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IMPROVING THE QUALITY AND EXTENDING THE SHELF LIFE OF CHILLED FRESH SAUSAGES USING NATURAL ADDITIVES AND THEIR EXTRACTS

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ABSTRACT

Natural additives are very important additives that can improve the oxidative and bacterial quality of meat products. Therefore, the main goal of the current study was to include green tea, clove and their extracts as well as fresh garlic and garlic powder during formulation of fresh sausages to improve the physico-chemical, sensory and bacterial quality of this product. Seven formulas were prepared; the 1st one was prepared from base batter and used as control and six formulas were prepared by addition of green tea powder 0.5%, green tea extract 0.03 %, clove powder 0.5 % clove extract 0.5%, fresh garlic 3 % and garlic powder 0.9 %. All formulas were processed into fresh sausages and chilled stored until appearance of signs of spoilage. Chilled sausages were examined for pH, thiobarbituric acid reactive substances, total volatile base nitrogen, bacterial counts and sensory attributes. A significant ($P < 0.05$) reduction in pH, TVB-N, TBARS and mesophilic and psychrotrophic counts were observed in all formulas treated with all natural additives. The sensory attributes of all formula treated with natural additives were acceptable. Green tea powder and clove powder were superior to their extracts as antioxidant and antibacterial agents and for extending the shelf life of fresh sausage. Moreover, fresh garlic was superior to garlic powder as antioxidant and antibacterial agents and for extending the shelf life of fresh sausage. Therefore, these natural additives can be safely used by meat processors to improve the quality and extend the shelf life of meat products.

Keywords: green tea, clove, garlic, sensory attributes, natural antioxidants

INTRODUCTION

Fresh sausage is one of the most popular meat products all over the world that are cheap, delicious, easily prepared and can solve the problems of shortage in fresh meat. Fresh sausage is mostly processed from meat trimmings which are relatively cheap as a raw material and are basically characterized by a high fat content and connective tissue with low functionality. Therefore, sausage manufacture is considered a mean of adding value to these low value cuts and increasing the utilization of carcass meat. There are more than 250 type of sausage sold around the world, which are named after the country of origin (**Dinstel, 2014**). Fresh sausages are coarsely comminuted, not heat treated products that are sold as uncooked, fresh (chilled) or frozen. It is composed of a mixture of meat, fat and spices stuffed into natural casing of animal small intestine. The lean portion can be made from edible red meat, poultry or both of them, while the fat portion is made from animal fat tissue to give a distinctive marbling appearance to the product (**Feiner, 2006**).

Fat is one of the most important raw materials in sausage products, as it represents a large percentage of sausage composition which may reach up to 30% and is important in the processing, textural, juiciness and sensory criteria of sausage products (**Baer and Dilger, 2014**). The high lipid contents of fresh sausage make the product more susceptible to lipid oxidation with subsequent changes in the organoleptic characteristics of the product. Lipid oxidation, the oxidative deterioration of the polyunsaturated lipids of food, leads to formation of hydroperoxides and short-chain aldehydes, ketones, and other oxidized compounds, which are considered to be responsible for causing flavor, texture, color and nutritional deterioration of meat and meat products. Several adverse health effects, due to the presence of free reactive oxygen species (ROS), such as superoxide radical, hydroxyl radical, peroxyl radical, during lipid oxidation, have been reported (**Nissen *et al.*, 2004**). Moreover, lipid oxidation is known to promote the occurrence of protein degeadation, resulting in loss of essential amino acids and detrimental effects on meat quality. It is well known that the addition of antioxidants could effectively delay lipid oxidation of meat products. Synthetic antioxidants are commonly used to retard lipid oxidation during the industrial production of meat products; however, these materials have been reported to have toxic effects (**McCarthy *et al.*, 2001**). As a result, natural antioxidants have been used instead of synthetic antioxidants and have been reported to be an effective method of controlling and retarding lipid oxidation. (**Armenteros *et al.*, 2016**). Natural antioxidants are primarily plant phenolic that may occur in all parts of plants, such as fruits, nuts, seeds, leaves, roots and barks (**Salejda *et al.*, 2011**). In the recent years many studies have been evaluating these natural substances as antioxidative additives in meat products leading to the development of novel food products (**Devatkal *et al.*, 2012**) and their effectiveness in extending shelf-life of the products has been widely documented (**Chouliara *et al.*, 2007**). From these natural additives green tea, clove and garlic have gained more interest from the public and scientific point of view due to their health promoting properties.

Green tea, clove and their extracts as well as fresh garlic and garlic powder have been used extensively as antioxidants in meat, however, their use during processing of fresh sausage still limited. Therefore the main goal of the current study was to include green tea, clove and their extracts as well as fresh garlic and garlic powder during formulation of fresh sausages to improve the physico-chemical, sensory and bacterial quality of this product. On the other hand, meat processors are concerned about the quality characteristics of processed products during storage. Therefore, the prepared sausage were stored at 4 °C until the signs of deterioration became evident. This work may encourage meat processors to use these natural antioxidants during processing of fresh sausages for production of high quality products.

MATERIAL AND METHODS

Experimental design

A three replicate based experiment (three independent replicates at different times) was carried out to investigate the effect of incorporation of natural additives on the physico-chemical, sensory characteristics and bacterial count of fresh sausages. Green tea powder 0.05 g/kg, clove powder 0.05 g/kg, green tea extract 0.003 g/kg, clove extract 0.05 g/kg, fresh garlic 0.3 g/kg and garlic powder 0.09 g/kg were added to fresh sausages during processing. Moreover, the prepared sausages at each replicate were stored at 4ºC until the signs of deterioration became evident.

Preparation of sausage ingredients

Imported deep frozen beef chucks (MINISTÉRIO DA AGRICULTURA, Brazil) were purchased from a local supplier in Benisuef, Egypt during the first third of shelf life. The chucks were kept frozen at -18 ºC until use. Beef fat was obtained after slaughter**,** washed and kept frozen at -18 ºC until use. Sodium tripolyphosphate and seasonings mix were obtained from Loba Chemie, Mumbai, India. Moreover, the sodium chloride and starch were obtained from a local market at Benisuef, Egypt.

Preparation of natural additives

Green tea and clove were purchased from local market, air dried and then ground into powders. Fresh garlic was purchased from local market, washed thoroughly and kept in refrigerator until used with few days. For preparation of garlic powder, the washed garlic was dried in hot air oven at 60 ˚C overnight and ground into powder. For preparation of green tea and clove extracts, 20 grams of air dried and ground green tea leaves or clove were soaked in 500 ml distilled water and boiled for 15 minutes. The boiled extracts were allowed to cool at room temperature then they were left at refrigerator at 4 ˚C overnight to allow the solute particles to settle. The aqueous phases were pipetted, labeled and kept in the refrigerator until use.

Sausage formulation and processing

A simple traditional formulation was prepared and used as a base batter. It consists of 63% lean beef meat, 20% beef fat, 1.8 % sodium chloride, 10 % water, 5 % starch, 0.3 % sodium tripolyphosphate and 0.05 % seasonings mix. Seven formulas were prepared; the 1st one was prepared from base batter and used as control. Six formulas were prepared by addition of green tea powder 0.5%, green tea extract 0.03 %, clove powder 0.5 % clove extract 0.5%, fresh garlic 3 % and garlic powder 0.9 %. For each replicate, the frozen beef of each formula was tempered to – 5 ºC, flaked using meat saw (Italians, Italy). The flaked lean beef and fat of each formula were ground through a 4.5-mm plate grinder (Seydelmann NW 114 E; Stuttgart, Deutschland, Germany). The ground lean beef and fat of each formula were mixed together with water, salt, bread crump, polyphosphates and seasonings for 5 minutes. The mixture of each formula was then stuffed into 18 Ø mm natural casing prepared from sheep small intestine using piston filler and linked to approximately 10-12 cm length then placed in plastic containers and stored at 4 °C. For each replicate, samples were withdrawn from each formula for analysis at 1st day, 3rd day, 6th day, 9th day and 12th day.

Sausages analysis

Physico-chemical examination

The pH, TVBN and TBA values were determined after processing and on 3 days interval during storage. For measurement of pH value, five grams from each of the chicken patties was homogenized with 20 ml distilled water for 10–15 s (**Kandeepan *et al.*, 2009**). The pH was measured using a pH meter (Lovibond Senso Direct) with a probe type electrode (Senso Direct Type 330) which was calibrated every two samples using two buffers 7.0 and 4.0. The Total Volatile Base Nitrogen (TVBN, mg/100 g sample) was measured according to the method of **Kearsley *et al.* (1983)** using a macro-Kjeldahl distillation method. Moreover, the thiobarbituric acid (TBA) value was measured by the method outlined by **Du and Ahn (2002)** and expressed as milligrams of malondialdehyde per kilogram of sample.

Microbiological examination

Ten grams from each fresh sausage samples were homogenized with 90-ml sterile Ringer's solution (Merck, Darmstadt, Germany) for 2 min. using Lab blender (400, Seward, model 6021, London UK). From the original solution, tenfold decimal dilution was prepared. The spreading technique and the standard plate count agar were used for enumeration of mesophilic bacterial count according to **Swanson *et al.* (2001)** and psychrotrophic bacterial count according to **Cousin *et al.* (1992).**

Sensory analysis

Sensory attributes of different sausage formulas were assessed according to the recommendations of **AMSA (1995).** Nine qualified individuals from both sexes in the age range of 25 to 40 years were selected from the members of the Department of Food Hygiene at Faculty of Veterinary Medicine, Beni-suef University, Egypt. The individuals received an introductory session related to descriptive profile of sensory analysis (appearance, flavor, juiciness, tenderness and overall acceptability, OA) preceding to testing so that each one could thoroughly discuss and explain each attribute to be assessed. A forced draught oven (Heraeus D-63450 Hanau, Germany) was used to cook sausage at 180 ºC to a core temperature 75 ºC and sasages were maintained warm in the oven until testing within 3-8 min. A needle thermocouple probe attached to a previously calibrated hand-held thermometer (Hanna HI 985091–1; Pasadena, TX, USA) was used to monitor the cooking temperature from the center of each sausage sample, rectangular pieces of approximately 1.5 cm - 2 cm were cut and served at room temperature. Each individual assessed three replicates of all formulas in a randomized order and asked to assign a numerical value between 1 and 5 for each attribute where 5 denote extremely acceptable and 1 denotes extremely unacceptable.

Statistical analysis

The SPSS program (v.22, IBM SPSS Inc.; Chicago, IL, USA) was used to statistically analyze the data. The statistical significance ($P < 0.05$) of the effect of different natural antioxidants was evaluated by one-way analysis of variance (ANOVA). The entire experimental design was followed in triplicate and differences between replicates were not significant ($P < 0.05$). The values in the tables are given in terms of mean values and standard error of the mean. Least squares differences (LSD) were used for comparison of mean values among different natural antioxidants.

RESULTS AND DISCUSSION

Meat processors and consumers prefere the use of natural additives that exhibit antioxidant, antimicrobial and health benefits to overcome the problems that may arise from the use of synthetic ingredients. Although many studies have been conducted using natural additives and their extracts in meat and meat products, the use of particular ingredients with specific concentrations in certain meat products is still limited. Fresh sausage is one of meat products which are under extensive consumption throughout the world. It is raw meat product with high fat contents; therefore, it may be subjected to oxidative degradation and consequently can be harmful to consumers. Therefore, the current study was designed to evaluate the antioxidant and antibacterial effect of green tea, clove and garlic as natural ingredients for improving the quality of fresh sausage during refrigerated storage.

The pH of fresh sausages

Addition of natural additives and their 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 (6.4) with objective signs of deterioration at the 6th day of storage. The pH values of sausage formulas treated with green tea extract, clove extract were higher than the acceptable limit at the 9th day of storage. However, pH values of formulas treated with green tea powder, clove powder or fresh garlic were marginally acceptable at the 12th day of storage at 4 ˚C (Table 1). These results were in good agreement with previous authors after treatment of different meat products with natural additives (**Liu *et al.*, 2009; Muthia *et al.*, 2014; Sharma *et al.*, 2015; Shokraneh, 2017**). However, a non-significant change in pH values were observed in chicken nuggets after addition of clove powder (**Kumar and Tanwar, 2011**).

Table 1 The pH values of fresh sausage treated with different natural additives during chilled storage at 4 ˚C.

Treatments	Storage time (days)				
	0-day	3rd day	6th day	9th day	12th day
Control	5.54 ±0.11[a]	6.18 ±0.15[a]	6.98 ±0.14[a]	-	-
Green tea powder 0.5%	4.79 ±0.14[b]	4.85 ±0.22[b]	5.48 ±0.12[b]	5.98 ±0.19[a]	6.42 ± 0.2[a]
Green tea extract 0.03%	5.06 ±0.12[b]	5.50 ±0.12[b]	5.83± 0.15[b]	6.62 ±0.20[a]	-
Clove powder 0.5 %	5.01 ±0.09[b]	4.88 ±0.14[b]	5.51 ±0.14[b]	6.05 ± 0.18[a]	6.46 ±0.17[a]
Clove extract 0.5 %	5.05 ±0.08[b]	5.64 ±0.08[c]	5.97± 0.16[b]	6.51 ±0.12[a]	-
Fresh garlic 3%	4.07± 0.06[c]	5.30 ±0.13[c]	5.86 ±0.18[b]	6.42± 0.23[a]	-
Garlic powder 0.9 %	4.95 ±0.12[b]	5.11 ±0.11[b]	5.80± 0.18[b]	6.37 ±0.22[a]	6.51 ±0.25[a]

[a-d]values with different superscripts within the same column are significantly different at P<0.05

Regarding the storage time, the pH values of control and all treated formulas increased during refrigerated storage. The pH values of control fresh sausages reached value 6.98 at the 6th day of storage with the objective signs of spoilage, however, pH values of sausages treated with natural additives and their extracts reached 6.42-6.51 by the 12th day of refrigerated storage at 4 ˚C. This result was in agreement with **Georgantelis *et al.* (2007)** and **Brannam (2008)** who reported an increase in pH during storage of meat products. This observation was attributed to an increase in the counts of psychrotrophic microorganisms during storage, which produce protease. Once protease production is started, the bacteria use amino acids as growth substrate instead of using glucose. The consumption of amino acids leads to elevation of pH due to the formation of ammonia and amines (**Scapin *et al.*, 2015**).

Total volatile base nitrogen (TVBN)

Protein degradation is one of the main causes for deterioration of meat quality including water-holding capacity, texture, flavor, color and biological functionality (**Lund *et al.*, 2011**). Therefore, the amount of total volatile base nitrogen was measured to evaluate the degree of protein degradation. The amount of carbonyl formation has been performed as an indicator for protein degradation by previous authors (**Chen *et al.*, 2016; Zhang *et al.*, 2017**)

The TVBN values of fresh sausages formulated with addition of natural additives and their extracts were significantly (P < 0.05) lower than those of control sausages after processing and during storage. The TVBN values of control fresh sausages reached value 28.5 mg/100g (above the acceptable limit, 20 mg/100g) at the 6th day of storage with the objective signs of spoilage. Sausage formulas treated with green tea extract, clove extract revealed TVBN values higher than the acceptable limit at the 9th day of storage, however, formulas treated with green tea powder, clove powder or fresh garlic revealed marginal TVBN values at the 12th day of storage (Table 2). These results indicated that natural additives and their extracts have the ability to protect sausages from protein degradation. This observation was in a good agreement with **Chen *et al.* (2016) and Zhang *et al*. (2017).**

Table 2 The total volatile base nitrogen (TVBN) values of fresh sausage treated with different natural additives during chilled storage at 4 ˚C.

Treatments	Storage time (days)				
	0-day	3rd day	6th day	9th day	12th day
Control	16.82 ±0.52[a]	18.79 ±0.42[a]	28.5 ±0.5[a]	-	-
Green tea powder 0.5%	10.5 ±0.45[c]	12.32 ±0.25[c]	16.4 ±0.34[c]	18.51 ±0.36[a]	19.59 ±0.55[a]
Green tea extract 0.03%	14.39 ±0.39[b]	16.8 ±0.49[b]	19.55 ±0.40[b]	22.1 ±0.46[a]	-
Clove powder 0.5 %	9.1 ±0.09[d]	12.1±0. 21[c]	15.4 ±0.25[d]	17.5 ±0.56[a]	19.33 ±0.43[a]
Clove extract 0.5 %	10.08 ±0.40[c]	14.2 ±0.33[d]	18.8 ±0.26[c]	21.46 ±0.44[a]	-
Fresh garlic 3%	11.5 ±0.81[ce]	14 ±0.25[d]	16,5 ±0.36[c]	18.57± 0.51[a]	19.78 ±0.55[a]
Garlic powder 0.9 %	12.6 ±0.22[c]	15.8 ±0.5[e]	17.8 ±0.71[e]	19.32 ±0.52[a]	-

*values represent the mean of three independent replicated ± SE

[a-d]values with different superscripts within the same column are significantly different at P<0.05

The effectiveness of natural additives and their extracts on protein degradation may be attributed to the phenolic compounds offered by these plants. The antioxidant activities of plant phenolics against protein degradation have previously been described in meat and meat products (**Estévez and Heinonen, 2010; Estévez *et al.*, 2007; Falowo *et al.*, 2014**). It has been reported that phenolic compounds can inhibit protein degradation by chelating metal ions, scavenging free radicals, blocking lipid oxidation and combining with proteins to prevent protein carbonylation (**Estévez, 2011; Maqsood *et al.*, 2012**).

The thiobarbituric acid reactive substances (TBARS)

The TBARS values of fresh sausages formulated with addition of natural additives and their 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 additives and their extracts were not significantly (P > 0.05) different. The TBARS values of control fresh sausages reached value 1.21 mg/kg (above the acceptable limit, 0.9 mg/kg) at the 6th day of storage with the objective signs of spoilage. Sausage formulas treated with green tea extract, clove extract revealed TBARS values higher than the acceptable limit at the 9th day of storage, however, formulas treated with green tea powder, clove powder or fresh garlic revealed marginal TBARS values at the 12th day of storage (Table 3). These results indicated that green tea, clove and garlic exerted antioxidant activities in fresh sausages. The antioxidant activities of these natural additives and their extracts have been observed previously in different meat products (**Naveena *et al.*, 2006; Kumar and Tanwar 2011; Gheisari and Ranjbar, 2012; Ibrahim *et al.*, 2013; Muthia *et al.*, 2014; Özvural *et al.*, 2016; Shokraneh, 2017).**

The antioxidant activity of natural additives 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 (**Perumalla and Hettiarachchy, 2011; Baghshahi et al., 2014; Wojdyło *et al.*, 2007**). The lower antioxidant activities of green tea extract or clove extract when compared with those of green tea powder or clove powder have been explained by the losses of volatile sulfur compounds during boiling or steam distillation. It has been recorded that garlic oil or steam-distilled garlic did not contain large amount of allicin, but contain various products of allicin transformation, none of which appears to have much biologic activity as either fresh garlic or garlic powder (**Miething, 1988**).

Table 3 The thiobarbituric acid reactive substances (TBARS) values of fresh sausage treated with different natural additives during chilled storage at 4 ˚C.

Treatments	Storage time (days)				
	0-day	3rd day	6th day	9th day	12th day
Control	0.27 ±0.03[a]	0.82 ±0.05[a]	1.21 ±0.08[a]	-	-
Green tea powder 0.5%	0.14 ±0.05[b]	0.32 ±0.04[b]	0.41 ±0.03[b]	0.66 ±0.05[b]	0.68 ±0.06[b]
Green tea extract 0.03%	0.14 ±0.03[b]	0.39 ±0.07[b]	0.74 ±0.05[c]	0.88 ±0.04[c]	-
Clove powder 0.5 %	0.22 ±0.02[b]	0.50 ±0.09[c]	0.60 ±0.02[d]	0.89 ±0.06[bc]	0.69 ±0.07[b]
Clove extract 0.5 %	0.15 ±0.04[b]	0.50 ±0.06[b]	0.75 ±0.03[c]	0.98 ±0.05[c]	-
Fresh garlic 3%	0.15 ±0.02[b]	0.41 ±0.04[b]	0.66 ±0.04 [cd]	0.80 ±0.04[c]	0.92 ±0.07[c]
Garlic powder 0.9 %	0.21 ±0.01[b]	0.41 ±0.03[b]	0.71 ±0.03[c]	0.85 ±0.05[c]	-

[a-d]values with different superscripts within the same column are significantly different at P<0.05

Microbiological quality

Incorporation of natural additives and their extracts during processing of fresh sausages resulted in significant (P < 0.05) reduction in total mesophilic count and psychrophilic count after processing and during storage. The total mesophilic and psychrophilic counts of fresh sausage formulated with green tea powder were significantly (P < 0.05) lower than those formulated with other natural additives or their extracts. The total mesophilic count of control fresh sausage reached 6.85 log cfu/g (above the acceptable limit 6 log cfu/g) by the 6th day of storage with the objective signs of spoilage. Sausage formulas treated with green tea extract, clove extract revealed bacterial counts higher than the acceptable limit at the 9th day of storage, however, formulas treated with green tea powder, clove powder or fresh garlic revealed marginal bacterial counts values at the 12th day of storage (Table 4 & 5). The microbial activities obtained in this study have been recorded previously by many authors for different natural additives and their extracts in meat and meat products (**Helander *et al.*, 1998; Elgayyar *et al.*, 2001; Lambert *et al.*, 2001; Naveena *et al.*, 2006; Mytle *et al.*, 2006, Kumar and Tanwar, 2011; Gheisari and Ranjbar, 2012; Ibrahim *et al.*, 2013; Sharma *et al.*, 2015**).

Table 4 The total mesophilic count (log cfu/g)* of fresh sausage treated with different natural additives during chilled storage at 4 ˚C.

Treatments	Storage time (days)				
	0-day	3rd day	6th day	9th day	12th day
Control	4.30 ±0.23[a]	5.69 ±0.35[a]	6.85 ±0.38[a]	-	-
Green tea powder 0.5%	0.95 ±0.63[c]	3.30 ±0.27[b]	3.69 ±0.35[b]	4.60 ±0.24[b]	5.60 ±0.37[b]
Green tea extract 0.03%	2.00 ±0.35[b]	3.48 ±0.24[b]	4.69 ±0.43[b]	6.48 ±0.35[b]	-
Clove powder 0.5 %	3.30 ±0.32[d]	4.60 ±0.19[c]	5.69 ±0.42[c]	5.60 ±0.56[c]	6.10 ±0.47
Clove extract 0.5 %	3.70 ±0.44[d]	4.00 ±0.26[d]	5.30 ±0.23[c]	6.68 ±0.25[c]	-
Fresh garlic 3%	2.30 ±0.62[b]	3.30 ±0.42[b]	4.60 ±0.31[d]	5.60 ±0.18[d]	5.95 ±0.32[c]
Garlic powder 0.9 %	2.30 ±0.45[b]	3.48 ±0.18[b]	4.69 ±0.27[d]	5.95 ±0.34[d]	-

[a-d]values with different superscripts within the same column are significantly different at P<0.05

Sensory evaluation

The sensory scores of fresh sausage formula treated with natural additives and their extracts as well as control one are presented in Table 6. The sensory scores (appearance, flavor, juiciness, tenderness and overall acceptability) of all treatment formulas were not (P > 0.05) significantly different from those of
At the 3rd day of processing, the sensory scores of all treated formulas were significantly (P < 0.05) higher than those of control formula, however, there were no significance (P > 0.05) differences among treated formulas. At the 6th day of storage, the sensory scores of all attributes for the control formula were lower than the acceptable score (2.5), however, the sensory scores of all treated formulas were above the acceptable score. The sensory scores of formulas treated with green tea and clove powders were significantly (P < 0.05) higher than other formulas. By the 9th day of storage, the sensory scores of fresh sausage formulas treated with green tea extract, clove extract or garlic powder were lower than the acceptable scores and the other formulas were above the acceptable scores. At the end of storage (12th day) the sensory scores of formulas treated with green tea powder, clove powder and fresh garlic were at critical values with all quality parameters, therefore, these formulas were not acceptable. Previously, different authors observed well acceptable sensory scores after incorporation of natural additives and their extracts (**Naveena *et al.*, 2006; Mytle *et al.*, 2006; Kumar and Tanwar, 2011; Ibrahim *et al.*, 2013; Zhang *et al.*, 2017**).

Table 5 The psychrotrophic count (log cfu/g)* of fresh sausage treated with different natural additives during chilled storage at 4 ˚C.

Treatments	Storage time (days)				
	0-day	3rd day	6th day	9th day	12th day
Control	5.60 ±0.33[a]	5.60 ±0.54[a]	6.60 ±0.33[a]	-	-
Green tea powder 0.5%	3.60 ±0.54[c]	3.47 ±0.53[b]	4.84 ±0.36[b]	5.77 ±0.45[b]	6.17 ±0.73[b]
Green tea extract 0.03%	2.00 ±0.43[b]	3.60 ±0.34[b]	4.69 ±0.42[b]	6.60 ±0.56[b]	-
Clove powder 0.5 %	3.47 ±0.19[d]	4.69 ±0.24[c]	5.77 ±0.26[c]	5.95 ±0.65[c]	6.19 ±0.38
Clove extract 0.5 %	4.69 ±0.36[d]	4.47 ±0.32[c]	5.69 ±0.27[d]	6.84 ±0.52[c]	-
Fresh garlic 3%	4.69 ±0.61[b]	4.69 ±0.72[d]	5.77 ±0.35[b]	6.90 ±0.43[d]	-
Garlic powder 0.9 %	4.77 ±0.62[b]	4.77 ±0.31[d]	5.60 ±0.21[b]	5.47 ±0.81[d]	6.65 ±0.35[c]

[a-d]values with different superscripts within the same column are significantly different at P<0.05 difference among treated formulas at 0-day of processing.

Table 6 The sensory attributes (1-5 scores) of fresh sausage treated with different natural additives during chilled storage at 4 ˚C.

Sensory attribute	Treatments*						
	C	GTP	GTE	CP	CE	FG	GP
0-day							
Appearance	5.00±0.00[a]	5.00 0.00[a]	5.00 0.00[a]	5.00 0.00[a]	5.00±0.00[a]	4.55±0.15[a]	5.00± 0.00[a]
Flavor	5.00± 0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	4.57±0.16[a]	5.00± 0.00 [a]
Juiciness	4.83±0.43 [a]	5.00±0.00[a]	5.00±0.00[a]	3.50±0.20[a]	3.54±0.20[a]	3.45±0.19[a]	4.75±0.23 [a]
Texture	4.58±0.2 [a]	5.00 0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.00±0.00[a]	5.00± 0.00 [a]
OA	4.75±0.23 [a]	5.00±0.00[a]	5.00 0.00[a]	4.80±0.23[a]	4.85±0.24[a]	4.85±0.24[a]	4.95±0.25[a]
3rd day							
Appearance	3.55±0.22[a]	4.80±0.1[b]	4.93±0.0[b]	4.80±0.1[b]	4.66±0.0[b]	4.10±0.2[b]	4.55±0.15[b]
Flavor	3.65±0.19[a]	4.63±0.09[b]	4.47±0.24[b]	4.45±0.28[b]	4.53±0.15[b]	4.40±0.20[b]	4.45±0.21 [b]
Juiciness	3.55±0.21[a]	4.75±0.37[b]	4.58±0.72[b]	4.36±0.19[b]	4.33±0.15[b]	4.77±0.14[b]	4.32±0.13[b]
Texture	3.25±0.09 [a]	4.76±0.14 [b]	4.57±0.29 [b]	4.53±0.15 [b]	4.76±0.18 [b]	4.15±0.17 [b]	4.67±0.11 [b]
OA	3.57±0.14[a]	4.70±0.15 [b]	4.70±0.20 [b]	4.40±0.21 [b]	4.26±0.14 [b]	4.50±0.28 [b]	4.55±0.18 [b]
6th day							
Appearance	1.56±0.25[a]	4.20±0.16[b]	3.46±0.15[c]	4.33±0.16[b]	3.50±0.04[c]	4.26±0.12[b]	3.70±0.14 [c]
Flavor	1.0±0.18 [a]	4.10±0.30[b]	3.26±0.15[c]	4.26±0.16[b]	3.23±0.16[c]	4.30±0.16[b]	3.66±0.17 [c]
Juiciness	_	4.23±0.09[b]	3.65±0.12[c]	4.26±0.11[b]	3.33±0.17[c]	4.50±0.28[b]	4.0±0.22 [c]
Texture	1.66±0.15[a]	4.26±0.14[b]	3.45±0.28[c]	4.33±0.18[b]	3.90±0.11[c]	4.36±0.23[b]	3.69±0.20 [c]
OA	1.0±0.12 [a]	4.0±0.29[b]	3.66±0.40[c]	4.33±0.16[b]	3.55±0.29[c]	4.44±0.12[b]	3.43±0.23 [c]
9th day							
Appearance	-	3.12±0.16[b]	2.0±0.28[c]	3.22±0.26[b]	2.39±0.18[c]	3.13±0.20[b]	2.46±0.17[c]
Flavor	-	3.11±0.18[b]	2.20±0.20 [c]	3.21±0.12[b]	2.4±0.15[c]	3.20±0.18[b]	2.58±0.15[c]
Juiciness	-	3.52±0.17[b]	2.34±0.23 [c]	3.0±0.09[b]	2.55±0.15[c]	3.11±0.11[b]	2.49±0.19[c]
Texture	-	3.45±0.05[b]	2.17±0.17 [c]	3.46±0.16[b]	2.45±0.25[c]	3.57±0.18[b]	2.45±0.20[c]
OA	-	3.15±0.20[b]	1.95±0.15 [c]	3.10±0.16[b]	2.0±0.09 [c]	3.36±0.26[b]	2.22±0.15[c]
12th day							
Appearance	-	2.76±0.17 [b]	-	2.57±0.15 [b]	-	2.49 ±0.13[b]	-
Flavor	-	2.52±0.13 [b]	-	2.51±0.22 [b]	-	2.52±0.13 [b]	-
Juiciness	-	2.56±0.17 [b]	-	2.52±0.13 [b]	-	2.57±0.15[b]	-
Texture	-	2.50±0.24 [b]	-	2.55± 0.19[b]	-	2.57± 0.19[b]	-
OA	-	2.56±0.22 [b]	-	2.57±0.18 [b]	-	2.65±0.18 [b]	-

[a-c]values with different superscripts within the same raw are significantly different at P<0.05
C: control; GTP: green tea powder; GTE: green tea extract; CP: clove powder; CE: clove extract; FG: fresh garlic; GP: garlic powder; OA: overall acceptability

Extension of shelf life by using natural additives and their extracts

From the results obtained in Tables 1-6 it is clear that addition of natural additives or their extracts extended the shelf life of fresh sausage with difference in the duration of extension. Regarding the control non-treated samples, all measured parameters were above the acceptable limits at the 6th day of storage with the appearance of objective signs of deterioration; therefore, control can be acceptable until the 3rd day of refrigerated storage. Sausages formulas formulated with addition of green tea extract, clove extract or garlic powder revealed values higher than the acceptable limits for pH, TVBN, TBARS and bacterial counts with sensory scores lower than the acceptable limits (2.5) at the 9th day of storage, therefore, these formulas can be acceptable until the 6th day of storage with extension of shelf life for 3 days (more than the control). However, sausage formulas treated with green tea powder, clove powder or fresh garlic revealed marginal (critical) limits for pH, TVBN, TBARS and bacterial counts with marginal critical sensory scores around 2.5 at the 12th day of refrigerated storage; therefore, these formulas can be acceptable until the 12th day of storage with 9 days extension in shelf life in comparison with control and 6 days extension in shelf life in comparison with green tea extract, clove extract and garlic powder.
Therefore, these natural additives and their extracts can extend the shelf life of fresh sausages. It has been reported previously that plant extracts with antimicrobial properties can be used to increase refrigerated meat products shelf-life (**Skandamis *et al*., 2002; Chouliara *et al.*, 2007**).

CONCLUSION

It could be concluded from the current study that natural additives and their extracts have the ability to protect fresh sausage from protein and lipid oxidation. They have antibacterial activities and they can extend the shelf life of meat products. Green tea powder and clove powder were superior to their extracts as antioxidant and antibacterial agents and for extending the shelf life of fresh sausage. Moreover, fresh garlic was superior to garlic powder as antioxidant and antibacterial agents and for extending the shelf life of fresh sausage. The sensory attributes of all formulas treated with natural additives were acceptable. Therefore, these natural additives could be safely used by meat processors to improve the quality and extend the shelf life of meat products.

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