Plant Archives Volume 20 No. 2, 2020 pp. 8821-8830 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

SHELF-LIFE EVALUATION OF RAW CHICKEN SAUSAGE INCORPORATED WITH GREEN TEA AND CLOVE POWDER EXTRACTS AT REFRIGERATED STORAGE

Robiel Kamel Moawad*, Ola Saleh Saleh Mohamed and Nahed M. Abdelmaguid

Food Technology Department, National Research Centre, Dokki, Egypt.

Abstract

Chicken sausage is one of the popular foodstuffs among meat products; they are highly perishable with a short shelf-life. The present study aimed to investigate the effect of incorporation green tea extract (GTE; 700 ppm) and clove powder extract (CPE; 500 ppm), on the quality characteristics and shelf-life extension of raw chicken sausages during 12 days of storage at 4±1°C under aerobic packaging. The optimum concentrations of GTE and CPE were established and added to chicken sausages. Raw sausage samples were refrigerated at 4±1°C to be periodically examined for their sensory quality, physical parameters, chemical indices and bacteriological status. Results indicated that as the time of cold storage progressed, the overall mean scores of chemical indices and microbiological parameters were increased, while sensory scores and physical parameters were decreased ($P<0.05$) irrespective of treatment**.** The obtained results showed that individually addition of tested antioxidants and antimicrobial compounds significantly ($P<0.05$) affected water holding capacity (WHC), cooking loss and cooking yields, lipid stability, microbiological loads, protein degradation and appeal to consumers as compared to control samples during the entire refrigerated storage period. CPE provided the highest significant ($P<0.05$) antioxidant and antimicrobial properties followed by GTE during cold storage. This study demonstrates the potential use of CPE and GTE to improve the microbial quality, retard lipid oxidation, maintain the quality indices and extended the shelf-life of treated chicken sausages by 3-6 days over that of control (6 days) as confirmed by microbiological, chemical indices and organoleptic analyses, and could be a good replacement for the synthetic antimicrobials and antioxidants currently used by the meat industry.

Key words: Antioxidant, Antimicrobial, GTE, CPE, WHC, Quality attributes, Shelf-life.

Introduction

Chicken sausage is one of the popular foodstuffs among meat products (Almeida, 2015). Chicken meat products 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.,* 2014). Color, microbial growth and lipid oxidation are important factors for the shelf-life and consumer acceptance of fresh chicken meat (Ding *et al.,* 2015). Lipid oxidation causes deterioration of meat by adversely affecting its color, flavor, sensorial quality, nutritional value and generation of toxic products such as malonaldehyde and cholesterol oxidation products (Garcia-Lomillo *et al.,* 2017).

Various synthetic chemicals are being used as antioxidant and antimicrobial agents to combat the above mentioned problems. However, the use of synthetic compounds is quite debatable because of their ill effects on human health (Zhang *et al.,* 2016). This has revived the search for natural preservatives having both antioxidant and antimicrobial activities such as clove powder and green tea extracts for maintaining chicken meat quality, extending shelf-life and preventing economic loss (Khare *et al.,* 2014). These compounds are classified as "Generally recognized as safe" (GRAS) food additives for human consumption (Aminzare, *et al.,* 2016).

Clove powder extract: Spices like Clove (*Eugenia caryophyllus*), a member of *Caryophyllaceae* family was the strongest antioxidant in retarding lipid oxidation (Tajik *et al.,* 2014). The superior antioxidant activity of clove may arise from its high content of eugenol and gallic acid (Shan *et al.,* 2009). Clove have also been

***Author for correspondence :** E-mail : rk_moawad@hotmail.com

reported to exhibit inhibitory effect on many food borne pathogens (Singh *et al.,* 2014). The inhibitory effect of spices on bacteria and molds is due to the presence of eugenol (Gulcin *et al.,* 2012 and Asha *et al.,* 2014). Clove powder is a natural preservative and flavoring substance that is not harmful when consumed in food products. Thus, the use of clove powder extract (CPE) is hence attractive for keeping the quality of refrigerated chicken sausage.

Green Tea extract: Green tea (*Camellia sinensis*) is well known for various health benefits associated with risk reduction of a wide range of chronic diseases, such as cancer, diabetes, and cardiovascular diseases. Green tea contains epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), and flavonoids including quercetin, kaempferol, and myricitin (Sharpe *et al.,* 2016). The tea catechins and other polyphenols are free radical scavengers, metal chelators, inhibitors of transcription factors, and enzymes as reported by (Aminzare *et al.,* 2016) in frozen broiler meat. Green tea extract, have been recognized to have diverse health benefits including antioxidant (due its high content of catechins), antimicrobial, anti-inflammatory, and anticarcinogenic properties (Gerolis *et al.,* 2017). Hence, there is a great interest to enhance safety and quality of refrigerated chicken sausage by using green tea extract (GTE).

Chicken sausages are considered as one of the popular foodstuffs among food products and widely spread all over the world. However, during storage, quality attributes of the product deteriorate due to lipid oxidation, microbial growth, and also may be due to chemical reactions. Sensory, microbiological and biochemical methods have been used to assess freshness and quality during handling and storage with the main attributes of freshness. Therefore, the present study was performed to investigate the antioxidant as well as the antimicrobial effectiveness of green tea and clove powder ethanolic extracts on the quality of raw chicken sausage during cold storage (4±1°C) until appearance of signs of spoilage, by evaluating certain sensorial attributes (color, odor and overall acceptability), chemical criteria (pH, total volatile basic nitrogen "TVB-N" and thiobarbutric acid reactive substances "TBARS", physical properties (water holding capacity "WHC", feder value (moisture/protein ratio) and processing yield "PY") and microbiological status (Total viable count "TVC", Psychrotrophic "PTC" and Enterobacteriaceae "EBC"). Proximate chemical composition of raw fresh chicken sausage was also determined.

Table 1: Constituents of chicken sausage, curing agents and spices mixture.

Component	g/kg	%	Component	g/kg	%
Chicken Meat	680	68.00	Sodium ascorbate	0.5	0.05
Cattle fat	150	15.00	Powdered rusk	15	1.5
Ice flakes	100	10.00	Potato starch	20	2.0
Sodium chloride	18	1.8	Sodium caseinate	10	1.0
Sodium tripolyphosphate	3.0	0.3	Spices mixture	6.00	0.6

Material and Methods

Chemicals, reagents and supplies:

Plate count agar (PCA), violet red bile glucose agar (VRBGA) and peptone water were purchased from Oxoid (Hampshire, UK). Methyl red, magnesium oxide, 2-thiobarbituric acid, bromocresol green, BHT and TCA were from Sigma-Aldrich (Germany). Sodium tripolyphosphate (STPP), sodium casinate, sodium ascorbate and All other solvents and chemicals used were of analytical grade or the highest grade available. Salt, seasonings, powdered rusk and potato starch were purchased from local market in Giza, Egypt.

Preparation of natural extracts

Green tea and clove were purchased from local market at Giza, air dried and then ground into powders. Powdered samples from the tested materials were macerated with 70% ethanol (1:10 w/v) in a closed conical flask for 24 h at room temperature in the dark. The extract centrifuged at 3000×g for 10 min at 20°C, the resultant was then filtered through Whatman No. 1 filter paper and the residue re extracted and filtered. The filtrate was concentrated separately in a rotary evaporator (Heidolph Instruments Germany) to remove the solvent at 38°C under reduced pressure. The dried crude extract residue was stored at -20°C until use.

Chicken source

Ten white Habard chickens (6-7 weeks old), with initial body weight around 2kg were purchased a live from a local poultry market in Giza. Chickens were slaughtered as per standard procedure. The dressed chicken carcasses were deboning manually and packed in low density polyethylene bags. Cattle fat and mutton casings were purchased from El-Moustafa butcher shop at Giza, Egypt. Chicken meat, sheep casings and cattle fat were immediately transported in ice box to the laboratory of Food Science and Technology, NRC, Egypt. Upon arrival to the laboratory, the external fat, bone and connective tissues were removed from chicken and lipid samples under aseptic conditions, chicken meat and cattle fat samples were then frozen separately at -20°C until processing into sausage on the day of purchase.

Spices mixture

Spices were obtained from local market at Dokki, Giza, Egypt. Each spice was powdered by the mill and then a mixture of the spices was prepared in the following percentages: Black pepper (30g), red pepper (8g), cumin (15g), nutmeg (8g), celery (15g), cloves (8g), ginger (8g) and coriander (8g). All ingredients were milled together and 0.6 % was taken for preparing chicken sausages.

Sausage Manufacture

Chicken sausage samples were prepared according to a simple traditional formulation. Starts by cutting chicken meat (Breast and thigh muscles) and cattle fat (after thawing), to small pieces (2-3cm^3), then minced individually through a 8-mm plates (coarse), minced chicken meat and fat portions needed to formulate 2 kg sausage from each group were mixed together with ice flakes (10%) reground through a 6-mm plate for 1 min to insure uniform meat (68%) with fat level (15%), after formulating process, the original mixture was split into 3 batches to which curing mixture (salt, 1.8%, spices mixture 0.6%, sodium ascorbate 0.05%, 0.3% sodium tripolyphosphate). Tea catechins extract 700 ppm and clove powder extract 500 ppm was added separately to second and third groups. After processing, samples from different batches were ground again (3 mm plates) to form meat emulsion. By the end of emulsion process powdered rusk 1.5% and potato starch (2%) were added and reground for 3 minutes and stored overnight at 4±1°C. Then each batch was stuffed separately (perfect filling) into previously cleaned and prepared sheep small intestine using piston filler. The rounds had been tight into fingers (approximately 10-12 cm length), rinsed in diluted vinegar solution as a decontaminator, packed separately in polyethylene bags, and chilled at 4±1°C. For each replicate, samples were withdrawn from each formula for analysis at 0, 1st day, 3rd day, 6th day, 9th day and 12th day.

Optimization the conditions of using CPE and TCE in chicken sausage

Initial studies were conducted to obtain the most effective treatments of each natural substance on the sensory and antimicrobial attributes of raw chicken sausage. CPE was used at 0, 200 and 500 ppm; and TCE was used at 0, 250, 500 and 700 ppm, mixed with the minced chicken meat which formed into sausage using piston filler. Sausage samples were package individually in polyethylene bags and stored for 8 days in a chilled storage (4±1°C). During this storage, the sensory (appearance and odor) of the raw sausages was conducted daily, whereas total viable count (TVC) was conducted at the initial day (0 day) and final days (day 8) of storage. The best concentration of natural substances was determined.

These extracts (GTE and CPE) were chosen because of their beneficial effects for human health, as well as for their notable antioxidant and antimicrobial properties. The concentrations of 700 ppm GTE and 500 ppm CPE solutions were chosen in accordance to the previous successful pretreatment studies achieved by Singh *et al.,* (2014) and Salem *et al.,* (2016), with the potential to extend the shelf life and improve the quality of perishable beef and poultry meat.

Chemical Assessments

Chemical analyses were made on finely ground chicken sausage samples. Analyses were conducted in triplicate. Proximate composition in terms of moisture, ash, crude lipid and total nitrogen of chicken sausages were determined according to the methods described in the AOAC, (1995). Water/protein ratios (Feder's numbers): Calculated according to the equation FN = *W*/*P* ratio (where FN is Feder's number, *W* is water, and *P* is protein), (Halagarda *et al.,* 2017). For pH determination 10 g of chicken sausage samples were homogenized in 90 mL distilled water for 1 min in a warring blender, and the pH value of the slurry was measured at room temperature using pH meter (JENWAY, 3510; UK). The total volatile basic nitrogen (TVB-N) expressed as mg TVB-N per 100 g sausage samples was determined according to the method described by Parvaneh (2007). A Thiobarbituric acid reactive substance (TBARS) as mg of malondialdehyde (MDA)/kg sausage samples was estimated according to Kilinc (2007).

Water-holding capacity (WHC)

(WHC) water holding capacity of chicken sausage was analyzed according to the method of Wardler *et al.,* (1993). Chicken sausage sample (8g) and (12ml) NaCl solution (0.6M) was mixed in a centrifuge tube and was centrifuged (4°C) at 10000 rpm for 15 minutes and supernatant was decanted and measured. Water holding capacity was reported as ml of 0.6M NaCl per100g of chicken sausage.

Formula

WHC% = (Total Volume of NaCl – Supernatant Volume)/ (Volume of NaCl) × 100.

Cooking yield (CY)

The PY of the sausages was determined in each treatment. The cooked (boiled and fried) and uncooked sausages were weighed, and the process yield was calculated as follows: the weight of the cooked sausage

sample divided by the weight of the uncooked sausage sample multiplied by 100 (Yang *et al.,* 2007). % CY= 100 – CL%.

% CL = [Weight of the cooked sausage/Weight of the uncooked sausage]x100.

Microbiological Analysis

Twenty five grams of chicken sausage samples were aseptically excised from chicken sausages and homogenized in 225 ml of sterile buffered 0.1% peptone water for 3 min. From this homogenate, decimal serial dilutions were made in the same sterile peptone water and used for microbiological analyses of the chicken sausage samples at appropriate time intervals during refrigerated storage. On each of the predetermined sampling days, 0.1 ml of each dilution was pipetted onto the surface of plate count agar to determine total viable counts (TVC) and psychrotrophic counts (PTC); while enterobacteriaceae counts (EBC) were determined by using violet red bile glucose agar. Then, all plates were prepared in triplicate and incubated for 2 days at 30°C for TVC and EBC; and 10 days at 5°C for PTC (Ozogul and Uçar, 2013). After specific incubation periods plates showing 25-250 colonies were counted. The number of colonies were multiplied by the reciprocal of the respective dilution and expressed as log CFU per gram.

Sensory assessment of raw chicken sausages

Modified acceptance test with 10 non-trained panel members of the laboratory staff was carried out using 9-points hedonic scales, following the procedures of AMSA (1995). The whole four raw chicken sausages were taken from each group at regular intervals and immediately packed in small white foam plates, then labeled and served to the panelists at room temperature in random order, to evaluate their appearance (the first impression when looks the product), odor (the intensity of sausage odor), and overall acceptability (it calculated to determine how much a person like or dislike the sausage samples through considering the average measures). The 9-points hedonic scales were 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. A score of 5 was taken as the lower limit of acceptability.

Statistical analysis

Results were expressed as means and standard deviation (M±SD) from triplicate determinations. Analysis of variance (ANOVA) was performed to compare the effect of TCE or CPE on chicken sausages quality. Significant differences were defined as $P<0.05$; according to PC-STAT (1985).

Results and Discussion

Proximate Analysis and feder value

Moisture, protein, fat, ash and carbohydrate content of the chicken sausage samples were 63.35, 17.42, 14.63, 2.14 and 2.46%, (on wet basis), respectively. These values were similar to those reported by other authors for fresh sausage (Singh *et al.,* 2014; Asha *et al.,* 2014; Zhang *et al.,* 2016 and Ali *et al.,* 2018). The analysis revealed that fresh raw sausage is rich in proteins, minerals and fat contents. The proximate composition reported in the different studies (Salem *et al.,* 2016 and Halagarda *et al.,* 2017) showed some degree of differences, especially for the lipid and moisture contents. The results also indicated that feder value (moisture/protein ratio) of chicken sausage was 3.64. Feder value in good quality products should not exceed 4.0 as reported by Pearson (1991).

Sensory evaluation of chicken sausage

Table 2 represents the mean score values for appearance, odor and overall acceptability of all examined chicken sausage samples. Results of table 2 reveal that no, significant ($P<0.05$) differences in the sensory scores were detected between raw control and treated chicken sausage samples at the beginning of storage (0 time). The present results indicate that these natural functional ingredients can be incorporated into chicken sausage without having a detrimental effect on product quality producing a healthy chicken meat product. Moreover, during refrigerated storage panel preference for treated samples over stored untreated control table 2, indicating that CPE and GTE are potent preservatives having better function, which enhanced the pleasant odor, lipid and microbial safety; essential for maintaining the sensory attributes of chicken meat products.

Table 2 also obvious that, the investigated sensorial scores decreased significantly ($P< 0.05$) in all sausage groups by increasing refrigerated storage time, probably due to microbial effect, oxidation of lipid and degradation of protein in the sausages. Similar to our findings, various researches reported significant reductions in the sensory scores during refrigerated storage of different meat products (Halagarda *et al.,* 2017 and Ali *et al.,* 2018 and Salem *et al.,* 2018).

As shown in table 2, decline of sensory attributes begin after the third day of storage with marked reduction of odor, appearance and overall acceptability values in the control sausage samples and rejection scores after the six day. In contrast, CPE showed the highest ($P<0.05$) appearance, odor, and overall acceptability values for 12 days at 4±1°C; followed by GTE was accepted after 9

Table 2: Sensory scores changes of raw sausage during refrigerated storage for 12 days.

Chicken sausage	Sensory criteria	Cold storage per day					
		0	1	3	6	9	12
Control (C)	Appear.Scores	9.00± 0.15^{a}	8.21 ± 0.23^{C}	6.85± 0.17^{c}	5.76± 0.10^{c}	3.14± 0.25^{c}	2.46± 0.15^{c}
GTE (0.07%)		8.84 ± 0.11^{a}	8.36 ± 0.17^{b}	7.14± 0.25^{b}	6.84± 0.15^{b}	5.45± 0.11^{b}	3.64± 0.24^{b}
CPE (0.05%)		8.90 ± 0.21^{a}	8.58 ± 0.14^{a}	8.00 ± 0.12^{a}	7.46 ± 0.36^{a}	6.30 ± 0.28^{a}	5.12± 0.18 ^{a}
Control (C)	OdorScores	8.72 ± 0.32^{a}	7.46± 0.35^{c}	6.52 ± 0.24^{c}	5.14± 0.12^{c}	3.00± 0.17^{c}	2.10± 0.21^{c}
GTE (0.07%)		8.63± 0.13^{a}	8.10 ± 0.11^{b}	7.35 ± 0.32^{b}	6.42 ± 0.25^{b}	5.12± 0.25^{b}	3.16± 0.32^{b}
CPE (0.05%)		8.70 ± 0.17^{a}	8.37 ± 0.24^{a}	7.84 ± 0.18^{a}	7.17 ± 0.15^{a}	6.00 ± 0.14^{a}	4.92± 0.26^{a}
Control (C)	OverallAccept.	8.86 ± 0.23^{a}	7.83 ± 0.31^{c}	6.55 ± 0.19^{c}	4.45± 0.11^{c}	3.07± 0.21^{c}	2.28± 0.18^{c}
GTE (0.07%)		8.73 ± 0.12^{a}	8.23 ± 0.14^{b}	7.24 ± 0.28^{b}	6.63 ± 0.20^{b}	5.28± 0.18^{b}	3.40± 0.28^{b}
CPE (0.05%)		8.80 ± 0.19^{a}	8.47 ± 0.17^{a}	7.92± 0.15^{a}	7.31± 0.26^{a}	6.15 ± 0.21^{a}	5.02± 0.22^{a}

Appearance, Odor and Overall Acceptability scores reflect the mean and standard deviation, (n=10). Mean values in the same column bearing the same superscript do not differ significantly (P<0.05). GTE: 700 ppm Green tea extract – CPE: 500 ppm clove powder extract.

days.

Decreases in sensory quality were assessed from the 3rd days of storage in samples stored at 4±1°C. Thereafter, almost all the samples with added extracts were evaluated more highly (P<0.05) than the control samples. Similar results have been reported in muscle origin foods (Halagarda *et al.,* 2017 and Ali *et al.,* 2018). ANOVA tests indicated that the use of extracts (CPE or GTE) had a significant effect on the sensory characteristics investigated. The longer the period of storage, the greater the differences in appearance and odor scores of samples containing CPE or GTE as compared to control samples. On advancement of storage period, the overall acceptability scores of sensory attributes were sharply decreased (P<0.05) irrespective of treatment, as a result of microbial spoilage, oxidation of lipid and degradation of protein in the chicken sausages.

The results table 2 clearly indicated that the discoloration, sliminess and off-odors were observed in the control sausage samples which became unfit for sale after day 6 of storage, while GTE and CPE treated sausage samples were just beginning to show only slight color change, putrid odors and sliminess after days 9 and 12, respectively. Similar extension trend of meat shelf-life was achieved during refrigerated storage by other authors (Halagarda *et al.,* 2017; Ali *et al.,* 2018 and Boruzi and Nour, 2019), who indicated that natural extracts have strong antioxidant and antimicrobial activities.

Physical properties of chicken sausage

Water-holding capacity (WHC)

Physical properties of chicken sausage as affected by CPE, GTE and cold storage at 4±1 ºC for 12 days are presented in table 3. WHC is considered as the most important technological properties as it affects the tenderness, juiciness, drip loss and cooking yield of meat and meat products (Marapana *et al.,* 2018). Data of table 3 indicate that raw fresh chicken sausage exhibits higher

Table 3: Physical properties of raw chicken sausage during cold storage at 4±1°C for 12 days.

Treatment/Day		0	1	3	6	9	12
Control (C)	WHC	13.39±0.12^{c}	12.84±0.17^{c}	11.25±0.14^{c}	10.46±0.65^{c}	9.64±0.19^{c}	8.87±0.37^{c}
GTE (0.07%)		13.75±0.74^{b}	13.14±0.52^{b}	12.56±0.32^{b}	11.17±0.35^{b}	10.56±0.45^{b}	10.12±0.48^{b}
CPE (0.05%)		14.16±0.37^{a}	13.85±0.10^{a}	13.15±0.27^{a}	12.32±0.18^{a}	11.73±0.15^{a}	11.28±0.16^{a}
Control (C)	C. Loss %	17.11±0.52^{a}	18.87±0.16^{a}	21.08±0.17^{a}	23.37±0.12^{a}	25.42±0.50^{a}	28.67±0.14^{a}
GTE (0.07%)		15.33±0.47^{b}	16.83±0.23^{b}	17.64±0.28^{b}	19.45±0.27^{b}	21.15±0.74^{b}	23.95±0.19^{b}
CPE (0.05%)		14.56±0.39^{c}	15.78±0.62^{c}	16.86±0.13^{c}	18.35±0.46^{c}	19.73±0.63^{c}	21.26±0.32^{c}
Control (C)	C. Yield %	82.89±0.64^{c}	81.13±0.56^{c}	78.92±0.72^{c}	76.63±0.63^{c}	74.58±0.18^{c}	71.33±0.47^{c}
GTE (0.07%)		84.67±0.76^{b}	83.17±0.15^{b}	82.36±0.38^{b}	80.55±0.28^{b}	78.85±0.26^{b}	76.05±0.53^{b}
CPE (0.05%)		85.44±0.36^{a}	84.22±0.24^{a}	83.14±0.15^{a}	81.65±0.11^{a}	80.27±0.29^{a}	78.74±0.66^{a}

All values reflect the mean and standard deviation are mean of triplicate determinations. GTE: 700 ppm Green tea extract – CPE: 500 ppm clove powder extract. There is no significant difference (P>0.05) between the values having the same superscripts in the same column.

WHC% = (Total Volume of NaCl – Supernatant Volume)/ (Volume of NaCl) × 100.

% CL = [Weight of the cooked sausage/Weight of the uncooked sausage]x100.

WHC as compared to refrigerated samples. This trend is similar to protein solubility changes and thus indicates that WHC increases with the increase in protein solubility and vice-versa. The WHC decrease as a function of storage time indicating some biochemical changes and protein denaturation associated with cold storage (Dai *et al.,* 2012 and Jukna *et al.,* 2012). On the other hand, Saleem *et al.,* (2017) reported that as the percentage of protein increases in chicken, WHC consequently increases.

From the same given results of table 3, it is apparent that WHC of chicken sausage samples treated with GTE and CPE are higher than that of control samples, in contrast WHC of CPE treated sausage is higher than that of GTE treated sausages. As the time of refrigerated storage increase the WHC of all samples decreased irrespective of treatment, with the control chicken sausages always being the lowest. Similar trend of changes was observed by other authors (El-Nashi *et al.,* 2015; Saleem *et al.,* 2017; Elbakheet *et al.,* 2018 and Marapana *et al.,* 2018).

Cooking loss and cooking yield %

Cooking loss and cooking yield of different prepared chicken sausage samples containing 700ppm GTE and 500 ppm CPE were evaluated and the results are illustrated in table 3. In general, cooking yield was significantly increased (P<0.05) and cooking loss were significantly decreased (P<0.05) in different prepared chicken sausage samples containing different concentrations of GTE and CPE during the storage period. Values of cooking loss were the lowest in CPE followed by GTE then control samples. In the contrary, cooking yield was the highest in CPE, followed by GTE then the control samples always being the lowest all over the storage period. Similar trend of cooking loss and cooking yield of chicken sausages was achieved by other authors (El-Nashi *et al.,* 2015; Elbakheet *et al.,* 2018 and Marapana *et al.,* 2018). These results could be correlated to the functional properties of GTE and CPE as a water binding material which was the most important factor in improving cooking characteristics of sausages products.

pH changes

Addition of natural extracts to fresh sausage during processing resulted in significant ($P < 0.05$) reduction in pH values when compared with control sausages after processing and during storage. The pH value of control non-treated sausage was above the acceptable limit (7.1) with objective signs of deterioration after the 6th day of storage. The pH values of sausage formulas treated with green tea extract, clove powder extract were higher than the acceptable limit at the 9th and 12th day of storage, respectively. However, pH values of formulas treated with clove powder extract were marginally acceptable at the 12th day of storage at 4±1^{o}C, followed by sausages treated with GTE till the ninth day table 4. These results were in good agreement with previous authors after treatment of different meat products with natural additives (Sharma *et al.,* 2015; Salem *et al.,* 2016; Shokraneh, 2017 and Marapana *et al.,* 2018).

Generally, the pH value of all chicken sausage samples slightly decreased during the first 3 days of storage, by more time of refrigeration storage pH values increased in different degrees within untreated and treated sausage samples as shown in table 4. This decrease indicates that some fermentation occurs during storage. The last pH values increase might have been due to the liberation of ammonia compounds as a result of enzyme activity or the proteolytic microbial flora present in the raw sausages (Ozogul *et al.,* 2013).

The increase in the storage time, produce significant increase in pH values (P<0.05), whatever the treatment

Table 4: Chemical indices of raw chicken sausage during cold storage at 4±1°C for 12 days.

Treatment/Day		0	1	3	6	9	12
Control (C)	pH	6.14±0.17^{a}	6.25±0.32^{a}	6.17±0.14^{a}	6.54±0.42^{a}	7.23±0.13^{a}	7.68±0.12^{a}
GTE (0.07%)		6.10±0.23^{a}	6.19±0.18^{a}	6.15±0.25^{a}	6.38±0.21^{b}	6.72±0.27^{b}	7.32±0.16^{b}
CPE (0.05%)		6.06±0.15^{a}	6.12±0.12^{a}	6.10±0.16^{a}	6.22±0.11^{c}	6.45±0.22^{c}	6.87±0.28^{c}
Control (C)	TVB-N	12.36±0.15^{a}	13.56±0.14^{a}	16.42±0.11^{a}	19.28±0.17^{a}	23.17±0.10^{a}	27.73±0.15^{a}
GTE (0.07%)		10.70±0.27^{b}	12.17±0.36^{b}	14.65±0.56^{b}	17.52±0.23^{b}	19.84±0.19^{b}	24.15±0.18^{b}
CPE (0.05%)		9.48±0.12^{c}	10.84±0.18^{c}	12.37±0.16^{c}	15.36±0.12^{c}	17.96±0.42^{c}	20.00±0.21^{c}
Control (C)	TBARS	0.18±0.13^{a}	0.35±0.15^{a}	0.67±0.11^{a}	0.85±0.18^{a}	1.36±0.23^{a}	1.23±0.17^{a}
GTE (0.07%)		0.14±0.24^{a}	0.23±0.21^{b}	0.46±0.17^{b}	0.70±0.42^{b}	0.92±0.16^{b}	1.17±0.14^{b}
CPE (0.05%)		0.10±0.17^{a}	0.16±0.12^{b}	0.28±0.32^{c}	0.53±0.10^{c}	0.74±0.19^{c}	0.89±0.28^{c}

All values reflect the mean and standard deviation are mean of triplicate determinations.
There is no significant difference (P>0.05) between the values having the same superscripts in the same column. Total volatile basic nitrogen (TVBN, as mg N/100g meat). Thiobarbituric acid reactive substances (TBARS, as mgMDA/kg meat. – GTE: 700 ppm Green tea extract – CPE: 500 ppm clove powder extract.

conditions. The pH values of control fresh sausages reached value 6.54 at the 6th day of storage, however, the highest values of pH were found in control samples followed by low constant values in GTE and CPE treated sausages till the 9 and 12 days of storage; respectively. Similar trends of pH changes have been observed (Singh *et al.,* 2014; Zang *et al.,* 2016; Ali *et al.,* 2018). However, the lower pH values of treated sausage samples reflect protection properties of GTE and CPE against microorganisms, which reduce the accumulation of basic substances (Singh *et al.,* 2014; Zang *et al.,* 2016 and Ali *et al.,* 2018). In this sense, although pH value cannot be considered as an important index to determine meat spoilage, it can be useful as a guideline for quality control of meat and meat products (Sharma *et al.,* 2015 and Shokraneh, 2017).

Total volatile base nitrogen (TVBN)

Protein degradation is one of the main causes for deterioration of chicken meat quality (Parvanch, 2007). Therefore, the amount of total volatile basic- nitrogen was measured as an indicator for protein degradation. The TVB-N values of fresh sausages formulated with addition of natural extracts were significantly ($P < 0.05$) lower than those of control sausages after processing and during storage. A sharp rise of TVB-N value was noticed in the control and treated sausages during refrigerated storage at 4±1°C. The TVB-N values of control fresh sausages reached value 23.17 mg/100g (above the acceptable limit, 20 mg/100g) after the 6th day of storage with the objective signs of spoilage. Sausage formulas treated with green tea extract and clove powder extract revealed TVBN values higher than the acceptable limit (Egyptian Standards, 2005), at the 9^{th} and 12^{th} days of storage, respectively table 4. These results indicated that natural extracts (GTE and CPE) have the ability to protect sausages from protein degradation. This observation was in a good agreement with Singh *et al.,* (2014), Shokraneh, (2017) and Ali *et al*., (2018).

The thiobarbituric acid reactive substances (TBARS)

TBARS value is used as an index of lipid stability in meat products during storage (Kilinc *et al.,*2007). Data presented in table 4 showed the changes that took place in TBARS values of raw chicken sausage samples during refrigerated storage for 12 days. The TBARS values of fresh sausages formulated with addition of natural extracts were significantly ($P < 0.05$) lower than those of control sausages after processing and during storage. The TBARS values of fresh sausages formulated with different natural extracts were not significantly ($P > 0.05$) different at the begging of cold storage (zero time). The increase in the storage time, produce significant increase in TBARS values ($P<0.05$), whatever the treatment conditions. This might be due to auto-oxidation of lipids over a period of low temperature storage and pro-oxidant nature of added salt. The TBARS values of control fresh sausages reached value 1.36 mg/kg (above the acceptable limit, 0.9 mg/kg) after the 6th day of storage with the objective signs of spoilage.

Sausage formulas treated with green tea extract and clove powder extract revealed TBARS values higher than the acceptable limit at the 9^{th} and 12^{th} days of storage, respectively table 3. These results indicated that green tea extract (GTE) and clove powder extract (CPE) have antioxidant activities in fresh sausages. The antioxidant activities of these natural extracts have been observed previously in different meat products (Singh *et al.,* 2014; Sharma *et al.,* 2015; Zang *et al.,* 2016 and Ali *et al.,* 2018). The antioxidant activity of natural extracts (GTE and CPE) have been attributed to their phenolic compounds which act by terminating the free radical chain reaction by donating hydrogen or electrons to free radicals and converting them to more stable products (Baghshahi *et al.,* 2014).

Microbiological quality

Safety and shelf-life of meat are limited by microbial growth (Ozogul and Uçar, 2013). The antimicrobial activity of GTE and CPE in chicken sausage samples stored at 4±1°C for 12 days, are shown in table 5. Incorporation of natural extracts during processing of fresh sausages resulted in significant ($P<0.05$) reduction in total viable count (TVC) and psychrophilic count (PTC) after processing and during storage. The total viable and psychrophilic counts of fresh sausage formulated with green tea extract and clove powder extract were significantly ($P < 0.05$) lower than those formulated without natural extracts (control samples). The increase in the storage time, produce significant proliferations in TVC ($P<0.05$), whatever the treatment conditions. The obtained results could be correlated with the results of TVB-N as reported in table 4. However, TVC reached and exceeded a value of 6 log cfu/g, considered as the upper microbiological limit for good quality chicken sausages, as defined by the Egyptian Standard, after the six day for the control samples, indicating a shelf life of about 6 days with the objective signs of spoilage.

The changes in Psychrotrophic count were approximately similar to those of TVC, with control also being the highest followed by samples treated with GTE and much lower counts was detected in samples treated

Table 5: TVC, PTC and EBC (as logCFU/g) of raw chicken sausages during cold storage at 4±1°C for 12 days.

Treatment/Day		0	1	3	6	9	12
Control (C)	TVC	4.15±0.24^{a}	4.62±0.12^{a}	5.17±0.14^{a}	5.82±0.13^{a}	6.35±0.12^{a}	7.14±0.17^{a}
GTE (0.07%)		4.00±0.17^{b}	4.25±0.19^{b}	4.86±0.18^{b}	5.23±0.19^{b}	5.87±0.15^{b}	6.48±0.14^{b}
CPE (0.05%)		3.91±0.13^{b}	4.10±0.25^{c}	4.47±0.45^{c}	4.90±0.27^{c}	5.36±0.24^{c}	5.92±0.36^{c}
Control (C)	PTC	3.78±0.42^{a}	3.95±0.16^{a}	4.45±0.13^{a}	5.34±0.35^{a}	6.26±0.17^{a}	6.89±0.15^{a}
GTE (0.07%)		3.65±0.11^{a}	3.80±0.27^{b}	4.17±0.27^{b}	4.85±0.12^{b}	5.43±0.13^{b}	6.37±0.27^{b}
CPE (0.05%)		3.57±0.18^{ab}	3.64±0.36^{c}	4.00±0.15^{c}	4.52±0.16^{c}	4.95±0.27^{c}	5.68±0.46^{c}
Control (C)	EBC	2.74±0.26^{a}	2.93±0.13^{a}	3.15±0.21^{a}	3.27±0.23^{a}	3.54±0.12^{a}	3.86±0.13^{a}
GTE (0.07%)		2.68±0.15^{a}	2.76±0.17^{b}	2.90±0.18^{b}	3.12±0.11^{b}	3.36±0.18^{b}	3.51±0.21^{b}
CPE (0.05%)		2.56±0.35^{ab}	2.61±0.23^{c}	2.74±0.42^{c}	2.92±0.36^{c}	3.10±0.26^{c}	3.28±0.10^{c}

All values reflect the mean and standard deviation are mean of triplicate determinations.
There is no significant difference (P>0.05) between the values having the same superscripts in the same column. TVC: Total Viable Count – PTC: psychrotrophic count –EBC: Enterobacteriaceae count . – GTE: 700 ppm Green tea extract – CPE: 500 ppm clove powder extract.

with CPE. The results indicate that GTE and CPE had antimicrobial activity which help for prolonging expiry of chicken sausages, leading to safer meat product (Asha *et al.,* 2014; El-Nashi *et al.,* 2015; Zang *et al.,* 2016 and Ali *et al.,* 2018). Generally, antibacterial effect of all extracts may be due to phenolic content which affected bacterial cell through changes in its membrane, changes in the surface charge of cells to less negative values; alteration in the cytoplasmic membrane permeability. With respect to Enterobacteriaceae table 5, considered as a hygiene indicator (Asha *et al.,* 2014), compared to control, Enterobacteriaceae grew in GTE or CPE treated sausage samples at a slower rate and never exceeding 10^3 CFU/g. At the end of the storage period (day 12), treated sausages exhibited much lower (P<0.05) counts as compared with control samples. These results are in accordance with those of (Singh *et al.,* 2014; Salem *et al.,* 2016; Ella *et al.,* 2018 and Marapana *et al.,* 2018). These findings could be attributed to antibacterial impacts of ethanolic extracts of GTE and CPE.

Conclusions

It could be concluded from the current study that natural green tea extract and clove powder extract (GTE and CPE) have the ability to protect fresh sausage from protein deterioration and lipid oxidation. They also improve the chemical criteria indices, water-holding capacity (WHC) and cooking yields of chicken sausages. They (GTE and CPE) have antibacterial activities and can extend the shelf life of meat products. Clove powder extract (CPE) was superior to green tea extract (GTE) as antioxidant and antibacterial agents and for extending the shelf life of fresh sausage. The sensory attributes of all formulas treated with natural extracts were acceptable. Therefore, these natural additives could be safely used by meat processors to improve the quality and extend the shelf life of chicken meat products.

Significance Statements

This study demonstrates the potential use of green tea extract (GTE) and clove powder extract (CPE) formulations to improve the microbial quality, retard lipid oxidation, maintain the quality indices and improve the sensory score of treated chicken sausages. Regarding the control non-treated samples, all measured parameters were above the acceptable limits after the 6th day of storage with the appearance and odor scores of objective signs of deterioration; therefore, control can be acceptable until the 6th day of refrigerated storage. Sausages formulas treated with GTE and CPE revealed values higher than the acceptable limits for pH, TVBN, TBARS, WHC, cooking yield and bacterial counts with sensory scores higher than the acceptable limits at the 9th and 12th days of storage, respectively. Therefore, these formulas can extend the shelf life for 3-6 days more than the control (6 days).

References

Ali, F., N. Abdel-Atty and E. Helmy (2018). Improving the quality and extending the shelf life of chilled fresh sausages using natural additives and their extracts. *J. Microbiol. Biotech. Food Sci.,* **7(6):** 580-585. doi: 10.15414/jmbfs.2018.7.6.580-585.

Almeida, A.L. (2015). A desconstrução de um vilão:entrevista cedida à Thaís Ito. *Revista Nacionalda Carne*, **457:** 50-55. Retrievedfromhttps://issuu.com/btsinforma/docs/revista_digital_457_carne.

Aminzare, M., M. Hashemi, H. Hassanzad Azar and J. Hejazi (2016). The Use of Herbal Extracts and Essential Oils as a Potential Antimicrobial in Meat and Meat Products; *A review. J. Human Environ. Health Pro.,* **1:** 63-74. https://doi. org/10.29252/jhehp.1.2.6.

AMSA (1995). Research guidelines for cookery, sensory

evaluation and instrumental tenderness measurements of fresh meat. Chicago, IL, USA: American Meat Science Association.

AOAC. (1995). Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists Inc., Arlington, Virginia, USA.

Asha, K., B. Sunil, G. George and Prejit (2014). Effect of clove on the bacterial quality and shelf life of chicken meat. *Journal of Meat Science and Technology,* **2(2):** | 37-39.

Baghshahi, H., A. Riasi, A.H. Mahdavi and A. Shirazi (2014). Antioxidant effects of clove bud (*Syzygium aromaticum*) extract used with different extenders on ram spermatozoa during cryopreservation. *Cryobiology*, **69:** 482-487. https://doi.org/10.1016/j.cryobiol.2014.10.009.

Boruzi, A.I. and V. Nour (2019). Antioxidant Effects of Walnut Leaves and Sweet Cherry Stems on Color, Lipid Oxidation and Sensory Quality of Cooked Pork Patties. *Not Bot Horti Agrobo,* **47(3):** 763-771. DOI: https://doi.org/10.15835/nbha47311458.

Dai, S.F., F. Gao, X.L. Xu, W.H. Zhang, S.X. Song and G.H. Zhou (2012). Effects of dietary glutamine and gamma-aminobutyric acid on meat color, pH, composition and water-holding characteristic in broilers under cyclic heat stress. *Poult Sci.,* **53(4):** 471-81.https://doi.org/10.1080/00071668.2012.719148.

Ding, Y., S.Y. Wang, D.J. Yang, M.H. Chang and Y.C. Chen (2015). Alleviative effects oflitchi (*Litchi chinensis* Sonn.) flower on lipid peroxidation and protein degradation in emulsified pork meatballs. *J. Food Drug Anal.,* **23:** 501-508. https://doi. org/10.1016/j.jfda.2015.02.004.

DOI: 10.29011/AFPT-101.100001

Egyptian standard (1972-2005). Egyptian standard for beef sausage. Egyptian Organization For Standardization and Quality Control, Ministry of Industry, Egypt.

Elbakheet, S.I., E.A. Elgasim and M.Z. Algadi (2018). Utilization of Wheat Germ Flour in the Processing of Beef Sausage. *Adv. Food Process Technol.,* **1(1):** 1-6.

Ella, K.L.C., N.A. Christian, G. Augustin, Aboubakar, A.B. Armand and M.C. Moses (2018). Production of chicken sausage (Cobb 500 (Smith, 1992)): caracterisation of Physicochemical, microbiological and sensorial qualities. *International Journal of Scientific Research and Management (IJSRM),* **6(8):** 17-23. DOI: 10.18535/ijsrm/v6i8.ft01.

El-Nashi, H.B., A.A. Abdel Fattah, N.R. Abdel Rahman and M.M. Abd El-Razik (2015). Quality characteristics of beef sausage containing pomegranate peels during refrigerated storage. *Annals of Agricultural Science,* **60(2):** 403-412.

Garcia-Lomillo, J., M.L. Gonzalez-San Jose, R. Del Pino-Garcia, M. Ortega-Heras and P. Muniz-Rodriguez (2017). Antioxidant effect of seasonings derived from wine pomace on lipid oxidation in refrigerated and frozen beef patties. *LWT - Food Sci. Technol.,* **77:** 85-91.

Gerolis, L.G.L., F.S. Lameiras, K. Krambrock and M.J. Neves (2017). Effect of gamma radiation on antioxidant capacity of green tea, yerba mate, and chamomile tea as evaluated by different methods. *Radiation Physics and Chemistry,* **130:** 177–185.

Gulcin, I., M. Elmastas and H.Y. Aboul-Enein (2012). Antioxidant activity of clove oil - a powerful antioxidant source. *Arabian Journal of Chemistry,* **5(4):** 489-499.

Halagarda, M., W. Kwdzior and E. Pyrzy Nska (2017). Nutritional Value and Potential Chemical Food Safety Hazards of Selected Traditional and Conventional Pork Hams from Poland. *Journal of Food Quality,* 1-10. https://doi.org/10.1155/2017/9037016.

Jukna, V., J. Klementavièiûtë, E. Meðkinytë-Kauðilienë, N. Peèiulaitienë, M. Samborskytë and L. Ambrasûnas (2012). Comparative evaluation of Ostrich, Turkey and Broiler meat. *Biotechnology in Animal Husbandry,* **28(2):** 385-392. https://doi.org/10.2298/BAH1202385J.

Khare, A.K., A.K. Biswas and J. Sahoo (2014). Comparison study of chitosan, EDTA, eugenol and peppermint oil for antioxidant and antimicrobial potentials in chicken noodles and their effect on colour and oxidative stability at ambient temperature storage. *LWT- Food Science and Technology,* **55:** 286-293.

Kilinc, B., S. Cakli, T. Dincer and A. Cadun (2007). Effects of phosphates treatment on the quality of frozen-thawed fish species. *J. Muscle Foods,* **20:** 377-391.

Marapana, R., H. Nayanarasi, S. Senanayaka, P. Perera and K. Kodagoda (2018). Effect of processing conditions on quality of chicken sausages stuffed in different casings. *Journal of Pharmacognosy and Phytochemistry,* **7(5):** 56-64.

Ozogul, Y. and Y. Uçar (2013). The effects of natural extracts on the quality changes of frozen chub mackerel (Scomber japonicas) burgers. *Food Bioprocess Technol.,* **6:** 1550 - 1560. DOI: 10.1007/s11947-012-0794-9.

Parvaneh, V. (2007). Quality Control and the Chemical Analysis of Food. 3rd Edn., University of Tehran Press, Tehran, Iran, pp: 49-68.

Pearson, D. (1991). The chemical analysis of food. Churchill: New York, London, pp: 374-410. *Cited from African J. Food Sci.,* **3(10):** 316-319.

Rao, V.N.M. and K. Blane (1985). Statistical Programs (PC-STAT) for Microcomputers. Version IA, Department of Food Science and Technology, The University of Georgia, Athens, GA., USA.

Saleem, M., R.R. Kaleri, D. Kumar, R.A. Mangi, L. Kumar, K. Ahmed, A. Memon, Z. Khoso and M.A. Jakhro (2017). Comparative Study on Water Holding Capacity (WHC) of Broiler and Layer Meat. *Journal of Basic and Applied Sciences,* **13:** 100-103.

Salem,A.M., D.F. Khater and S.A. El Barody (2016). Effect of rosemary and green tea extracts on overall quality and

shelf-life of meat during storage. *Benha Veterinary Medical Journal,* **31(1):** 17-23, (http://www.bvmj.bu.edu.eg).

Shan, B., Y.Z. Cai, J.D. Brooks and H. Corke (2009). Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. *Journal of the Science of Food and Agriculture,* **89(11):** 1879-1885.

Sharma, H., S.K. Mendiratta, R.K. Agrawal and G. Kandeepan (2015). Effect of Clove and Holybasil Essential Oil on Sensory and Microbiological Quality of Fresh Chicken Sausages. *Journal of Meat Science and Technology,* **3:** 37-41.

Sharpe, E., F. Hua, S. Schuckers, S. Andreescu and R. Bradley (2016). Effects of brewing conditions on the antioxidant capacity of twenty-four commercial green tea varieties. *Food Chemistry,* **192:** 380-387.

Shokraneh, N., P. Ariaii, F.R. Ghahroudi, F. Hasannia and S. Sabaghpour (2017). The Effect of Coating with Green Tea Extract and Collagen Fiber on Quality Attributes of Vacuum Packaged Sausage. *Journal of Food Biosciences and Technology,* **7:** 23-30.

Singh, P., J. Sahoo, M.K. Chatli and A.K. Biswas (2014). 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). *International Food Research Journal,* **21(4):** 1363-1373.

Tajik, H., A. Farhangfar, M. Moradi and S.M. Razavi Rohani (2014). Effectiveness of clove essential oil and grape seed extract combination on microbial and lipid oxidation characteristics of raw buffalo patty during storage at abuse refrigeration temperature. *J. Food Process Preserv.,* **38(1):** 31-38. https:// doi.org/10.1111/j.1745-4549.2012.00736.x.

Wardler, T.P. and L. Young Soung (1993). Determination of water holding capacity of poultry meat. *Journal of Poultry Sciences,* **53(2):** 987-990.

Yang, H.S., S.G. Choi, J.T. Jeon , G.B. Park and S.T. Joo (2007). Textural and sensory properties of low fat pork sausages with added hydrated oatmeal and tofu as texture modifying agents. *Meat Sci.,* **75:** 283– 289.

Zhang, H., J. Wu and X. Guo (2016). Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. *Food Science and Human Wellness,* **5:** 39-48. https://doi.org/10.1016/j.fshw.2015.11.003.