meat emulsion stored at 4±1°C

Treatments	Refrigerated storage (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
	Standard Plate Count (log_{10}cfu/g)				
C	4.65 ± 0.09^{Aa}	4.83 ± 0.08^{ABb}	4.85 ± 0.05^{Bb}	4.92 ± 0.02^{Bb}	4.97 ± 0.04^{Bb}
T_1	4.18 ± 0.09^{Aa}	4.31 ± 0.07^{ABa}	4.42 ± 0.08^{ABCa}	4.51 ± 0.10^{BCa}	4.63 ± 0.10^{Ca}
T_2	4.18 ± 0.09^{Aa}	4.41 ± 0.11^{ABa}	4.58 ± 0.09^{Ba}	4.62 ± 0.10^{Ba}	4.64 ± 0.09^{Ba}
T_3	3.61 ± 0.72^{Aa}	4.43 ± 0.06^{ABa}	4.41 ± 0.05^{ABa}	4.48 ± 0.09^{ABa}	4.72 ± 0.11^{Bab}
	Coliform Count (log_{10}cfu/g)				
C	2.38 ± 0.05^{Aa}	2.49 ± 0.09^{ABb}	2.61 ± 0.09^{ABa}	2.68 ± 0.12^{BCa}	2.90 ± 0.07^{Cb}
T_1	1.38 ± 0.44^{Aa}	1.54 ± 0.49^{ABa}	2.35 ± 0.09^{Ba}	2.44 ± 0.08^{Ba}	2.47 ± 0.09^{Ba}
T_2	1.87 ± 0.39^{Aa}	2.37 ± 0.10^{ABb}	2.46 ± 0.10^{ABa}	2.51 ± 0.11^{Ba}	2.63 ± 0.10^{Ba}
T_3	1.72 ± 0.35^{Aa}	2.22 ± 0.11^{Bab}	2.43 ± 0.09^{Ba}	2.56 ± 0.07^{Ba}	2.64 ± 0.05^{Ba}
	Yeast and Mould Count (log_{10}cfu/g)				
C	1.51 ± 0.48^{Aa}	2.28 ± 0.06^{Ba}	2.36 ± 0.09^{Ba}	2.50 ± 0.08^{Ba}	2.66 ± 0.09^{Ba}
T_1	1.10 ± 0.49^{Aa}	1.80 ± 0.37^{ABa}	2.18 ± 0.09^{Ba}	2.23 ± 0.11^{Ba}	2.52 ± 0.10^{Ba}
T_2	1.05 ± 0.47^{Aa}	1.38 ± 0.44^{ABa}	1.82 ± 0.37^{ABa}	2.15 ± 0.07^{Ba}	2.43 ± 0.14^{Ba}
T_3	1.05 ± 0.47^{Aa}	1.72 ± 0.35^{ABa}	1.87 ± 0.38^{ABa}	1.92 ± 0.38^{ABa}	2.40 ± 0.10^{Ba}

Mean ± S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P < 0.05$). C = Control (without natural preservatives), T_1 = 0.2% CP, T_2 = 3% GiP and T_3 = 2% GaP.

having better function in maintaining the colour of CME. There was no significant effect of treatment on the colour scores in the beginning of storage. As the storage period advanced, both colour as well as odour scores declined in all the CME batches. Odour scores were significantly higher ($P < 0.05$) in T_1 batch on day 1 which was maintained till day 9 i.e. end of storage. But there was no significant difference of odour scores of C, T_2 and T_3 batches on all the storage intervals. On all the storage days, odour scores in T_3 batch were marginally lower than T_2 batch. This may be due to organosulfur compounds derived from garlic in T_3 batch which enhanced colour, lipid and microbial safety (Yin and Cheng, 2003). The observed odour scores were in agreement with the indicators for lipid oxidation (TBA, PV, and FFA) in raw chicken meat emulsion. Das *et al.* (2011) also proposed no significant difference in colour and odour scores of control and curry leaf powder treated raw ground goat meat (stored at 4±1°C for 9 days). Throughout the storage period, both colour and odour scores declined linearly. Similar trends were observed by Verma and Sahoo (2000) in tocopherol preblended ground chevon (stored at 4±1°C for 9 days).

Microbiological profile

Standard Plate Count (SPC) was non-significantly higher in control CME than T_1, T_2 and T_3 batches at the beginning of the storage on day 1. On subsequent storage intervals, the control samples exhibited significantly higher ($P < 0.05$) microbial load as compared to treated emulsion batches till the end of the storage. Among the three different treated batches, there was no significant difference on day 3, 5, 7 and 9. However, at the end of the storage T_1 showed the lowest microbial load. This indicates that all three natural preservatives are effective in checking the microbial growth during the storage period and among them CP was proved to be a preferred preservative ingredient. Total coliform count showed no significant variation between control and treated batches at the beginning of the storage with marginally higher microbial load in control batch. There was hardly any significant variation in coliform count on day 5 and 7 whereas on day 9, control samples showed significantly higher ($P < 0.05$) coliform count as compared to T_1, T_2 and T_3. It was further noticed that coliform count did not vary significantly among three treated batches with marginally lower values in CP treated batches. Throughout the storage period, comparatively lower values of both SPC and coliform counts were detected in CP batch than GiP and GaP batches which is in accordance with the findings of Leuschner and Lelsch (2003) who revealed that out of ground clove, fresh garlic and red chilli, ground clove exhibited strongest antimicrobial systems in broth model systems. *Staphylococcus* spp. was not detected in any of the CME batch throughout the storage period of 9 days. Yeast and mould count did not show any significant difference among different batches but control batch had non-significantly higher load as compared to treated batches. On day 3, 5, 7 and 9, yeast and mould count did not bring any significant variation among different emulsion batches. However, it always remained highest in all the storage intervals for control emulsion batch. This indicates that natural preservatives used in the present study could not inhibit yeast and mould successfully. These findings are in agreement with the results of Naveena *et al*. (2001), who reported that there was no significant difference in total plate count, yeast and mould count and proteolytic counts between control and ginger treated spent hen meat samples. Bali *et al.* (2011) also observed that in chicken sausages (incorporated with garlic and coriander and stored at 4±1°C for 21 days) total plate count of garlic treated sausages was lower than control and coriander batches, and yeast and mould were not detected initially but after 7 days onwards there was significant increase in all the groups throughout the storage period.

Conclusions

Clove powder, ginger and garlic are natural preservatives having antioxidant and antimicrobial activity in complex food systems. The results in this comparative study demonstrated that addition of 0.2% clove powder in chicken meat emulsion produced better results in terms of physico-chemical characteristics, oxidative stability and microbiological parameters than 3% ginger and 2% garlic paste during refrigerated storage under aerobic packaging. Therefore, meat industry can effectively utilize 0.2% clove powder to improve colour and minimize oxidation-induced deteriorative changes in raw chicken meat emulsion without compromising the sensory attributes.

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