FTB Food Technology & Biotechnology

preliminary communication
ISSN 1330-9862
https://doi.org/10.17113/ftb.62.02.24.8244

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

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Received: 6 June 2023
Accepted: 5 March 2024

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SUMMARY

Research background. Ginseng is a medicinal plant that has anti-inflammatory, antidiabetic, anticancer, antiobesity, cardioprotective, antioxidant and antimicrobial properties. However, previous reports lack information on the effects of ginseng extract on the shelf life and quality characteristics of muscle foods. Thus, it is essential to determine the effects of ginseng extract on the meat model system to gain valuable insights to improve the shelf life and quality of muscle foods.

Experimental approach. After determining the *in vitro* antioxidant activity of ginseng extract, the antioxidant effect of ginseng extract on cooked ground beef was investigated. *In vitro* antioxidant activity was determined using Fe(III) reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and total phenolic content (TPC) analyses, while lipid oxidation, chemical, microbiological and textural changes were determined during 30 days of storage. Cooking loss, proximate composition and textural features were measured after thermal processing. The pH, CIE colour parameters, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxide (LPO), total aerobic mesophilic bacteria, total coliform bacteria, yeast and mould counts were determined during refrigerated storage.

Results and conclusions. The mean values for FRAP expressed as Fe(II) equivalents ((4.7±0.2) mmol/g), DPPH (IC_{50}=(12.11±0.09) mg/mL) and TPC expressed as gallic acid equivalents ((146.0±2.4) mg/g) showed a potential antioxidant capacity of ginseng extract. The addition of ginseng extract increased the cooking loss ($p<0.05$), but it did not affect the proximate composition of ground beef. It also caused a decrease in pH ($p<0.05$). Ground beef samples containing 1 % or more ginseng extract had lower TBARS values than control ($p<0.05$). In addition, LPO values of ground beef with ginseng extract were lower than the control after 30 days of storage ($p<0.05$). Total aerobic mesophilic bacteria, total coliform bacteria, yeast and mould were not found in any of the groups, except in the control, which had 3.35 log CFU/g total aerobic mesophilic bacteria at the end of storage.

Novelty and scientific contribution. The results show that ginseng extract has an important activity in controlling lipid oxidation and can be used in the meat industry to extend shelf life and microbial stability.

Keywords: ginseng extract; antioxidant capacity; lipid oxidation; meat

INTRODUCTION

Lipid oxidation and microbial growth are common quality defects in the food industry that affect not only consumer preference but also safety. To eliminate these quality defects, the use of food additives attracts the attention of the food industry (*1*,*2*). The quality parameters of food products are maintained by the addition of food additives (*3*). Synthetic food additives, which have antimicrobial and antioxidant effects, are widely used by the food industry. However, synthetic food additives such as nitrite, butylated hydroxyanisole and butylated hydroxytoluene are being questioned due to their toxicity and carcinogenic effects. The use of some of these food additives is restricted or prohibited by law because of their harmful effects on consumer health. Furthermore, the use of

natural sources as food additives is increasing due to the expectations of consumers, legal agencies and the food industry regarding healthy food (*4*).

Ginseng (*Panax ginseng* C.A. Meyer) is a highly regarded therapeutical plant in the countries of East Asia. Besides its use as a traditional medicine for treatments of chronic metabolic syndromes, diabetes and cardiovascular disorders, ginseng also has antioxidant, anti-inflammatory, anticancer, antiobesity and antiviral properties. The pharmacological benefits of ginseng are attributed to phenolics, quinones, saponins, flavonoids, tannins, coumarins and alkaloids (*5*,*6*). Ginsenosides, such as saponin, are the main bioactive compounds of ginseng and responsible for its biological properties. Kwon *et al.* (*7*) have reported that the ginsenosides obtained from ginseng are divided into polar and less polar ginsenosides. The polarity of ginsenosides has been reported to determine the pharmacological activity, which decreases with the increasing degree of polarity (*6*).

The antioxidant activity of ginseng has been associated with the presence of biological molecules such as phenolic acids (*8*), polyacetylene (*9*), polysaccharide (*10*), saponin (*11*) and ginsenoside (*12*). Hussain *et al.* (*13*) stated that the importance of flavonoids for antioxidant activity should not be ignored. It has been found that ginseng increased the activity of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase in rats (*14*). Guo *et al.* (*10*) stated that polysaccharides obtained from the stem of ginseng had higher antioxidant activity than those extracted from the root. Differences in harvesting and germination conditions of ginseng plants, different extraction methods and postextraction applications have been reported to have an effect on the antioxidant ability of ginseng extracts (*15*,*16*). Due to the large molecular mass of phenolic acids and 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 properties are formed (*12*). An increase in the nitric oxide binding activity and hydroxyl radical scavenging activity of phenolic compounds containing maltol has been reported, as well as a reduced rate of lipid oxidation due to the Fe^{3+} chelating activity of maltol itself (*17*).

Besides the antioxidant activity, ginsenosides obtained from ginseng are also responsible for the antibacterial and antifungal activities of ginseng (*12*). The antimicrobial activity of ginseng is explained by several mechanisms such as 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*).

The aim of this study is to evaluate the *in vitro* antioxidant activity of ginseng extract and to demonstrate its effects on the inhibition of lipid oxidation, chemical, microbiological and textural properties of cooked ground beef during 30 days of refrigerated storage.

MATERIALS AND METHODS

Materials

The roots of ginseng (*Panax ginseng* C.A. Meyer) plant were purchased locally (Tokay Herbs & Spices Store, Isparta, Turkey). The ginseng roots were chopped into small pieces and then ground into powder using a grinder (Arzum, Istanbul, Turkey). Ginseng powder was then stored at –80 °C. The 24-hour *postmortem* beef (*Longissimus thoracis et lumborum*) from approx. 1.5–2-year-old Simmental cattle was obtained from a local meat supplier, transferred to the laboratory under cold chain, ground, vacuum packaged and then kept in a freezer (–20 °C) until use.

Ginseng extraction

A mass of 20 g of ginseng powder was macerated for 2 days with 100 mL of ethanol (80 %; Merck, Darmstadt, Germany) in the dark. The ethanolic ginseng extract was filtered and the filtrate was held at 40 °C in a vacuum rotary evaporator (Hei-VAP; Heidolph Instruments, Schwabach, Germany) until all alcohol was removed (*19*).

Antioxidant capacity assays

FRAP anaylsis

FRAP was determined in the ginseng extract according to Ou *et al.* (*20*). The FRAP reagent was prepared by mixing 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 Fe(III) chloride (Merck, Darmstadt, Germany) (10:1:1). A volume of 100 µL of ginseng extract and 3 mL FRAP reagent were added and the absorbance was determined at 593 nm (T8+ UV/VIS spectrometer; PG Instruments Ltd., Leicestershire, UK). The results were reported as Fe(II) equivalents in mmol/g.

DPPH scavenging anaylsis

The scavenging activity of the ginseng extract was measured according to Dorman *et al.* (*21*). Different volumes of ginseng extract (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 measured at 517 nm against a blank after 15 min of incubation in the dark at room temperature. Results were expressed as percentage of inhibition and IC_{50} value.

Anaylsis of total phenolic content

A volume of 1 mL of ginseng extract and 5 mL of 0.2 M 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 at room temperature for 30 min. To determine the TPC, the absorbance was measured at 720 nm. The results were reported in mg/g of gallic acid equivalents (*22*).

Meat sample preparation and design of experimental groups

Ground beef was thawed at 4 °C for 12 h. After adding 10 % pure water and 2 % NaCl, the thawed ground beef was divided into equal portions for the experimental groups. Experimental groups were formed according to the tested ginseng extract amounts (**Table S1**). After the addition of ginseng extract, the samples were filled in plastic centrifuge tubes with screw caps. For each experimental group, 50 g of ground beef was carefully filled into each of these tubes so that there was no air gap. In addition to experimental groups, an extra tube was filled to monitor the core temperature. After each filled tube had been put into a water bath set to 60 °C, the 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 cooking. After cooking, the liquid part was removed from the tubes that were cooled at room temperature and the mass was recorded again. Cooking loss was 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 the cooked sample.

Physicochemical composition

Moisture, protein, fat and ash were determined according to AOAC methods (*23–26*). The pH was measured with a pH meter (Hanna Instruments, Leighton Buzzard, UK). After homogenization of 5 g sample in 50 mL distilled water, the pH was determined. Colour 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 (PCE Instruments, Southampton, UK) (*27*). The colour device was calibrated with the dark and white field standards before the measurements. Texture measurements were made by a texture analyzer (CT3; Brookfield, Middleboro, MA, USA) at room temperature. A 36 mm thick probe, 50 kg load cell, 0.5 cm thick sample, 0.35 mm penetration (70 % compression), 2 mm/s probe velocity before and after the test and 5 mm/s probe velocity during the test were implemented as analysis conditions. Hardness, cohesiveness, springiness, gumminess, chewiness, adhesiveness and resilience parameters were determined in meat samples.

Analysis of TBARS and LPO

Thiobarbituric acid reactive substances (TBARS) were analysed in accordance with the method described by Kilic and Richards (*28*) for monitoring the progress of lipid oxidation in meat samples. Propyl gallate and EDTA were added to the extraction solution of trichloroacetic acid (TCA) to avoid the formation of TBARS during the analysis. A mass of 2 g meat sample was stired in the extraction solution (12 mL). Meat samples were homogenized for 15 s and the homogenate was filtered through Whatman no. 1 filter paper. A volume of 1 mL of the obtained filtrate was taken and mixed with thiobarbituric acid (TBA) solution and then vortexed at 250×*g*. Then, the mixture was warmed up at 100 °C for 40 min. Then, the tubes were cooled in cold water. After cooling, the samples were centrifuged (Rotofix 32A; Hettich, Schwerin, Germany) at 2000×*g* 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 concentrations were espressed as malondialdehyde in µmol/kg.

A method of detection of lipid hydroperoxide (LPO) described by Kılıç *et al.* (*29*) was used for the analysis of lipid hydroperoxide. Briefly, 1 g sample was homogenized for 30 s in 5 mL chloroform/methanol (1:1). After that, 3 mL NaCl (0.5 %) were added and vortexed for 30 s. Then, this mixture was subjected to centrifugation (Rotofix 32A; Hettich) for 10 min at 2000×*g* to achieve phase separation. After that, lower phase (2 mL) was taken and added to the 1.3 mL cold methanol/chloroform (1:1) mixture and vortexed. After adding 25 µL Fe(II) chloride (18 mM) and 25 µL ammonium thiocyanate (4.38 M), the samples were held at room temperature for 20 min and then their absorbance values were measured at 500 nm.

Microbiological analysis

To determine total aerobic mesophilic bacteria (TAMB), total coliform bacteria (TCB), yeast and mould (YM) counts, under aseptic conditions, 10 g meat samples were weighed into homogenizer bags and 90 mL of physiological saline were added. After homogenizing for 1 min, serial dilutions were prepared from this dilution and incubated at 30 °C for 48 h on a plate count agar (Merck, Darmstadt, Germany) for TAMB, at 37 °C for 48 h on eosin methylene blue agar (Merck) for TCB and at 25 °C for 72 h on potato dextrose agar (Merck) for YM. Colony counts were obtained at the end of the incubation and expressed as CFU/g (*30*).

Statistical analysis

The experiments were conducted in two replicates and the analyses were performed in three parallel experiments. The significant differences among the results were determined using the MiniTab® 19.1.1 package program (*31*). After applying the analysis of variance (one-way ANOVA) to the antioxidant capacity assays (DPPH, FRAP and TPC) of the ginseng extract and the post-production analyses (cooking loss, moisture, protein, fat and ash content, and texture analysis), the differences among the experimental groups were determined by Duncan's multiple range test. As for pH, instrumental colour, TBARS, LPO and microbiological analysis, the

statistical factorial design was six ginseng extract amounts (0, 0.1, 0.5, 1, 1.5 and 2 %) × six storage times (0, 3, 5, 7, 15 and 30 days) for cooked ground beef samples. The independent variables (ginseng extract dose and storage time) and replications were designed 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 colour, TBARS, LPO, total aerobic mesophilic bacteria, total coliform bacteria, and yeast and mould counts. In this model, the results were tested using the restricted maximum likelihood (REML) method with a confidence interval of 95 %.

RESULTS AND DISCUSION

The results of the antioxidant capacity assay

The results of the DPPH radical scavenging assay (data not shown) showed that IC_{50} and inhibiton percentage of the ginseng extract were (12.11±0.09) mg/mL and (70.2±0.8) %, respectively. Previous studies report on the binding activity of DPPH radical by the ginseng extract. Chung *et al.* (*32*) stated that DPPH values of ginseng extracted with methanol were between 18.08 and 25.61 %. Moreover, Lee *et al.* (*33*) reported 51.0 and 86.2 % DPPH radical binding activity in the ethanolic extract of red ginseng and puffed red ginseng, respectively. Ganguly *et al.* (*34*) stated that IC_{50} values of ginseng extract were 32.80 and 38.83 µg/mL in methanol and methanol/chloroform/water solvents, respectively. Jiang *et al.* (*35*) found the IC_{50} value of 12 mg/mL in the essential oil obtained from the leaves of ginseng. Zhao *et al.* (*36*) found that DPPH binding activity varied between 50 and 95 % and IC_{50} values were between 0.150 and 0.155 mg/mL in oligosaccharides obtained from the ginseng extract. Hussain *et al.* (*13*) also found that the inhibition value in the ginseng extract ranged from 53.12 to 62.84 %.

The total phenolic content (TPC) of ginseng extract, expressed as gallic acid equivalents (GAE) on dry mass basis, was (146.0±2.4) mg/g in our study. Lee *et al.* (*33*) found that the TPC, expressed as tannic acid equivalents, of ethanolic ginseng extract was 6.72 µg/g. Ganguly *et al.* (*34*) found that the total phenolic content, expressed as GAE, of ginseng extract in methanol and methanol/chloroform/water (1:1:1) was 97.38 and 109.65 µg/mg, respectively. Shahriar *et al.* (*37*) found that the TPC value, as GAE, after chloroform extraction of ginseng was 60.99 µg/mg. Pal *et al.* (*38*) reported that total phenolic content, as GAE, of ginseng extract in three solvents (methanol, chloroform and water) was 42, 66.72 and 88.58 µg/mg, respectively, and the reason for this difference in each solvent was explained by the polarity of the polyphenolic substances. Zhao *et al.* (*36*) found that the total phenolic content, as GAE, of four different oligosaccharides extracted from ginseng ranged from 1.91 to 3.51 µg/mg. Ryu *et al.* (*39*) stated that the breakdown of ginsenosides into large molecular structures and their conversion into small molecules increased the total phenolic content of ginsenosides approximately threefold.

In our study, the FRAP activity of ginseng extract, expressed as Fe^{2+}, was (4.7±0.2) mmol/g. Lee *et al.* (*33*) found the FRAP activity, expressed as ascorbic acid equivalents, of ginseng extract in the range of 1.04–2.34 µg/g. Variation in the antioxidant activity of ginseng extract in previous studies is thought to be associated with the differences in the type and 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 affect the content of antioxidants such as polyphenols, flavonoids and saponins in ginseng extract (*40*).

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

Cooking loss results

The cooking loss results obtained in our study are shown in **Table 1**. The values for cooking loss were between 22.6 and 26.3 %. It was found that the addition of ginseng extract had a considerable effect on cooking loss ($p<0.05$). Although the groups containing ginseng extract had similar cooking loss values among themselves, they were higher than control ($p<0.05$). Our findings are supported by Kim *et al.* (*41*), who also found that the addition of ginseng to the pork sausage recipe resulted in an increase in cooking loss. An increased cooking loss due to the addition of ginseng associated with the change in meat pH was also reported (*41*). Similarly, in our study, the pH of the meat was found to decrease (**Table 2**) at the tested high ginseng extract amounts (G15 and G20).

Table 1. Effect of ginseng extract on the proximate composition and cooking loss of cooked ground beef

Group	*w*/%				
	Moisture	Protein	Fat	Ash	Cooking loss
Control	(69.0±1.1)^{a}	(27.3±1.5)^{a}	(3.4±0.5)^{a}	(3.4±0.1)^{abc}	(22.6±0.6)^{b}
G01	(68.2±2.0)^{a}	(28.8±3.2)^{a}	(3.5±0.4)^{a}	(3.17±0.08)^{c}	(25.8±1.4)^{a}
G05	(67.8±1.5)^{a}	(28.2±0.5)^{a}	(3.7±0.8)^{a}	(3.4±0.1)^{abc}	(26.3±1.2)^{a}
G10	(67.2±0.7)^{a}	(28.2±1,9)^{a}	(3.6±0.5)^{a}	(3.6±0.4)^{a}	(25.1±0.7)^{a}
G15	(67.7±0.6)^{a}	(29.0±2.2)^{a}	(3.9±0.9)^{a}	(3.5±0.3)^{ab}	(26.2±0.6)^{a}
G20	(68.6±1.1)^{a}	(29.2±1.0)^{a}	(3.8±0.6)^{a}	(3.3±0.3)^{bc}	(25.6±0.6)^{a}

Results are expresse as mean value±standard error. Values with different letters in superscript within a column are significantly different ($p<0.05$). G01 to G20=ginseng extract added to ground beef at 0.1, 0.5, 1.0, 1.5 and 2.0 %

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

Parameter	Group	*t*(storage)/day					
		0	3	5	7	15	30
*L**	Control	$(55.5\pm0.8)^{c-g}$	$(53.4\pm0.6)^{h-m}$	$(54.1\pm0.9)^{e-l}$	$(55.9\pm0.6)^{cde}$	$(53.4\pm0.3)^{g-m}$	$(58.7\pm1.0)^{a}$
	G01	$(55.2\pm0.8)^{c-h}$	$(53.3\pm0.5)^{h-n}$	$(55.8\pm0.7)^{c-f}$	$(57.0\pm1.0)^{abc}$	$(55.2\pm1.0)^{c-h}$	$(58.5\pm0.9)^{a}$
	G05	$(55.3\pm1.0)^{c-h}$	$(54.4\pm0.9)^{d-k}$	$(56.341.1)^{bcd}$	$(51.1\pm1.0)^{opq}$	$(57.0\pm1.6)^{abc}$	$(56.1\pm1.0)^{b-e}$
	G10	$(56.3\pm1.0)^{bcd}$	$(54.8\pm1.4)^{d-h}$	$(54.6\pm1.0)^{d-j}$	$(52.8\pm0.5)^{i-o}$	$(58.1\pm0.9)^{ab}$	$(54.8\pm0.6)^{d-i}$
	G15	$(53.7\pm1.0)^{f-m}$	$(51.3\pm1.2)^{n-q}$	$(52.5\pm1.0)^{k-p}$	$(52.2\pm0.8)^{l-q}$	$(55.3\pm0.8)^{c-h}$	$(51.7\pm0.7)^{m-q}$
	G20	$(47.1\pm116)^{s}$	$(50.2\pm1.1)^{qr}$	$(52.7\pm0.8)^{j-p}$	$(49.0\pm0.8)^{rs}$	$(50.6\pm0.6)^{pqr}$	$(51.2\pm1.0)^{opq}$
*a**	Control	$(11.9\pm1.4)^{b-e}$	$(14.6\pm1.4)^{a}$	$(12.4\pm1.6)^{a-d}$	$(13.8\pm0.5)^{ab}$	$(12.5\pm2.0)^{abc}$	$(9.5\pm1.7)^{e-l}$
	G01	$(12.1\pm0.9)^{bcd}$	$(12.5\pm1.3)^{abc}$	$(11.5\pm2.3)^{b-g}$	$(11.5\pm1.2)^{b-g}$	$(8.8\pm2.4)^{h-m}$	$(6.8\pm1.0)^{m}$
	G05	$(11.7\pm0.7)^{b-f}$	$(11.8\pm0.8)^{b-e}$	$(8.8\pm0.5)^{h-m}$	$(11.7\pm1.7)^{b-f}$	$(8.2\pm0.6)^{j-m}$	$(7.1\pm0.6)^{lm}$
	G10	$(12.8\pm0.5)^{abc}$	$(12.5\pm0.7)^{abc}$	$(10.0\pm0.7)^{d-k}$	$(11.1\pm1.1)^{c-h}$	$(8.4\pm0.2)^{i-m}$	$(7.6\pm0.7)^{klm}$
	G15	$(10.6\pm0.4)^{c-j}$	$(10.8\pm0.4)^{c-i}$	$(10.8\pm0.6)^{c-i}$	$(8.7\pm0.2)^{h-m}$	$(8.7\pm0.5)^{h-m}$	$(7.7\pm0.4)^{klm}$
	G20	$(10.6\pm1.1)^{c-j}$	$(10.5\pm0.4)^{c-j}$	$(9.3\pm1.1)^{f-l}$	$(9.1\pm0.7)^{g-m}$	$(9.2\pm0.3)^{g-m}$	$(7.6\pm0.6)^{klm}$
*b**	Control	$(2.9\pm0.5)^{f-i}$	$(1.2\pm0.2)^{j}$	$(2.4\pm0.6)^{g-j}$	$(1.6\pm0.1)^{ij}$	$(3.7\pm0.5)^{c-g}$	$(4.8\pm0.1)^{bc}$
	G01	$(3.2\pm0.5)^{d-h}$	$(2.1\pm0.8)^{hij}$	$(4.3\pm0.6)^{b-e}$	$(2.9\pm1.5)^{f-i}$	$(4.7\pm0.6)^{bc}$	$(6.4\pm0.2)^{a}$
	G05	$(2.4\pm0.2)^{hij}$	$(2.4\pm0.8)^{g-j}$	$(4.2\pm0.6)^{b-f}$	$(2.8\pm1.0)^{f-i}$	$(5.3\pm0.3)^{ab}$	$(6.2\pm0.4)^{a}$
	G10	$(2.7\pm0.5)^{ghi}$	$(1.64\pm0.2)^{ij}$	$(3.1\pm0.2)^{d-h}$	$(3.1\pm0.8)^{e-h}$	$(4.4\pm0.5)^{bcd}$	$(4.5\pm0.6)^{bcd}$
	G15	$(2.5\pm0.5)^{ghi}$	$(2.6\pm0.24)^{ghi}$	$(2.7\pm0.2)^{ghi}$	$(4.3\pm0.4)^{b-e}$	$(4.4\pm0.8)^{b-e}$	$(4.6\pm0.4)^{bc}$
	G20	$(2.630.2)^{ghi}$	$(2.5\pm0.3)^{ghi}$	$(4.8\pm0.4)^{bc}$	$(4.0\pm0.6)^{b-f}$	$(4.4\pm0.4)^{bcd}$	$(4.89\pm0.6)^{bc}$
pH	Control	$(6.03\pm0.02)^{b-h}$	$(6.07\pm0.02)^{a-h}$	$(6.09\pm0.01)^{a-f}$	$(5.98\pm0.05)^{b-h}$	$(6.02\pm0.05)^{a-h}$	$(6.00\pm0.03)^{d-h}$
	G01	$(6.07\pm0.02)^{a-h}$	$(6.11\pm0.02)^{a-d}$	$(6.14\pm0.04)^{ab}$	$(6.03\pm0.02)^{b-h}$	$(6.06\pm0.02)^{a-h}$	$(6.00\pm0.02)^{b-h}$
	G05	$(6.09\pm0.07)^{a-h}$	$(6.15\pm0.05)^{a}$	$(6.09\pm0.04)^{a-e}$	$(6.04\pm0.02)^{a-h}$	$(6.06\pm0.02)^{a-h}$	$(6.02\pm0.03)^{b-h}$
	G10	$(5.99\pm0.03)^{c-h}$	$(6.12\pm0.05)^{a-g}$	$(6.10\pm0.03)^{b-h}$	$(6.00\pm0.03)^{b-h}$	$(6.03\pm0.01)^{b-h}$	$(6.01\pm0.03)^{b-h}$
	G15	$(5.97\pm0.03)^{fgh}$	$(6.08\pm0.01)^{a-h}$	$(6.05\pm0.01)^{a-h}$	$(5.95\pm0.04)^{gh}$	$(5.99\pm0.02)^{d-h}$	$(5.98\pm0.01)^{e-h}$
	G20	$(5.98\pm0.01)^{fgh}$	$(6.05\pm0.01)^{a-h}$	$(6.04\pm0.02)^{a-h}$	$(6.00\pm0.05)^{h}$	$(6.06\pm0.06)^{b-h}$	$(6.00\pm0.03)^{d-h}$

Results are expressed as mean value±standard error. Values with different letters in superscript within each column are significantly different ($p<0.05$). G01 to G20=ginseng extract added to ground beef at 0.1, 0.5, 1.0, 1.5 and 2.0 %

The results of physicochemical composition analysis

Physicochemical composition of the experimental groups is shown in **Table 1**. The moisture content in the control was 69.0 %, while in the experimental groups containing ginseng extract it varied between 67.2 and 68.6 %. The results showed that groups containing ginseng extract were found to have similar moisture content to the control, which means that the addition of ginseng extract to the formulation did not affect the moisture content. Protein content determined in the experimental groups ranged from 27.3 to 29.7 % and did not reveal a significant difference between the groups with or without the ginseng extract. The amount of fat in the experimental groups varied between 3.5 and 3.9 % and it did not show any differences among the experimental groups either. The ash content of the experimental groups was determined in the range of 3.2–3.6 %. Although the ash content of the groups containing ginseng extract was similar to that of control, ash content of G10 group (3.6 %) was found to be higher ($p<0.05$) than that of G01 (3.2 %).

The results showed that the amount of ginseng extract and storage time had an effect on the pH values of the samples ($p<0.0001$), while their interaction (**Table S1**) was not a factor. Therefore, only the main effects (ginseng extract amount and storage time) and not their interaction will be discussed. In general, there was a decrease in pH values in the groups with ginseng extract of 1.5 % or more ($p<0.05$; **Table 2**). Regardless of the storage time, the pH values of the control (6.04±0.01) and groups G01 (6.07±0.01), G05 (6.06±0.01) and G10 (6.04±0.01) were similar. In addition, groups G15 (6.01±0.01) and G20 (6.01±0.01) were found to have similar pH values. Although the pH values of groups G15 and G20 were statistically lower than those of the other groups, these differences may not be significant in practical applications. Ibrahim *et al.* (*42*) also found that the use of ginseng extract in lamb patties resulted in lower pH values than the control. Regardless of ginseng extract, pH values increased ($p<0.05$) during the first 5 days of storage and then started to decrease ($p<0.05$) during the rest of the storage (day 0: 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 and day 30: 6.00±0.01). Ibrahim *et al.* (*42*) found that the increase in pH values during storage of lamb patties was due to ammonia produced by protein oxidation or degradation by proteolysis. It is also believed that the pH decrease observed after 5 days of storage is related to the activity of lactic acid bacteria. The decrease in pH of stored muscle foods may be related to the activity of lactic acid bacteria, which metabolise the carbohydrates in muscle food and convert them into lactic acid (*43*,*44*).

The effect of the amount of ginseng extract and storage time on the CIE colour values of the experimental groups is shown in **Table 2**. The analysis of variance revealed that the

effect of the ginseng extract amount, storage time and their interaction on the CIE $L^*a^*b^*$ values was significant ($p<0.0001$). The lowest L^* values on the day of processing (t=0) were obtained in group G20 ($p<0.05$). Although the L^* values of groups G01, G05, G10 and G15 on the day of processing were similar to those of the control, the L^* values of groups G10 and G15 were found to differ from each other ($p<0.05$). The results indicated that the L^* values tended to increase ($p<0.05$) throughout storage, except for those of groups G05, G10 and G15, which were constant during the same period. After 30 days of storage, the lowest L^* values were obtained in groups G15 and G20, while the highest L^* values were found in the control and G01 ($p<0.05$). In general, the higher the amount of ginseng extract, the lower the L^* value after 30 days of storage ($p<0.05$). There were no significant differences between the a^* and b^* values between the control group and the groups with the added ginseng extract on the day of processing. A decrease in a^* values and an increase in b^* values were observed in all experimental groups throughout the storage ($p<0.05$). After 30 days of storage, the a^* values of the groups containing ginseng extract were similar to each other, but lower ($p<0.05$) than tose of the control. On the other hand, the highest ($p<0.05$) b^* values were found in groups G01 and G05, while the other experimental groups had similar b^* values. Kim *et al.* (*41*) found that the addition of ginseng increased the b^* values of pork sausage, while the L^* and a^* values decreased. However, Cho *et al.* (*45*) claimed that the addition of ginseng powder did not affect the colour parameters of pork.

The results of the texture analysis of the experimental groups in **Table 3** show no significant differences among the experimental groups in terms of hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess and chewiness. Kim *et al.* (*41*) reported that the addition of ginseng reduced only the hardness parameters of pork sausage, but did not affect other parameters.

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

Group	Hardness/N	Adhesiveness/mJ	Resilience	Cohesiveness
Control	(5.4±0.7)^a	(0.3±0.4)^a	(0.16±0.02)^a	(0.58±0.06)^a
G01	(5.6±1.7)^a	(0.2±0.1)^a	(0.19±0.07)^a	(0.61±0.06)^a
G05	(5.8±0.7)^a	(0.08±0.05)^a	(0.20±0.06)^a	(0.61±0.06)^a
G10	(5.9±0.6)^a	(0.2±0.2)^a	(0.18±0.02)^a	(0.58±0.03)^a
G15	(5.0±1.0)^a	(0.2±0.2)^a	(0.17±0.03)^a	(0.65±0.04)^a
G20	(5.3±0.6)^a	(0.2±0.1)^a	(0.16±0.01)^a	(0.6±0.2)^a
Group	**Springiness**	**Gumminess/N**	**Chewiness/N**	
Control	(0.81±0.06)^a	(3.1±0.5)^a	(17.7±3.)^a	
G01	(0.86±0.08)^a	(3.4±0.9)^a	(20.2±6.6)^a	
G05	(0.9±0.1)^a	(3.5±0.3)^a	(22.1±3.9)^a	
G10	(0.85±0.04)^a	(3.4±0.4)^a	(20.4±3.2)^a	
G15	(0.84±0.08)^a	(3.2±0.5)^a	(18.7±3.9)^a	
G20	(0.88±0.05)^a	(3.3±0.7)^a	(20.6±5.2)^a	

Results are expresses as mean value±standard error. Values with different letters in superscript in the same column are significantly different ($p<0.05$). G01 to G20=ginseng extract added to ground beef at 0.1, 0.5, 1.0, 1.5 and 2.0 %

TBARS and LPO values

The effect of ginseng extract amount and storage time on TBARS and lipid hydroperoxide (LPO) values of the experimental groups is shown in **Table 4**. The results indicate that TBARS values of all experimental groups containing ginseng extract were lower than of control on the day of processing ($p<0.05$). The TBARS values gradualy increased in all experimental groups throughout the storage ($p<0.05$). The interaction between the amount of ginseng extract and storage time showed that the TBARS values of control and groups G01 gradually increased during each storage day ($p<0.05$). In the meantime, TBARS values of G05 increased during the first 5

Table 4. Effect of ginseng extract on thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide (LPO) values in cooked ground beef during 30 days of storage at 4 °C

Parameter	Group	*t*(storage)/day					
		0	3	5	7	15	30
TBARS *b*(malonaldehyde)/(μmol/kg)	Control	(6.8±0.7)^q	(20.8±0.8)^{lm}	(24.3±0.9)^{hij}	(27.9±0.5)^{ef}	(36.2±0.9)^b	(39.4±1.2)^a
	G01	(4.8±0.4)^r	(23.4±0.7)^{ijk}	(26.2±0.7)^{fg}	(31.7±0.7)^{cd}	(34.89±0.03)^b	(40.4±0.8)^a
	G05	(4.7±0.4)^r	(21.8±0.3)^{kl}	(25.4±0.9)^{gh}	(26.6±0.7)^{fg}	(35.70±0.02)^b	(40.5±0.6)^a
	G10	(3.6±0.2)^r	(19.7±0.4)^{mn}	(21.1±0.3)^{lm}	(25.2±1.0)^{gh}	(30.72±0.01)^d	(32.8±0.8)^c
	G15	(4.3±0.8)^r	(18.6±0.6)^n	(18.4±0.8)^{no}	(25.6±1.0)^{gh}	(28.44±0.02)^e	(30.9±1.0)^d
	G20	(4.2±0.6)^r	(14.8±0.6)^p	(16.6±0.8)^{op}	(23.0±0.4)^{jk}	(25.8±0.06)^{gh}	(28.9±1.8)^e
LPO *b*(lipid hydroperoxide)/(μmol/kg)	Control	(18.8±0.9)^m	(35.2±1.9)^l	(41.2±1.9)^l	(80.7±1.6)^j	(299.1±8.5)^e	(454±12)^a
	G01	(18.0±1.0)^m	(36.2±1.3)^l	(41.7±1.1)^l	(85.2±2.1)^j	(314.6±3.1)^d	(363.0±9.7)^c
	G05	(16.1±0.5)^m	(35.2±0.6)^l	(38.8±1.4)^l	(60.8±2.2)^k	(324.1±7.6)^d	(381.4±9.3)^b
	G10	(17.8±0.4)^m	(35.0±0.8)^l	(40.3±2.5)^l	(44.4±1.2)^l	(266.2±6.0)^g	(285.3±8.1)^f
	G15	(18.4±0.7)^m	(35.0±1.1)^l	(40.2±2.9)^l	(43.5±1.7)^l	(186.4±4.5)^i	(256.4±7.9)^g
	G20	(19.1±1.5)^m	(35.0±1.4)^l	(39.88±5.43)^l	(44.0±1.2)^l	(178.7±2.3)^i	(216.8±3.5)^h

Results are expressed as mean value±standard error. For each tested parameter in the table, the values with different letters in superscript are significantly different ($p<0.05$). G01 to G20=ginseng extract added to ground beef at 0.1, 0.5, 1.0, 1.5 and 2.0 %

days of storage, remained stable during the period between days 5 and 7 and then increased again during the rest of the storage. Furthermore, TBARS values of groups G10, G15 and G20 remained constant during the storage between days 3 and 5, and then increased during the remaining storage time ($p<0.05$). After 30 days of storage, the highest TBARS values were obtained in the control and groups G01 and G05, while the lowest TBARS (26.79 % reduction) value was determined in group G20 ($p<0.05$). Generally, TBARS values decreased with increasing ginseng extract amount ($p<0.05$). Papuc *et al.* (*46*) pointed out that dried plants and essential oils successfully delay lipid oxidation in muscle food and that this effect is due to the fact that polyphenols are good electron and proton donors. It has also been found that bioactive components such as triterpenes and saponins in ginseng can prevent chain reactions that occur during lipid oxidation (*47*). Lipid oxidation in cooked muscle foods can be influenced by pH, which affects the activities of prooxidants, especially haem iron. The iron-catalyzed oxidation has been reported to decrease with an increase in pH from 2 to 10 and myoglobin-catalyzed oxidation decreased with an increase in pH (*48*). Since the differences in pH among the groups in the present study were negligible from a practical point of view, pH is thought to be insignificant factor modulating the development of lipid oxidation. In addition, Ibrahim *et al.* (*42*) found that ginseng extract had higher antioxidant activity than jojoba and ginger extracts, and the lowest TBARS values after 9 days of storage were found in the groups with added ginseng extract. In another previous study in which ginseng extract was added to the meat emulsion model, the researchers found that the use of 2.5 % ginseng extract prevented lipid oxidation of meat products (*46*). On the other hand, Cho *et al.* (*45*) found that the addition of ginseng powder could maintain TBARS content in pork chops stored at 4 °C for 15 days constant for up to 5 days, but lipid oxidation could not be prevented during the remaining storage time.

The results regarding the effects of ginseng extract amount and storage time on LPO values of the experimental groups showed that there were no significant differences among the experimental groups on the day of processing. It was found that the LPO values of all experimental groups increased progressively during storage ($p<0.05$). On the other hand, the LPO values of the groups containing 1 % or more ginseng extract increased during the first 3 days of storage, remained stable between day 3 and 7 and then showed an increasing trend again during the rest of the storage ($p<0.05$). After 30 days of storage, the highest LPO values were obtained in the control group, while the lowest LPO (52.25 % reduction) values were determined in group G20 ($p<0.05$). Generally, LPO values decreased with increasing ginseng extract amount, but the same trend was not observed in groups G01 and G05. The LPO values of G01 were lower than the LPO values of G05 ($p<0.05$).

The results of microbiological analysis

The results of the microbiological analysis (**Table 5**) showed that the total aerobic mesophilic bacteria (TAMB) in the raw meat material before heat treatment was 4.60 log CFU/g, while after cooking it was <1 log CFU/g in all experimental groups. After 30 days of storage, the TAMB in the control group was 3.4 log CFU/g, but in the other experimental

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

N/(CFU/g)		*t*(storage)/day					
	Group	0	3	5	7	15	30
TAMB	Control	<1	<1	<1	<1	<1	$(3.4\pm1.0)^{a}$
	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	$<1^{b}$
TCB	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
YM	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

Values with different letters in superscript within the same column are significantly different ($p<0.05$). TAMB=total aerobic mesophilic bacteria, TCB=total coliform bacteria, YM=yeasts and moulds, CFU=colony forming units. G01 to G20=ginseng extract added to ground beef at 0.1, 0.5, 1.0, 1.5 and 2.0 %

groups containing ginseng extract, it was <1 log CFU/g. Ibrahim *et al*. (*42*) also pointed out that the use of ginseng extract reduced the load of aerobic mesophyll bacteria in lamb patties. While the TAMB in the experimental groups containing ginseng extract was below the detection limit in our study, Ibrahim *et al*. (*42*) reported the TAMB in the experimental group containing ginseng extract of 2.51 log CFU/g. An increasing trend in TAMB in the control group was observed during refrigerated storage. Furthermore, Kim *et al*. (*41*) found that the total aerobic plate count in pork sausage decreased with increaseing ginseng extract amount.

The total coliform bacteria (TCB) was 3.69 log CFU/g in raw meat material (data not shown), but after heat treatment, it was <1 log CFU/g in all experimental groups (**Table 5**). In all experimental groups, TCB was also <1 log CFU/g during the entire storage period. On the other hand, the yeast and mould (YM) count in the raw meat material before heat treatment was 3.30 log CFU/g, but after heat treatment, it was <1 log CFU/g in all experimental groups. The YM count determined in all experimental groups was <1 log CFU/g until the end of storage. Ibrahim *et al*. (*42*) found that ginseng extract had a higher influence on TAMB than YM count in lamb patties. Overall, the inhibited microbial growth in the experimental groups containing ginseng extract could be due to the antimicrobial activity of the components of ginseng extract used in our study. The components of ginseng extract, such as ginsenosides, have been reported to interact with microorganisms and prevent microbial growth by inhibiting microbial motility and quorum sensing, reducing biofilm formation, disrupting cell wall structure and reducing bacterial adhesion due to stimulation of the immune system (*18*).

CONCLUSIONS

The *in vitro* results on antioxidant capacity showed that the ginseng extract had iron ion reducing and free radical scavenging activities. The results showed that the addition of ginseng extract caused a decrease in pH and an increase in cooking loss in ground beef. Furthermore, the addition of ginseng extract caused a decrease in brightness and redness values, but an increase in yellowness values in cooked ground beef. Ginseng extract did not affect the texture parameters and the proximate composition of cooked ground beef. The used ginseng extract showed the ability to inhibit lipid oxidation in cooked ground beef, and this effect increased with increasing amount of ginseng extract. The results indicated that aerobic mesophilic bacteria were more inhibited at the end of storage in cooked ground beef with ginseng extract than in those prepared without it. The growth of yeasts, moulds and coliform bacteria was not observed in any of the experimental groups during 30 days of storage, regardless of the addition of ginseng extract. In conclusion, the study results show that ginseng extract can be used as a natural preservative to ensure oxidative and microbial stability in the ready-to-eat meat products. Further research is needed to determine the effects of ginseng extract on the sensory properties of the meat products, to ensure that the food meets consumer demands.

ACKNOWLEDGEMENTS

The authors are grateful to Coordinatorship of Scientific Research Projects of Suleyman Demirel University and TUBITAK for the financial support.

FUNDING

This study was supported by Coordinatorship of Scientific Research Projects of Suleyman Demirel University under the project number FDK-2018-6089 and TUBITAK PhD Student Scholarship Programme (scholarship number: 1649B031800481).

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 writing of the manuscript.

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