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Assessment of *In Vitro* Antioxidant Capacity of Ginseng Extract and Its Efficiency on Lipid Oxidation Inhibition and Physicochemical Properties in Cooked Ground Beef During Refrigerated Storage

Running head: Antioxidant Efficiency of Ginseng Extract in Ground Beef

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SUMMARY

Research background. Ginseng is a medicinal herb that has anti-inflammatory, anti-diabetic, anti-cancer, anti-obesity, cardio protective, antioxidant and antimicrobial features. However, there is lack of information in previous reports regarding effects of ginseng extract (GE) on the shelf life and quality features of muscle foods. Thus, it is essential to determine the effects of GE on meat model system to provide a valuable insight for improving the shelf life and quality in muscle foods.

Experimental approach. After determining *in vitro* antioxidant activity of GE, the antioxidant effect of GE on cooked ground beef was investigated. *In vitro* antioxidant activity was assigned by ferric

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reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and total phenolic content (TPC) analyzes, whereas lipid oxidation formation, chemical, microbiological and textural changes were determined during 30 days storage. Cooking loss (CL), proximate composition and textural features were measured after thermal processing. pH, CIE color parameters, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxide (LPO), total aerobic mesophilic bacteria, total coliform bacteria, yeast and mold counts were evaluated during refrigerated storage.

Results and conclusions. The mean FRAP ((4.66±0.21) mmol Fe^{+2}/g GE), DPPH (IC_{50}=(12.11±0.09) mg/mL) and TPC ((146.00±2.40) mg GAE/g GE) values of GE exhibited potential antioxidant capacity. GE addition increased CL ($p<0.05$). GE incorporation did not influence the proximate composition of ground beef. GE addition caused a decline in pH ($p<0.05$). Ground beef samples containing 1 % or higher GE had lower TBARS in comparison with control ($p<0.05$). Furthermore, LPO levels of ground beef containing GE were found to be lower than those found in control after 30 days storage ($p<0.05$). Total aerobic mesophilic bacteria, total coliform bacteria, yeast and mold were not evidenced in all groups except control which had 3.35 log CFU/g total aerobic mesophilic bacteria at the end of storage.

Novelty and scientific contribution. Results revealed that GE has an important activity in controlling lipid oxidation and may be implemented in the meat industry to provide prolonged shelf life and microbial stability.

Keywords: ginseng extract; antioxidant; lipid oxidation; meat

INTRODUCTION

Lipid oxidation and microbial growth are frequently encountered quality deteriorations in the food industry and adversely affect not only a consumer preference but also safety. In the elimination of these quality defects, the usage of food additives attracts the attention of the food industry (*1*,*2*). The quality parameters of food products are maintained via incorporation of food additives (*3*). Synthetic food additives possess antimicrobial and antioxidant activity are widely used by the food industry. However, synthetic food additives like nitrite, butylated hydroxyanisole and butylated hydroxytoluene are questioned due to their toxicity and carcinogenic effects. The use of some of these food additives is legally restricted or prohibited because of their harmful influences on consumer health. Furthermore, the usage of natural sources as food additives has increasing trend due to expectation of consumers, legal agencies and the food industry for healthy food (*4*).

Ginseng (*Panax ginseng* C.A. Meyer) is an reputable therapeutical plant in Countries in Eastern Asia. Besides ginseng is utilized as a traditional medicine for treatments of chronic metabolic syndromes, diabetes and cardiovascular disorders, it has also antioxidant, anti-inflammatory, anticancer, antiobesity and antiviral features. Pharmacologic benefits of ginseng are attributed to phenolics, quinones, saponins, flavonoids, tannins, coumarins and alkaloids (*5*,*6*). Ginsenosides as a saponin, are main bioactive compounds of ginseng and responsible for biological properties of ginseng. Kwon *et al*. (*7*) have reported that ginsenosides obtained from ginseng are divided into polar or less polar ginsenosides. Polarity of ginsenosides was reported to determine the pharmacological activity which decreases as the degree of polarity increases (*6*).

The antioxidant activity of ginseng has been associated with the existance of biological molecules such as phenolic acid (*8*), polyacetylene (*9*), polysaccharide (*10*), saponin (*11*) and ginsenoside (*12*). Hussain *et al*. (*13*) stated that the importance of flavonoids in antioxidant activity should not be ignored. Researcher found that ginseng increased antioxidant enzymes like glutathione peroxidase and superoxide dismutase in rats (*14*). Guo *et al*. (*10*) stated that polysaccharides obtained from the stem of ginseng showed higher antioxidant activity compared to those obtained from the root. Differences in harvesting and germination conditions of ginseng plants, different extraction methods and post-extraction applications were reported to have an influence on the antioxidant capability of ginseng extracts (*15*, *16*). Due to the large molecular weight of phenolic acids or ginsenosides in the structure of ginseng, some applications such as thermal application are used to increase its bioavailability. As a result of thermal application, maltol compounds with phenolic characteristics are formed (*12*). An increase in nitric oxide binding activities and hydroxyl radical scavenging activities of phenolic structures containing maltol and a decrease in acceleration of lipid oxidation due to Fe^{+3} chelating activity of maltol itself have been reported (*17*).

Besides the antioxidant activity, it has been stated that ginsenosides obtained from ginseng is also responsible for the antibacterial and antifungal activities of ginseng (*12*). Antimicrobial activity of ginseng has been explained via several mechanisms like inhibition of microbial motility and quorum sensing, reduction of biofilm formation, disruption of cell wall structure and reduction of bacterial adhesion due to stimulation of the immune system (*18*).

This research aimed to evaluate *in vitro* antioxidant activity of ginseng extract and reveal its impacts on lipid oxidation inhibition, chemical, microbiological and textural features of cooked ground beef during 30 days refrigerated storage.

MATERIALS AND METHODS

Materials

Roots of Ginseng (*Panax ginseng* C.A. Meyer) plant were purchased locally (Tokay Herbs & Spices Store, Isparta, Turkey). Ginseng roots were chopped into small fragments and then ground to make a powder by a grinder (Arzum, Istanbul, Turkey). Ginseng powders were then stored at -80 °C. 24-hour post mortem beef (*M. longissimus thoracis et lumborum*) from approximately 1.5–2 years old Simmental cattles was obtained from a local meat supplier, transferred to the laboratory under cold chain, ground, vacuum packaged and then kept in a deep freezer (-20 °C) until used.

Ginseng extraction

Twenty grams of ginseng powder was macerated for 2 days with 100 mL of ethanol (80 % *V/V*; Merck, Darmstadt, Germany) in the dark. The ethanolic ginseng extract (GE) was filtered and the filtrate was held at 40°C in vacuum rotary evaporator (Heidolph Instruments Hei-Vap, Schwabach, Germany) until all of alcohol was removed (*19*).

Antioxidant capacity assays

FRAP anaylsis

FRAP was determined in GE according to Ou *et al*. (*20*). FRAP reagent was prepared by mixing of 300 mmol acetate buffer (pH 3.6), 10 mmol 2,4,6-tripyridyl-s-triazine (TPTZ) (Merck, Darmstadt, Germany) in 40 mmol HCl (Merck, Darmstadt, Germany), and 20 mmol ferric chloride (Merck, Darmstadt, Germany) (10:1:1). 100 µL GE and 3 mL FRAP reagent were added and absorbance values were determined at 593 nm (T8+ UV/VIS Spectrometer, PG Instruments Ltd., Leicestershire, U.K.). The results were reported as mmol Fe^{2+} equivalents/g.

DPPH scavenging anaylsis

The scavenging activity of GE were measured according to Dorman *et al*. (*21*). GE at different amounts (20, 40, 60, 80 and 100 µL) were added to 600 µL of DPPH reagent (0.1 mmol) and total volume was completed to 6 mL with ethanol. Absorbance of the mixture was recorded at 517 nm against a blank after 15 min incubation in dark at room temperature. Results were expressed as % inhibition and IC_{50} value.

TPC anaylsis

One mL GE and 5 mL 0.2 N Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) were mixed for 3 min. A 7.5 % sodium carbonate (Merck, Darmstadt, Germany) was put into the mixture and then kept for 30 min at room temperature. To determine TPC, absorbance was obtained at 720 nm. The results were reported as mg gallic acid equivalents/g (*22*).

Meat sample preparation and design of experimental groups

Ground beef removed from the freezer was thawed at 4 °C for 12 h. After adding 10 % pure water and 2 % NaCl, thawed ground beef was divided into equal portions for the experimental groups. Experimental groups were formed according to tested GE doses (Table S1). After GE was incorporated, the filling process was carried out in plastic centrifuge tubes with screw caps. For each experimental group, a 50 g ground beef sample was carefully filled into each of these tubes to have no air gap. In addition to experimental groups, one extra tube was filled for monitoring of the core temperature. After each filled tube was put into a water bath set at 60 °C, water bath temperature was increased to 85 °C. Then, the core temperature for the experimental groups was tracked with a thermocouple. The cooking process was terminated when the core temperature reached 74 °C. After removal of the cookout liquid, cooked samples were stored at 4 °C for 30 days.

Cooking loss analysis

The mass of the raw sample was recorded before production. After the cooking process, the liquid part was removed from the tubes that were cooled at room temperature and the mass was recorded again. Cooking loss is calculated according to the formula shown below.

$$\text{Cooking loss}=((m_r-m_c)/m_r)\cdot 100 \qquad /1/$$

where m_r is the mass of the raw meat sample and m_c is the mass of cooked sample.

Physicochemical composition

Moisture, protein, fat, and ash levels were determined according to AOAC method (*23*). pH measurement was performed with a pH meter (Hanna Instruments, Bedfordshire, U.K.). After homogenization of 5 g sample in 50 mL distilled water, pH was determined. Color measurements were performed in triplicate from samples stored at 4 °C. CIE *L**, *a** and *b** values were determined with Precise Color Reader TCR 200 (BAMR Ltd., Claremont, South Africa) instrument (*24*). The color device was calibrated with the dark and white field standards before the measurements. Texture measurements were made by a texture analyzer (Brookfield, CT3, Middleboro, MA, USA) under room

temperature conditions. A 36 mm probe thickness, 50 kg load cell, sample 0.5 cm thickness, 0.35 mm penetration (70 % compression), 2 mm/s probe velocity before and after test, and 5 mm/s probe velocity during test were implemented as analysis conditions. Hardness/N, cohesiveness, springiness, gumminess/N, chewiness, adhesiveness/mJ and resilience parameters were determined in meat samples.

Analysis of TBARS and LPO

TBARS analysis was implemented in accordance with method explained by Kilic and Richards (*25*) for tracking progress of lipid oxidation in meat samples. Propyl gallate and EDTA were added to the extraction solution of trichloroacetic acid (TCA) to avoid TBARS formation during the analysis. 2 g meat sample was stired in the extraction solution (12 mL). Meat samples were homogenized for 15 seconds and the homogenate was filtered on Whatman 1 filter paper. 1 mL of the filtrate obtained was taken and mixed with thiobarbituric acid (TBA) solution and then vortexed. Then, the mixture was warmed up at 100 °C for the duration of 40 min. Then, tubes were cooled in cold water. After the cooling, the samples were centrifuged at 4000 rpm for 10 min. Absorbance values were recorded at 532 nm versus a blank including TCA extraction solution (1 mL) and TBA solution (1 mL). TBARS levels were reported as μmol MDA/kg.

LPO method explained by Kılıç *et al*. (*26*) was used for the analysis of lipid hydroperoxide. Briefly, 1 g sample was homogenized for 30 seconds in 5 mL chloroform/methanol (1:1). After that, 3 mL NaCl (0.5 %) was added and then vortexed for 30 seconds. Then, this mixture was subjected to centrifugation for 10 min at 2000 g to achieve phase separation. After that, lower phase (2 mL) was taken and added to the cold methanol/chloroform (1.3 mL; 1:1) mixture and vortexed. After adding 25 μL iron (II) chloride (18 mM) and 25 μL ammonium thiocyanate (4.38 M), the samples were held at room temperature for 20 min, and their absorbance values were measured at 500 nm.

Microbiological analysis

To determine total aerobic mesophilic bacteria (TAMB), total coliform bacteria (TCB), yeast and molt (YM) counts, under aseptic conditions, 10 g meat samples were weighed into homogenizer bags and 90 mL of physiological saline was added. After homogenizing for 1 min, serial dilutions were prepared from this dilution and incubated at 30 °C for 48 h on Plate Count Agar (Merck, Darmstadt, Germany) for TAMB, at 37 °C for 48 h on Eosin Methylene Blue Agar (Merck, Darmstadt, Germany) for TCB, and at 25 °C for 72 h on Potato Dextrose Agar (Merck, Darmstadt, Germany) for YM. Colony counts were obtained at the end of the incubation and presented as CFU/g (*27*).

Statistical analysis

The study was performed in two replications and the analyzes were performed in three parallels. The significant differences among results were determined using the MiniTab® 19.1.1 (U.S.A.) package program (*28*). After applying the analysis of variance (One-way ANOVA) to antioxidant *capacity assays* (DPPH, FRAP and TPC) of GE and the post-production analyzes (cooking loss, moisture, protein, fat and ash contents, and texture analysis), the differences among the experimental groups were determined by Duncan multiple comparison test. As far as pH, instrumental color, TBARS, LPO and microbiological analysis are concerned, statistical desing of experiments was six GE doses (0, 0.1, 0.5, 1, 1.5 and 2 %) x six storage times (0, 3, 5, 7, 15 and 30 days) for cooked ground beef samples as a factorial arrangement. The independent variables (GE dose and storage time) and replications were desinged as fixed and random effects, respectively. The main effects and their interactions related to the independent variables were determined. The dependent variables were pH, instrumental color, TBARS, LPO, total aerobic mesophilic bacteria, total coliform bacteria, yeast and molt counts. In this model, the results were tested using restricted maximum likelihood (REML) method with a 95 % confidence interval.

RESULTS AND DISCUSION

Antioxidant capacity assay results

DPPH results (data not shown) indicated that IC_{50} and inhibiton % of GE were 12.11±0.09 mg/mL and 70.21±0.82 % respectively. Several previous studies are available regarding DPPH radical binding activity of GE. Chung *et al*. (*28*) stated that DPPH values of ginseng extracted with methanol was between 18.08 % and 25.61 %. Moreover, Lee *et al*. (*30*) reported 51-86,2 % DPPH radical binding activity in ginseng ethanolic extract. Ganguly *et al*. (*31*) stated that IC_{50} values of GE were 32.80 and 38.83 µg/mL in methanol and methanol:chloroform:water solvents, respectively. Jiang *et al*. (*32*) found 12 mg/mL IC_{50} value in the essential oil obtained from leaves of ginseng. Zhao *et al*. (*33*) found that DPPH binding activity varied between 50-95 % and IC_{50} values were between 0.150-0.155 mg/mL in oligosaccharides obtained from GE. Hussain *et al*. (*13*) also found that the inhibition value in GE ranged from 53.12% to 62.84 %.

TPC value of GE was 146.00±2.40 mg gallic acid equivalent/g dry matter in our study. Lee *et al*. (*30*) indicated that TPC value of ginseng ethanolic extract was found to be 6.72 µg tannic acid equivalent/g. Ganguly *et al*. (*31*) stated that total phenolic content of GE in methanol and methanol:chloroform:water (1:1:1) was 97.38 and 109.65 µg GAE/mg extract, respectively. Shahriar

et al. (*34*) stated that TPC value in chloroform extraction of ginseng was 60.99 µg GAE/mg extract. Pal *et al*. (*35*) reported that total phenolic content levels of GE in three solvents (methanol, chloroform and water) were 42, 66.72 and 88.58 µg GAE/mg extract, respectively and the reason for this difference in each solvent was explained with the polarity of the polyphenolic substances. Zhao *et al*. (*33*) found that the total phenolic content of 4 different oligosaccharides extracted from ginseng ranged from 1.91 to 3.51 µg GAE/mg. Ryu *et al*. (*36*) stated that the breakdown of ginsenosides in large molecule structures and their conversion into small molecules increased the total phenolic content of ginsenosides approximately three folds.

In our study, the FRAP activity of GE was determined as 4.66±0.21 mmol Fe^{+2}/g GE. Lee *et al*. (*30*) found the FRAP activity of GE in the range of 1.04-2.34 µg ascorbic acid equivalent/g extract. Variation in previous studies regarding antioxidant activity of ginseng extract is thought to be associated with the differences in the type and the parts of ginseng plant used and the applied extraction conditions such as the type of extraction solvent, extraction time and temperature. All these factors greatly influence contents of antioxidants like polyyphenols, flavonoids and saponins in ginseng extract (*37*).

Effect of ginseng extract on inhibition of lipid oxidation, chemical, microbiological and textural features of cooked ground beef during refrigerated storage

Cooking loss results

Cooking loss results obtained in our study are presented in Table 1. Cooking loss values were found to be ranged from 22.63 to 26.31 %. It was determined that GE addition had a considerable effect on cooking loss values ($p<0.05$). While the groups containing GE showed similar cooking loss values among themselves, the groups containing GE had a higher cooking loss values than control ($p<0.05$). Our findings are supported by Kim *et al*. (*38*) who also stated that ginseng addition in the formulation of pork sausage caused an increase in cooking loss. Researchers (*38*) also reported that increased cooking loss due to ginseng addition was associated with the change in meat pH. Similarly, in our study, it was determined that there was a decrease in meat pH (Table 2) at tested high GE doses (G15 and G20).

Physicochemical composition

Physicochemical composition of the experimental groups is presented in Table 1. The moisture content in control was 69.03 %, whereas, the moisture content in the experimental groups containing

GE varied between 67.22-68.56 %. Results revealed that groups containing GE were found to have similar moisture content with control, which means that GE incorporation into formulation had no impact on the moisture content. Protein contents determined in experimental groups raged from 27.33 to 29.7 % and did not revealed a significant difference among the experimental groups formulated with or without GE. The amount of fat in the experimental groups varied between 3.48 and 3.89 %, and did also not show any difference among the experimental groups. The ash content of the experimental groups was determined in the range of 3.17-3.62 %. Although the ash contents of the groups containing GE were similar to those of control, ash content of G10 group (3.62 %) was found to be higher ($p<0.05$) than that of G01 (3.17 %).

Results revealed that GE dose (GD) and storage time (ST) had a influence on pH values of the samples ($p<0.0001$), whereas GDxST interaction (Table 2) was not a factor. Therefore, only main effects (GD and ST) will be discussed rather than GDxST interaction. In general, there was a decline in pH values in groups with GE dose of 1.5 % or more ($p<0.05$). Regardless of ST, pH values of control (6.04±0.01), G01 (6.07±0.01), G05 (6.06±0.01)and G10 (6.04±0.01) groups were found to be similar. In addition, it was determined that G15 (6.01±0.01) and G20 (6.01±0.01) groups were similar in terms of pH values. Although the pH values of the G15 and G20 groups determined in this study are statistically lower than the other groups, it is thought that these differences may not be significant in practical applications. Ibrahim *et al*. (*39*) also stated that the usage of GE in lamb patties created lower pH values compared to control. Regardless of GD, pH values increased ($p<0.05$) during first 5 days of storage and then started to decrease ($p<0.05$) again during rest of the storage (Processing day; 6.00±0.01 day 3: 6.08±0.01 day 5 6.08±0.01 day 7 6.01±0.01 day 15 6.03±0.01 day 30 6.00±0.01). Ibrahim *et al*. (*39*) stated that the elevation in pH values during the storage of lamb patties was due to ammonia resulting from protein oxidation or degradation of proteins by proteolysis. Furthermore, pH decline determined after 5 days of storage is thought to be related with the activity of lactic acid bacteria. pH drop in stored muscle foods has been reported to be possibly associated with the activity of lactic acid bacteria, which metabolize the carbohydrates in muscle foods and convert them into lactic acid (*40*,*41*).

The effect of GD and ST on CIE color values of the experimental groups is presented in Table 2. According to the analysis of variance, the impact of GD, ST and GDxST interaction on the CIE $L^*a^*b^*$ values was found to be significant ($p<0.0001$). The lowest L^* values on processing day were obtained in G20 group ($p<0.05$). Although L^* values of G01, G05, G10 and G15 groups on processing day were similar to control, L^* values of G10 and G15 groups were found to be different from each other ($p<0.05$). Results indicated that L^* values presented an increasing ($p<0.05$) trend throughout

storage except those of G05, G10 and G15 groups which were completely constant throughout the same period of time. After 30 days of storage, the lowest *L** values were obtained in G15 and G20 groups, whereas the highest *L** values were in control and G01 ($p<0.05$). In general, as GE dose increased, *L** values decreased after 30 days storage ($p<0.05$). There was no considerable differences regarding *a** and *b** values among control and GE incorporated groups on processing day. A decrease in *a** values and an increase in *b** values were observed in all experimental groups throughout storage ($p<0.05$). After 30 days storage, *a** values of groups containing GE were found to be similar among themselves but lower ($p<0.05$) than that of control. On the other hand, the highest ($p<0.05$) b* values were found in G01 and G05 groups, whereas the rest of the experimental groups had similar *b** values. Kim *et al*. (*38*) found that ginseng addition elevated *b** values of pork sausage while decreasing the *L** and *a** values. However, Cho *et al*. (*42*) stated that ginseng powder addition did not influence the color parameters of pork.

Texture analysis results belonging to the experimental groups are shown in Table 3. Texture analysis results showed no significant differences among the experimental groups in terms of hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess and chewiness. Kim *et al*. (*38*) reported that ginseng addition decreased only the hardness parameters of pork sausage, but did not affect other parameters.

TBARS and LPO

Impacts of GE dose and storage time on TBARS and LPO values of the experimental groups are shown in Table 4. Results indicated that TBARS values of all experimental groups containing GE were lower than control on processing day ($p<0.05$). Results revealed that TBARS values gradualy rised in all experimental groups throughout storage period ($p<0.05$). GDxST interaction revealed that TBARS values of control and G01 groups gradually increased during each storage day ($p<0.05$). In the meantime, TBARS values of G05 increased during the firts 5 days storage, remained stable during the period between day 5 and day 7 and then showed increasing trend again during the rest of the storage. Furthermore, TBARS values of G10, G15 and G20 groups remained constant during to the storage period between day 3 and day 5, and then presented an increasing pattern during the remaining storage days ($p<0.05$). After 30 days of storage, the highest TBARS values were obtained in control, G01 and G05 groups, whereas the lowest TBARS (26.79 % reduction) values were determined in G20 group ($p<0.05$). Generally, TBARS values decreased as incorporated GE dose increased ($p<0.05$). Papuc *et al*. (*43*) indicated that dried plants and essential oils are successful in retarding lipid oxidation in muscle foods and this effect is due to the fact that polyphenols are good

electron and proton donors. It has also been stated that bioactive components such as triterpenes and saponins in ginseng can prevent chain reactions that occur during lipid oxidation (*44*). It has also been stated that lipid oxidation in cooked muscle foods can be influenced by pH which affects the activities of pro-oxidants, especially haem iron. It has been reported that iron-catalyzed oxidation has been reported to decrease with increase in pH from 2 to 10 and myogloblin-catalyzed oxidation decrease with increase in pH (*45*). Since pH differences determined among the groups in the present study were negligible in practical stand point, pH is thought to be insignificant factor to modulate lipid oxidation development. Furthermore, Ibrahim *et al*. (*39*) stated that GE had higher antioxidant activity compared to jojoba and ginger extracts, and the lowest TBARS values after 9 days storage were found in GE added groups. In another previous study in which GE was added into the meat emulsion model, researchers stated that use of 2.5 % GE prevented lipid oxidation of meat products (*43*). On the other hand, Cho *et al*. (*42*) stated that incorporation of ginseng powder was able to kept TBARS constant up to 5 days in pork chops stored at 4 °C for 15 days, but lipid oxidation could not be prevented during the rest of storage period.

Results regarding the impacts of GE dose and storage time on LPO levels of the experimental groups showed that there was no meaningful variation among the experimental groups on processing day. It was determined that LPO values of all experimental groups progressively elevated throughout storage ($p<0.05$). On the other hand, LPO values of groups containing 1 % or more GE increased during firts 3 days storage, remained stable during the period between day 3 and day 7 and then showed increasing trend again during the rest of the storage ($p<0.05$). After 30 days storage, the highest LPO values were obtained in control, while the lowest LPO (52.25 % reduction) values were determined in G20 group ($p<0.05$). Generally, as the amount of GE dose increased, LPO values decreased, but the same trend was not observed in G01 and G05 groups. LPO values of G01 were lower than LPO values of G05 ($p<0.05$).

Microbiological analysis

Microbiological analysis outcomes (Table 5) indicated that TAMB was 4.60 log CFU/g in raw meat material before heat treatment, whereas it was <1 log CFU/g in all experimental groups after cooking process. After 30 days of storage, even though TAMB in control was 3.35 log CFU/g, TAMB was <1 log CFU/g in rest of the other experimental groups containing GE. Ibrahim *et al*. (*39*) also pointed out that the usage of GE reduced the aerobic mesophyll bacteria load in lamb patties. While TAMB in experimental groups containing GE was below the detection limit in our study, Ibrahim *et al*. (*39*) reported that TAMB in the experimental group containing GE was 2.51 log CFU/g. As far as

experimental group without GE is concerned, researchers also observed an increasing trend in TAMB in control throughout refrigerated storage period. Furthermore, Kim *et al*. (*38*) stated that as GE dose increased, the total aerobic plate count in pork sausage decreased. As far as TCB is concerned, TCB was 3.69 log CFU/g in raw meat material, but after heat treatment, it was <1 log CFU/g in all experimental groups. TCB in all experimental groups was also <1 log CFU/g throughout whole storage periods. On the other hand, YM count in raw meat material was 3.30 log CFU/g before heat treatment, but after heat treatment, YM count was <1 log CFU/g in all experimental groups. YM count determined in all experimental groups were <1 log CFU/g until the end of storage. Ibrahim *et al*. (*39*) stated that GE was more effective on TAMB rather than YM count in lamb patties. Overall, inhibited microbial growth in the experimental groups containing GE could be the resulted from antimicrobial activity of the components in GE used in our study. The components of GE such as ginsenosides was reported to interact with the microorganism and prevent the microbial growth via inhibition of microbial motility and quorum sensing, reduction of biofilm formation, disruption of cell wall structure and reduction of bacterial adhesion due to stimulation of the immune system (*18*).

CONCLUSIONS

In vitro antioxidant capacity results demonstrated that GE had iron ion reducing and free radical scavenging activities. Results revealed that GE addition caused a decline in pH and an elevation in cooking loss in ground beef. Furthermore, GE addition resulted in decreased brightness and redness values, but increased yellowness values in cooked ground beef. GE incorporation did not affect the textural parameters and the proximate composition of cooked ground beef. The use of GE exibited capability of inhibiting lipid oxidation in cooked ground beef and this effect increased as incorporated GE dose increased. Results indicated that aerobic mesophilic bacteria were inhibited more at the end of storage in GE incorporated cooked ground beef compared to those produced without GE. In addition, yeast, mold and coliform bacteria growth was not observed during 30 days storage in all experimental groups regardles of GE incorporation. In conclusion, study results showed that GE has the ability to be utilized as a natural food preserver to provide oxidative and microbial stability in the ready to eat meat products. Moreover, further research is needed to determine the effects of GE on the sensory properties of the meat products for designing foods to meet consumer demands.

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CONFLICT OF INTEREST

Authors have no disclosable conflict of interests regarding this article.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at: www.ftb.com.hr.

AUTHORS' CONTRIBUTION

A. Soyuçok, B. Kılıç and G. B. Kılıç participated in the research design and conducting experiments. B. Kılıç and G. B. Kılıç contributed to the manuscript writing.

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Table 1. Effects of ginseng extract on proximate composition and cooking loss values of cooked ground beef

Group	*w*(moisture)/%	*w*(protein)/%	*w*(fat)/%	*w*(ash)/%	*w*(cooking loss)/%
Control	(69.03±1.12)^{a}	(27.33±1.49)^{a}	(3.40±0.49)^{a}	(3.42±0.11)^{abc}	(22.63±0.62)^{b}
G01	(68.15±2.00)^{a}	(28.78±3.21)^{a}	(3.48±0.39)^{a}	(3.17±0.08)^{c}	(25.80±1.43)^{a}
G05	(67.79±1.49)^{a}	(28.22±0.50)^{a}	(3.73±0.81)^{a}	(3.41±0.13)^{abc}	(26.31±1.23)^{a}
G10	(67.22±0.68)^{a}	(28.20±1,93)^{a}	(3.64±0.52)^{a}	(3.62±0.39)^{a}	(25.07±0.70)^{a}
G15	(67.73±0.59)^{a}	(29.05±2.18)^{a}	(3.89±0.88)^{a}	(3.47±0.32)^{ab}	(26.20±0.58)^{a}
G20	(68.56±1.06)^{a}	(29.17±0.97)^{a}	(3.83±0.60)^{a}	(3.33±0.26)^{bc}	(25.62±0.58)^{a}

Mean±Std. error; $^{a\text{-}c}$Within a column, values superscripted with different letters are significantly different ($p<0.05$).

Table 2. Effects of ginseng extract on CIE *L*a*b** color and pH values in cooked ground beef during 30 days storage at 4 °C

			t(Storage)/day				
	Groups	Processing day	3	5	7	15	30
*L**	Control	(55.52±0.78)$^{c-g}$	(53.38±0.56)$^{h-m}$	(54.12±0.88)$^{e-l}$	(55.93±0.64)^{cde}	(53.45±0.29)$^{g-m}$	(58.66±0.95)^{a}
	G01	(55.21±0.80)$^{c-h}$	(53.29±0.48)$^{h-n}$	(55.78±0.66)$^{c-f}$	(57.04±1.03)^{abc}	(55.23±0.99)$^{c-h}$	(58.53±0.92)^{a}
	G05	(55.29±0.96)$^{c-h}$	(54.41±0.88)$^{d-k}$	(56.39±1.06)^{bcd}	(51.14±1.03)^{opq}	(56.98±1.60)^{abc}	(56.09±0.97)$^{b-e}$
	G10	(56.29±0.97)^{bcd}	(54.83±1.39)$^{d-h}$	(54.60±1.02)$^{d-j}$	(52.76±0.51)$^{i-o}$	(58.10±0.88)^{ab}	(54.82±0.65)$^{d-i}$
	G15	(53.74±0.98)$^{f-m}$	(51.29±1.21)$^{n-q}$	(52.47±1.04)$^{k-p}$	(52.16±0.85)$^{l-q}$	(55.27±0.84)$^{c-h}$	(51.72±0.66)$^{m-q}$
	G20	(47.08±1.06)^{s}	(50.15±1.09)^{qr}	(52.71±0.79)$^{j-p}$	(49.04±0.85)^{rs}	(50.65±0.65)^{pqr}	(51.19±0.99)^{opq}
*a**	Control	(11.91±1.44)$^{b-e}$	(14.63±1.39)^{a}	(12.37±1.65)$^{a-d}$	(13.84±0.53)^{ab}	(12.49±2.04)^{abc}	(9.49±1.70)$^{e-l}$
	G01	(12.12±0.87)^{bcd}	(12.50±1.32)^{abc}	(11.49±2.33)$^{b-g}$	(11.53±1.23)$^{b-g}$	(8.85±2.42)$^{h-m}$	(6.82±0.96)^{m}
	G05	(11.72±0.74)$^{b-f}$	(11.84±0.75)$^{b-e}$	(8.76±0.52)$^{h-m}$	(11.69±1.68)$^{b-f}$	(8.17±0.63)$^{j-m}$	(7.13±0.55)^{lm}
	G10	(12.82±0.53)^{abc}	(12.51±0.70)^{abc}	(9.96±0.69)$^{d-k}$	(11.11±1.09)$^{c-h}$	(8.41±0.16)$^{i-m}$	(7.58±0.71)^{klm}
	G15	(10.60±0.39)$^{c-j}$	(10.84±0.43)$^{c-i}$	(10.81±0.65)$^{c-i}$	(8.69±0.20)$^{h-m}$	(8.73±0.52)$^{h-m}$	(7.66±0.43)^{klm}
	G20	(10.61±1.06)$^{c-j}$	(10.46±0.42)$^{c-j}$	(9.27±1.12)$^{f-l}$	(9.13±0.72)$^{g-m}$	(9.17±0.32)$^{g-m}$	(7.63±0.57)^{klm}
*b**	Control	(2.89±0.49)$^{f-i}$	(1.16±0.15)^{j}	(2.43±0.55)$^{g-j}$	(1.64±0.13)^{ij}	(3.72±0.52)$^{c-g}$	(4.77±0.14)^{bc}
	G01	(3.16±0.54)$^{d-h}$	(2.13±0.79)^{hij}	(4.28±0.55)$^{b-e}$	(2.93±1.48)$^{f-i}$	(4.74±0.57)^{bc}	(6.39±0.24)^{a}
	G05	(2.37±0.22)^{hij}	(2.44±0.81)$^{g-j}$	(4.17±0.63)$^{b-f}$	(2.83±1.02)$^{f-i}$	(5.34±0.33)^{ab}	(6.24±0.40)^{a}
	G10	(2.68±0.49)^{ghi}	(1.64±0.15)^{ij}	(3.14±0.24)$^{d-h}$	(3.07±0.80)$^{e-h}$	(4.44±0.46)^{bcd}	(4.46±0.65)^{bcd}
	G15	(2.51±0.51)^{ghi}	(2.55±0.24)^{ghi}	(2.66±0.23)^{ghi}	(4.31±0.42)$^{b-e}$	(4.35±0.82)$^{b-e}$	(4.57±0.36)^{bc}
	G20	(2.63±0.25)^{ghi}	(2.51±0.29)^{ghi}	(4.85±0.45)^{bc}	(4.03±0.59)$^{b-f}$	(4.42±0.43)^{bcd}	(4.89±0.36)^{bc}
pH	Control	(6.03±0.02)$^{b-h}$	(6.07±0.02)$^{a-h}$	(6.09±0.01)$^{a-f}$	(5.98±0.05)$^{b-h}$	(6.02±0.05)$^{a-h}$	(6.00±0.03)$^{d-h}$
	G01	(6.07±0.02)$^{a-h}$	(6.11±0.02)$^{a-d}$	(6.14±0.04)^{ab}	(6.03±0.02)$^{b-h}$	(6.06±0.02)$^{a-h}$	(6.00±0.02)$^{b-h}$
	G05	(6.09±0.07)$^{a-h}$	(6.15±0.05)^{a}	(6.09±0.04)$^{a-e}$	(6.04±0.02)$^{a-h}$	(6.06±0.02)$^{a-h}$	(6.02±0.03)$^{b-h}$
	G10	(5.99±0.03)$^{c-h}$	(6.12±0.05)$^{a-g}$	(6.10±0.03)$^{b-h}$	(6.00±0.03)$^{b-h}$	(6.03±0.01)$^{b-h}$	(6.01±0.03)$^{b-h}$
	G15	(5.97±0.03)^{fgh}	(6.08±0.01)$^{a-h}$	(6.05±0.01)$^{a-h}$	(5.95±0.04)^{gh}	(5.99±0.02)$^{d-h}$	(5.98±0.01)$^{e-h}$
	G20	(5.98±0.01)^{fgh}	(6.05±0.01)$^{a-h}$	(6.04±0.02)$^{a-h}$	(6.00±0.05)^{h}	(6.06±0.06)$^{b-h}$	(6.00±0.03)$^{d-h}$

Mean±Std. error; $^{a-s}$For each of tested parameters in the table, values superscripted with different letters are significantly different ($p<0.05$)

Table 3. Effects of ginseng extract on textural characteristics of cooked ground beef

Group	Hardness/N	Adhesiveness/mJ	Resilience	Cohesiveness
Control	(5.44±0.74)^{a}	(0.33±0.40)^{a}	(0.16±0.02)^{a}	(0.58±0.06)^{a}
G01	(5.57±1.71)^{a}	(0.20±0.14)^{a}	(0.19±0.07)^{a}	(0.61±0.06)^{a}
G05	(5.76±0.66)^{a}	(0.08±0.05)^{a}	(0.20±0.06)^{a}	(0.61±0.06)^{a}
G10	(5.88±0.57)^{a}	(0.15±0.19)^{a}	(0.18±0.02)^{a}	(0.58±0.03)^{a}
G15	(4.96±0.97)^{a}	(0.25±0.17)^{a}	(0.17±0.03)^{a}	(0.65±0.04)^{a}
G20	(5.32±0.56)^{a}	(0.20±0.12)^{a}	(0.16±0.01)^{a}	(0.63±0.15)^{a}
	Springiness	Gumminess/N	Chewiness/N	
Control	(0.81±0.06)^{a}	(3.13±0.46)^{a}	(17.73±3.4)^{a}	
G01	(0.86±0.08)^{a}	(3.35±0.91)^{a}	(20.20±6.6)^{a}	
G05	(0.91±0.11)^{a}	(3.48±0.29)^{a}	(22.13±3.9)^{a}	
G10	(0.85±0.04)^{a}	(3.42±0.41)^{a}	(20.38±3.2)^{a}	
G15	(0.84±0.08)^{a}	(3.19±0.50)^{a}	(18.68±3.9)^{a}	
G20	(0.88±0.05)^{a}	(3.33±0.66)^{a}	(20.55±5.2)^{a}	

Mean±Std. error; $^{a\text{-}b}$Within a column, values superscripted with different letters are significantly different ($p<0.05$)

Table 4. Effects of ginseng extract on TBARS and LPO values in cooked ground beef during 30 days storage at 4 °C

			t(storage)/day				
	Groups	Processing day	3	5	7	15	30
TBARS	Control	(6.83±0.73)^{q}	(20.76±0.81)^{lm}	(24.26±0.88)^{hij}	(27.92±0.52)^{ef}	(36.27±0.87)^{b}	(39.45±1.75)^{a}
	G01	(4.75±0.45)^{r}	(23.36±0.66)^{ijk}	(26.16±0.71)^{fg}	(31.70±0.68)^{cd}	(34.89±0.03)^{b}	(40.43±0.84)^{a}
	G05	(4.70±0.36)^{r}	(21.83±0.33)^{kl}	(25.35±0.87)^{gh}	(26.56±0.66)^{fg}	(35.70±0.02)^{b}	(40.51±0.61)^{a}
	G10	(3.61±0.18)^{r}	(19.66±0.36)^{mn}	(21.13±0.29)^{lm}	(25.06±1.05)^{gh}	(30.72±0.01)^{d}	(32.75±0.77)^{c}
	G15	(4.27±0.83)^{r}	(18.63±0.65)^{n}	(18.35±0.75)^{no}	(25.57±0.96)^{gh}	(28.44±0.02)^{e}	(30.88±0.99)^{d}
	G20	(4.21±0.62)^{r}	(14.78±0.60)^{p}	(16.59±0.85)$^{o-p}$	(23.03±0.43)^{jk}	(25.79±0.06)^{gh}	(28.88±1.82)^{e}
LPO	Control	(18.75±0.94)^{m}	(35.18±1.86)^{l}	(41.17±1.88)^{l}	(80.72±1.63^{j}	(299.09±8.54)^{e}	(454.00±12.69)^{a}
	G01	(17.97±1.00)^{m}	(36.19±1.32)^{l}	(41.66±1.12)^{l}	(85.16±2.09^{j}	(314.55±3.09)^{d}	(363.01±9.66)^{c}
	G05	(16.11±0.54)^{m}	(35.17±0.56)^{l}	(38.77±1.41)^{l}	(60.85±2.25^{k}	(324.11±7.65)^{d}	(381.42±9.30)^{b}
	G10	(17.85±0.40)^{m}	(35.04±0.80)^{l}	(40.30±2.53)^{l}	(44.36±1.17^{l}	(266.18±6.05)^{g}	(285.27±8.08)^{f}
	G15	(18.41±0.67)^{m}	(34.98±1.06)^{l}	(40.15±2.92)^{l}	(43.50±1.74^{l}	(186.38±4.47)^{i}	(256.37±7.91)^{g}
	G20	(19.08±1.46)^{m}	(34.96±1.45)^{l}	(39.88±5.43)^{l}	(43.97±1.16^{l}	(178.69±2.28)^{i}	(216.79±3.54)^{h}

Mean±Std. error; $^{a\text{-}r}$For each of tested parameters in the table, values superscripted with different letters are significantly different ($p<0.05$)

Table 5. Effects of ginseng extract on microbiological counts of cooked ground beef during 30 days storage at 4 °C

			t(storage)/day				
	Groups	Processing day	3	5	7	15	30
N(TAMB)/(CFU/g)	Control	<1	<1	<1	<1	<1	3.35±0.95[a]
	G01	<1	<1	<1	<1	<1	<1[b]
	G05	<1	<1	<1	<1	<1	<1[b]
	G10	<1	<1	<1	<1	<1	<1[b]
	G15	<1	<1	<1	<1	<1	<1[b]
	G20	<1	<1	<1	<1	<1	<1[b]
N(TCB)/(CFU/g)	Control	<1	<1	<1	<1	<1	<1
	G01	<1	<1	<1	<1	<1	<1
	G05	<1	<1	<1	<1	<1	<1
	G10	<1	<1	<1	<1	<1	<1
	G15	<1	<1	<1	<1	<1	<1
	G20	<1	<1	<1	<1	<1	<1
N(YM)/(CFU/g)	Control	<1	<1	<1	<1	<1	<1
	G01	<1	<1	<1	<1	<1	<1
	G05	<1	<1	<1	<1	<1	<1
	G10	<1	<1	<1	<1	<1	<1
	G15	<1	<1	<1	<1	<1	<1
	G20	<1	<1	<1	<1	<1	<1

[a-b]Within a column, values superscripted with different letters are significantly different ($p<0.05$). TAMB=total aerobic mesophilic bacteria, TCB=total coliform bacteria, YM=yeasts and mould, CFU=colony forming units

Table S1. Applied experimental design, main and interaction effects of ginseng extract doses and storage time on pH, CIE color, TBARS and LPO of cooked ground beef samples stored at 4 °C

Experimental group						
Control	No ginseng extract					
G01	*w*(ginseng extract)/0.1 %					
G05	*w*(ginseng extract)/0.5 %					
G10	*w*(ginseng extract)/1 %					
G15	*w*(ginseng extract)/1.5 %					
G20	*w*(ginseng extract)/2 %					
Independent variable	Dependent variable					
	pH	CIE *L**	CIE *a**	CIE *b**	TBARS	LPO
GD	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ST	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
GD*ST	0.170	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Initial letters used to form abbreviations are; GD: Ginseng extract dose, ST: Storage time