

## Production of Probiotic Doogh Enriched with Red Ginseng Extract

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### ABSTRACT

Nowadays, Ginseng is used in production of functional foods because of its beneficial effects in prevention and treatment of cancers, diabetes, central nervous system disorders, etc. Ginsenosides, the largest and most prominent compounds in Ginseng, are responsible for the known health effects of Ginseng plant. The present study aimed to produce a functional beverage and evaluate the quality characteristics of this health beverage. The effects of adding red Ginseng extract at different levels (0, 0.5, 1 and 2 g L<sup>-1</sup>) on pH, titratable acidity, color values, the viability of *Lactobacillus acidophilus* and *Bifidobacterium lactis* and overall acceptability of probiotic Doogh containing mint powder (1 g L<sup>-1</sup>) were investigated during 21-day storage at 4°C. The results showed that with increasing the level of red Ginseng extract up to 2 g L<sup>-1</sup>, no significant difference was observed in the pH and titratable acidity of probiotic Doogh samples (P > 0.05); color values of the Doogh samples changed significantly (P < 0.05); viability of the probiotic bacteria increased significantly (P < 0.05); and the overall acceptability decreased significantly (P < 0.05). In general, Doogh sample containing 1 g L<sup>-1</sup> red Ginseng extract gained the highest overall acceptability score in comparison with the other samples.

**Keywords:** Doogh acceptability, Functional food, Ginsenoside, Probiotic bacteria, Sinbiotic Doogh.

### INTRODUCTION

Doogh is a traditional Iranian drink based on fermented milk. Doogh can be produced by adding water and salt into yoghurt. The popularity of this fermented milk-based beverage arises from its typical organoleptic characteristics along with several health benefits [11]. Incorporation of probiotics and natural prebiotics to Doogh may lead to more beneficial health impacts for the host. Prebiotics are non-digestible food components that can facilitate the growth and activity of selective bacteria in the colon [17]. To obtain the claimed probiotic benefits, survival of bacteria during processing and shelf life is the most important criterion [33]. The most

famous species of probiotic microorganisms are *Lactobacillus* and *Bifidobacterium* [26]. Functional properties of these bacteria include improvement of the immune system, gastrointestinal complications and lactose intolerance symptoms, as well as reduction of cholesterol, and anti-tumor effects [5]. Since the early civilizations, it was understood that certain foods have additional functional effects such as prevention and treatment of a variety of diseases [1]. *Mentha* spices are widely used for their flavoring and medicinal properties throughout the world. *Mentha piperita* is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, biliary tract disorders and liver complaints [31]. Peppermint is on the FDA's (Food and Drug

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Administration.) GRAS (generally recognized as safe) list, and whole herb peppermint has few side effects [14].

The root of Ginseng, as one of the oldest plants used in traditional medicine in China and East Asian countries, has proven countless health effects. There are usually two species of Ginseng, which are used as dietary supplement and food component: Asian Ginseng (*Panax ginseng* C.A. Meyer) and American Ginseng (*Panax quinquefolius* L). It has been reported that both species of Ginseng have many pharmacological effects on human beings that will be mentioned in the following paragraphs.

Effective amount of Ginseng to achieve functional effects is 300 mg of standardized extract for two to three times per day [6, 19, 38]. Also, the recommended doses for a positive effect on learning and memory systems are 100 and 200 mg of Ginseng extract for two to three times per day [30]. Positive effects of Ginseng on health are primarily attributed to Ginsenosides, which are assigned to the *Panax* genus and are known as triterpenoids saponin glycosides. The amount of Ginsenosides in a root varies between 0.7 and 20% [2, 12], and almost 30 Ginsenosides have been discovered in Ginseng [29]. Ginseng contains many phenolic compounds that are involved in plant antioxidant activity [8]. American Ginseng root extract can resist against pasteurization, though almost 25% of its active ingredients are destroyed during pasteurization. Additionally, it is reported that Ginsenosides found in Ginseng extract have sufficient stability against Ultra-High Temperature (UHT) heat treatment; as a result, Ginseng is an appropriate compound for milk products with memory and learning increasing effects in human [35].

A lot of studies have been conducted on the pharmacological properties of Ginseng extract such as lipid-lowering [21], anti-diabetic [2, 37], anti-fatigue [23], anti-depressive, and immune-modulatory [18, 20] activities, improving working memory and perceptual systems [30], stimulation and

inhibition of central nervous system [28], and inhibiting the growth of tumor cells [40, 13], especially in female reproductive system [27]. Additionally, Kim *et al.* [22] confirmed the use of Ginseng as an antioxidant supplement. So far, the production of dairy products containing Ginseng extract including probiotic fermented milk [16], low-lactose milk with the potential to improve learning and memory function in old age [35], and probiotic yoghurt [10] have been investigated. Chung *et al.* [9] evaluated the effect of adding sweeteners to Ginseng tea and adding Ginseng extract to chocolate on consumer acceptance, in addition to determination of the sensory acceptability of commercial food products containing Ginseng. Nevertheless, in recent research, some results obtained can be estimated, the product is different than the commercially available ones. Doogh is a traditional Iranian drink based on fermented milk. Addition of Ginseng extract to Doogh is no favorite; mainly due to the presence of saponins compounds, and bitter, sweet, and earthy tastes in it, which are a bit like licorice flavors; and these tastes are unfamiliar to Iranian consumers. In contrast, mint powder, in addition to having many functional properties, enjoys desirable flavor; therefore, it plays an important role in covering the taste and color of Ginseng in Doogh prodced.

Extract of Ginseng has been found in some medicinal and nutritional sports supplements; however, especially in Iran, people do not consume these compounds and, so, do not benefit from its beneficial effects. Taking certain herbal extracts, especially Ginseng, due to their special flavor is not desirable for the majority of people; hence, they can be used in combination with other foods; e.g., Ginseng can be added to flavored drinks. Since Doogh is considered as a popular fermented beverage in Iran, by adding Ginseng extract to Doogh, in addition to producing a novel product falling in the category of functional foods, we can also contribute to community health. Meanwhile, foods containing both

probiotic bacteria and prebiotics are known to sin-biotics; so, the probiotic Doogh containing Ginseng extract produced in the present research is sin-biotic. It is to be noted that mint powder has desirable flavor; therefore, it is expected to play an important role in covering the taste and color of Ginseng in Doogh.

The aim of this study was to produce a probiotic Doogh containing mint powder enriched with Ginseng extract and also study the effect of adding different concentrations of Ginseng extract on some properties of the product during the 21-day cold storage in order to determine the optimal concentration of Ginseng extract.

## MATERIALS AND METHODS

### Starter Culture

Pouches of commercial lyophilized ABY-3 starter culture (containing *Lactobacillus acidophilus* La5, *Bifidobacterium lactis* BB-12 and yogurt bacteria) were supplied by Chr. Hansen Company (Horsholm, Denmark). The cultures were used in a freeze dried Direct Vat Set (DVS) and maintained according to the manufacturer's instructions at -18 °C until used.

### Additives

The root of South Korean red Ginseng, mint powder and refined edible salt procured from the local market were used for the studies. DKXG and GSE stabilizers were purchased from Tate and Lyle (Germany); DKXG is combination of xanthan gum, sodium citrate and gellan gum and GSE is combination of carboxy methyl cellulose, locust bean gum, and pectin.

### Aqueous Ginseng Extract Preparation

To prepare aqueous Ginseng extract, red Ginseng root was grinded and powdered and

then soaked in water (four times the weight of powder) for 3 to 5 hour periods at 40°C and evaporated under vacuum at 45°C (up to 60% dry matter).

### Doogh Samples Preparation

Cow's milk with 3.35% fat, 3.05% protein, 9.19% solids non-fat, pH 6.65 and titratable acidity 14.43 (°D) was pasteurized at 85°C for 30 minutes. After cooling down of the milk up to the fermentation temperature (42°C), it was inoculated with ABY-3 according to the manufacturer's instructions, and incubated until its pH reached 4.8±0.02. After fermentation, the prepared yoghurt was held in the refrigerator (4°C).

The yogurt was diluted by drinking water (50% v/v and NaCl 0.7% w/v) to produce Doogh. Then DKXG (0.2% w/v) and GSE (0.2% w/v) and powdered mint (1 g L<sup>-1</sup>) were added to the mixture and stirred. Doogh samples were enriched with 0, 0.5, 1 and 2 g L<sup>-1</sup> Ginseng extract (treatments T1, T2, T3 and T4, respectively). Then, they were filled into 250-mL PET bottles and stored at 4°C for 21 days.

### Doogh Samples Analysis

The prepared Doogh samples (10 mL) were titrated with 0.1N NaOH (Merck), using 0.5 % phenolphthalein as an indicator. The pH of the samples was measured at room temperature using a pH meter (Metrohm, Germany) [3].

A Hunterlab (Colorflex EZ, USA) was used to determine the whiteness (L\*), red/greenness (a\*), and yellow/blueness (b\*) values of the probiotic Doogh containing mint enriched with Ginseng extract [25].

MRS-bile agar medium was used for determining the viability of *L. acidophilus* (La5) and *B. lactis* (Bb12) (MRS agar by Merck, Darmstadt, Germany and bile by Sigma-Aldrich, Inc., Reye, USA). One mL of each sample was diluted with 9 mL of



sterile 0.1% w/v peptone water (Oxoid, Hampshire, UK), and mixed uniformly with a vortex mixer. Subsequent serial dilutions were made. The counts of the viability of *L. acidophilus* (La5) were taken after incubating the plates aerobically at 37°C for 72 hours; and for the selective enumeration of *L. lactis* (Bb12), the plates were incubated at 37°C for 3 days under anaerobic conditions using a GasPak system (Merck, Darmstadt, Germany) [34].

A group of eight trained panelists from Tehran Pegah (Dairy Company, Iran) were asked to determine the Doogh scores with a 5-point hedonic scale for external appearance (color), flavor, taste and texture using standard questionnaire. The scores (1= Dislike very much, 2= Dislike a little, 3= Neither like nor dislike, 4= Like a little, and 5= Like very much) were given by the expert panelists for each of the mentioned properties. Since the final index assessment is overall acceptability; therefore, in this research, only overall acceptability results have been reported.

The Doogh samples were taken for the analysis after 1, 7, 14 and 21 days of storage.

### Statistical Analysis

A factorial experiment was used in a randomized complete block design (for sensory test) and completely randomized design (for pH, acidity, color and microbial

tests). Kruskal-Wallis nonparametric test was used to analyze the data obtained from the sensory tests. The experiment consisted of two factors including Ginseng extract factor (4 levels) and time factor (4 levels, days 1, 7, 14 and 21). Each treatment had three replicates. The statistical significance of the data was determined using Duncan's test.  $P$ -value  $< 0.05$  was considered sufficient to reject the null hypothesis. Statistical analysis was performed by running the SAS 9.1 software.

## RESULTS AND DISCUSSION

### Acidity and pH of Sin-Biotic Doogh Samples during Cold Storage

As shown in Table 1, adding Ginseng extract, with brix 60%, had no effect on the pH of sin-biotic doogh samples containing mint powder ( $P > 0.05$ ). This is in accordance with the findings of Hekmat *et al.* [16], who found a partial reduction of pH in probiotic fermented milk enriched with Ginseng extract. The results obtained by the measurement of acidity (Table 2) indicated no significant difference between the different treatments ( $P > 0.05$ ); the addition of Ginseng extract reduced acidity, but this decline was not enough to cause a statistically significant difference.

During the storage time, a decrease in the pH and an increase in the acidity of the samples were observed ( $P < 0.05$ ), and seemingly Ginseng extract had no effect on

**Table 1.** pH changes of probiotic Doogh enriched with red Ginseng extract during cold storage (Mean±Standard deviation).

Treatment <sup>a</sup>	Day			
	1	7	14	21
T1	4.56±0.012 <sup>a</sup>	4.44±0.015 <sup>b</sup>	4.39±0.015 <sup>c</sup>	4.34±0.013 <sup>d</sup>
T2	4.56±0.010 <sup>a</sup>	4.43±0.011 <sup>b</sup>	4.38±0.008 <sup>c</sup>	4.33±0.015 <sup>d</sup>
T3	4.56±0.015 <sup>a</sup>	4.43±0.009 <sup>b</sup>	4.39±0.016 <sup>c</sup>	4.33±0.008 <sup>d</sup>
T4	4.55±0.006 <sup>a</sup>	4.43±0.010 <sup>b</sup>	4.38±0.010 <sup>c</sup>	4.33±0.005 <sup>d</sup>

<sup>a</sup> Probiotic Doogh enriched with T1= 0 g l<sup>-1</sup> red Ginseng extract, T2= 0.5 g l<sup>-1</sup> red Ginseng extract, T3= 1 g l<sup>-1</sup> red Ginseng extract, T4= 2 g l<sup>-1</sup> red Ginseng extract. <sup>a-d</sup> Means with different subscripts differ significantly ( $P < 0.05$ ).

**Table 2.** Titratable acidity (°D) changes of probiotic Doogh enriched with red Ginseng extract during cold storage (Mean±Standard deviation).

Treatment <sup>a</sup>	Day				
	1	7	14	21	
T1	42.07±0.603 <sup>a</sup>	34.17±0.473 <sup>d</sup>	36.97±0.541 <sup>c</sup>	39.57±0.510 <sup>b</sup>	42.07±0.603 <sup>a</sup>
T2	41.90±0.557 <sup>a</sup>	34.07±0.465 <sup>d</sup>	36.77±0.503 <sup>c</sup>	39.43±0.650 <sup>b</sup>	41.90±0.557 <sup>a</sup>
T3	41.47±0.666 <sup>a</sup>	33.60±0.458 <sup>d</sup>	36.30±0.458 <sup>c</sup>	38.93±0.551 <sup>b</sup>	41.47±0.666 <sup>a</sup>
T4	41.37±0.490 <sup>a</sup>	33.57±0.404 <sup>d</sup>	36.23±0.450 <sup>c</sup>	38.90±0.600 <sup>b</sup>	41.37±0.490 <sup>a</sup>

<sup>a</sup> Probiotic Doogh enriched with T1= 0 g l<sup>-1</sup> red Ginseng extract, T2= 0.5 g l<sup>-1</sup> red Ginseng extract, T3= 1 g l<sup>-1</sup> red Ginseng extract, T4= 2 g l<sup>-1</sup> red Ginseng extract. <sup>a-d</sup> Means with different subscripts differ significantly (P< 0.05).

them. The reason is that lactic bacteria in the samples continued to ferment lactose and convert it to lactic acid and other metabolites and, so, decreased pH [7, 4]. However, reduction in pH and increase in acidity were higher in the first day until the seventh day of storage because lactic bacteria's growth had been limited and the rate of producing lactic acid had also been reduced. This finding is consistent with the results of Yousefi *et al.* [39], who reported reduction of the pH and increase in the acidity of yoghurt samples containing different concentrations of fruit-based additives during storage, as well as a sharply higher pH and increased acidity in the first 6 days of storage in comparison to the other days.

#### Color Values of Sinbiotic Doogh Samples during Cold Storage

Whiteness (L\*), red/greenness (a\*), and yellow/blueness (b\*) values of the Doogh samples are shown in Table 3. Adding Ginseng extract to the probiotic Doogh samples increased their L\* value. The L\* value showed no significant difference with the control sample by increasing Ginseng extract up to 0.5 g L<sup>-1</sup> (P> 0.05). However, by increasing the extract to 1 and 2 g L<sup>-1</sup>, the L\* values demonstrated significant differences with the control sample (P< 0.05). This represents an increase in the brightness or transparency of the samples with increasing the amount of the extract.

This could be due to the presence of colored compounds in the extract that increases the brightness and transparency of the product. Additionally, there was statistically significant difference between the L\* values of the samples during 21 days of storage at the intervals of 7 days. However, this difference was not significant between samples enriched with 0 and 0.5 g/l Ginseng extract. In general, it can be understood that during storage, the L\* value had a reduction trend, indicating the decrease in the product's lightening and darkening over time. This occurred because of changes in the structure of proteins in the Doogh due to decrease in pH and increase in acidity as well as changes in the color compounds of the extract over time. This is in accordance with other studies, which have shown that L\* value decreased in processed berry fruit yoghurts with high pressure during storage, influenced by the color migration from the pieces of fruit to yoghurt [25] and that the brightness value of packaged cheese with modified atmosphere decreased during the storage [32].

Adding Ginseng extract led to increase in a\* value, which is a negative numerical factor (becomes more positive number). The increase was negligible for the sample containing 0.5 g L<sup>-1</sup> Ginseng extract; and as a result, samples enriched with 0 and 0.5 g/l Ginseng extract were not significantly different in terms of a\* value. But, with the increasing concentration of extract to 1 and 2 g L<sup>-1</sup>, the difference increased significantly

**Table 3.** Changes in  $L^*$ ,  $a^*$  and  $b^*$  values of probiotic Doogh enriched with red Ginseng extract during cold storage (Mean±Standard deviation) <sup>a</sup>.

Treatment <sup>a</sup>	Day					
	1	7	14	21		
$L^*$	T1	84.86±0.011 <sup>l</sup>	85.62±0.006 <sup>c</sup>	85.37±0.018 <sup>f</sup>	85.13±0.021 <sup>i</sup>	84.86±0.011 <sup>l</sup>
	T2	84.89±0.014 <sup>l</sup>	85.64±0.010 <sup>c</sup>	85.39±0.050 <sup>f</sup>	85.15±0.025 <sup>i</sup>	84.89±0.014 <sup>l</sup>
	T3	84.95±0.023 <sup>k</sup>	85.71±0.017 <sup>b</sup>	85.45±0.012 <sup>e</sup>	85.21±0.026 <sup>h</sup>	84.95±0.023 <sup>k</sup>
	T4	85.05±0.020 <sup>j</sup>	85.80±0.014 <sup>a</sup>	85.54±0.015 <sup>d</sup>	85.32±0.046 <sup>g</sup>	85.05±0.020 <sup>j</sup>
$a^*$	T1	-1.85±0.011 <sup>l</sup>	-1.37±0.009 <sup>d</sup>	-1.57±0.012 <sup>g</sup>	-1.70±0.019 <sup>i</sup>	-1.85±0.011 <sup>l</sup>
	T2	-1.85±0.030 <sup>l</sup>	-1.36±0.021 <sup>d</sup>	-1.56±0.015 <sup>g</sup>	-1.68±0.016 <sup>i</sup>	-1.85±0.030 <sup>l</sup>
	T3	-1.78±0.019 <sup>k</sup>	-1.28±0.017 <sup>c</sup>	-1.46±0.015 <sup>f</sup>	-1.61±0.004 <sup>h</sup>	-1.78±0.019 <sup>k</sup>
	T4	-1.54±0.028 <sup>g</sup>	-1.05±0.004 <sup>a</sup>	-1.21±0.024 <sup>b</sup>	-1.37±0.007 <sup>d</sup>	-1.54±0.028 <sup>g</sup>
$b^*$	T1	3.61±0.010 <sup>p</sup>	6.40±0.018 <sup>g</sup>	5.46±0.025 <sup>k</sup>	4.56±0.011 <sup>n</sup>	3.61±0.010 <sup>p</sup>
	T2	4.32±0.020 <sup>o</sup>	7.14±0.021 <sup>d</sup>	6.19±0.024 <sup>h</sup>	5.27±0.013 <sup>l</sup>	4.32±0.020 <sup>o</sup>
	T3	5.04±0.019 <sup>m</sup>	7.85±0.023 <sup>b</sup>	6.92±0.015 <sup>e</sup>	5.95±0.015 <sup>i</sup>	5.04±0.019 <sup>m</sup>
	T4	5.66±0.027 <sup>j</sup>	8.46±0.028 <sup>a</sup>	7.52±0.035 <sup>c</sup>	6.61±0.026 <sup>f</sup>	5.66±0.027 <sup>j</sup>

<sup>a</sup> Probiotic Doogh enriched with T1= 0 g l<sup>-1</sup> red Ginseng extract, T2= 0.5 g l<sup>-1</sup> red Ginseng extract, T3= 1 g l<sup>-1</sup> red Ginseng extract, T4= 2 g l<sup>-1</sup> red Ginseng extract. <sup>a-d</sup> Means with different subscripts differ significantly (P< 0.05).

due to increase in the amounts of the red color compounds importing into the probiotic Doogh samples through increasing the amount of consumed extract. During the storage,  $a^*$  value showed decreasing trend so that with increased shelf-life,  $a^*$  value tended to negative numbers. This could be due to decomposition of red color compounds and oxidation of pigments in the extract influenced by decrease in antioxidant activity and increase in the amount of green color [25]. On every day of storage, sample enriched with 2 g/l Ginseng extract (T4) had the highest (the most positive) amount of  $a^*$  value, and the lowest amount (the most negative) of  $a^*$  value belonged to sample without Ginseng extract (T1).

Adding Ginseng extract to the probiotic Doogh led to the increase of  $b^*$  value so that the sample lacking Ginseng extract (T1) showed the lowest  $b^*$  value while the sample having the highest amount of extract (T4) showed the highest  $b^*$  value. As a result, increase in the amount of extract led to increase in  $b^*$  value, representing more yellow color of the samples. Cold storage of the products reduced their  $b^*$  value and

increased their blue color, which is equal to darkening of the products because of changes in the color compounds of the extract such as phenolic compounds. On every day of storage, the highest  $b^*$  value was observed in sample enriched with 2 g/l Ginseng extract (T4), and with reducing the amount of extract, this value decreased, too. The results of this research are consistent with the findings of Marcia *et al.* [25] who reported that  $b^*$  value in berry frozen yogurt processed by high pressure decreased during the storage period due to the migration of color from the fruit pieces into the yoghurt.

#### Viability of Probiotic Bacteria in Sin-Biotic Doogh Samples during Cold Storage

The results of investigating the viability of *Lactobacillus acidophilus* (La5) and *Bifidobacterium lactis* (Bb12) revealed that addition of Ginseng extract increased the survival rate of these two bacteria in the probiotic Doogh containing powdered mint. These results are consistent with the findings of Cimo *et al.* [10], who found increase in the viability of *Lactobacillus rhamnosus* GR-1 in the presence of aqueous extract of American Ginseng due to prebiotic

compounds including oligo-saccharides and ginsenosides. Ginseng has ginsenosides (that can only be absorbed through de-glycosylation by the intestinal flora [24]) and also 9 oligosaccharides with a polymerization degree of 2 to 10 [36]; therefore, it is considered a prebiotic compound.

Haddadin [15] found that the survival rate of *Lactobacillus acidophilus* and *Bifidobacterium Infantis* increased in Doogh enriched with olive leaf extract due to the presence of the phenolic compounds in it. According to the same reference, reducing oxidative stress and creating a favorable environment. So, it can be expected that with increasing Ginseng extract in the treatments, as a result of increasing the concentrations of prebiotic and phenolic compounds, the survival rate of *Bifidobacterium lactis* and *Lactobacillus acidophilus* would increase.

According to Table 4, the lowest counting *Bifidobacterium lactis* (Bb12) was related to sample without Ginseng extract, T1, (in the twenty-first day of storage) and the highest

counting belonged to sample enriched with 2 g/l Ginseng extract, T4, (on the seventh day of storage). According to Table 5, the lowest counting *Lactobacillus acidophilus* (La5) was related to T1 (in the first day of storage) and the highest counting was observed in sample enriched with 1 g/l Ginseng extract, T3, (in the seventh day of storage). In general, in the 21 days of storage, the highest and the least viability of probiotic bacteria were observed in samples enriched with 2 and 0 g/l Ginseng extract, T4 and T1, respectively.

### Overall Acceptance of Enriched Sin-Biotic Doogh Samples

The highest overall acceptance score was obtained in sample without Ginseng extract, T1, (in the first day of storage) and the lowest score belonged to sample enriched with 2 g/l Ginseng extract, T4, (after twenty first days of storage) (Table 6). Overall acceptance results demonstrated that, in general, sensory scores in the samples with

**Table 4.** *Bifidobacterium lactis* (Bb12) viability (log CFU ml<sup>-1</sup>) changes of probiotic Doogh enriched with red Ginseng extract during cold storage (Mean±Standard deviation).

Treatment <sup>a</sup>		Day			
		1	7	14	21
Bb12	T1	7.97±0.02 <sup>ef</sup>	8.07±0.01 <sup>b</sup>	8.02±0.01 <sup>cde</sup>	7.94±0.01 <sup>i</sup>
	T2	8.00±0.02 <sup>ef</sup>	8.07±0.02 <sup>b</sup>	8.01±0.03 <sup>def</sup>	7.96±0.03 <sup>hi</sup>
	T3	8.02±0.01 <sup>cde</sup>	8.08±0.03 <sup>a</sup>	8.05±0.02 <sup>ab</sup>	7.99±0.02 <sup>fg</sup>
	T4	8.04±0.03 <sup>bc</sup>	8.09±0.02 <sup>a</sup>	8.06±0.01 <sup>ab</sup>	8.03±0.01 <sup>cd</sup>

<sup>a</sup> Probiotic Doogh enriched with T1= 0 g l<sup>-1</sup> red Ginseng extract, T2= 0.5 g l<sup>-1</sup> red Ginseng extract, T3= 1 g l<sup>-1</sup> red Ginseng extract, T4= 2 g l<sup>-1</sup> red Ginseng extract. <sup>a-1</sup> Means with different subscripts differ significantly (P < 0.05).

**Table 5.** *Lactobacillus acidophilus* (La5) viability (log CFU ml<sup>-1</sup>) changes of probiotic Doogh enriched with red Ginseng extract during cold storage (Mean±Standard deviation).

Treatment <sup>a</sup>		Day			
		1	7	14	21
La5	T1	6.10±0.02 <sup>ef</sup>	6.35±0.03 <sup>b</sup>	6.30±0.02 <sup>cde</sup>	6.17±0.01 <sup>i</sup>
	T2	6.15±0.01 <sup>ef</sup>	6.38±0.02 <sup>b</sup>	6.31±0.01 <sup>def</sup>	6.16±0.01 <sup>hi</sup>
	T3	6.27±0.02 <sup>cde</sup>	6.51±0.01 <sup>a</sup>	6.46±0.01 <sup>ab</sup>	6.28±0.03 <sup>fg</sup>
	T4	6.29±0.02 <sup>bc</sup>	6.50±0.01 <sup>a</sup>	6.47±0.01 <sup>ab</sup>	6.40±0.02 <sup>cd</sup>

<sup>a</sup> Probiotic Doogh enriched with T1= 0 g l<sup>-1</sup> red Ginseng extract, T2= 0.5 g l<sup>-1</sup> red Ginseng extract, T3= 1 g l<sup>-1</sup> red Ginseng extract, T4= 2 g l<sup>-1</sup> red Ginseng extract. <sup>a-1</sup> Means with different subscripts differ significantly (P < 0.05).

**Table 6.** Overall acceptability changes of probiotic Doogh enriched with red Ginseng extract during cold storage (Mean±Standard deviation).

Treatment <sup>a</sup>	Day			
	1	7	14	21
T1	4.00±0.18 <sup>a</sup>	3.92±0.15 <sup>ab</sup>	3.83 ±0.36 <sup>ab</sup>	3.69±0.30 <sup>bc</sup>
T2	3.83±0.40 <sup>ab</sup>	3.50±0.18 <sup>cd</sup>	3.75±0.34 <sup>abc</sup>	3.67±0.36 <sup>bc</sup>
T3	3.88±0.30 <sup>ab</sup>	3.88±0.53 <sup>ab</sup>	3.88±0.17 <sup>ab</sup>	3.75±0.24 <sup>abc</sup>
T4	3.38±0.38 <sup>d</sup>	3.75±0.24 <sup>abc</sup>	3.75±0.29 <sup>abc</sup>	3.92±0.15 <sup>ab</sup>

<sup>a</sup> Probiotic Doogh enriched with T1= 0 g l<sup>-1</sup> red Ginseng extract, T2= 0.5 g l<sup>-1</sup> red Ginseng extract, T3= 1 g l<sup>-1</sup> red Ginseng extract, T4= 2 g l<sup>-1</sup> red Ginseng extract. <sup>a-d</sup> Means with different subscripts differ significantly (P< 0.05).

higher concentrations of Ginseng extract increased over time, while T1, which did not contain the extract, obtained lower score than the treatment containing the highest dose of extract. This suggests that Ginseng extract covers undesirable flavor created in the product during storage due to the presence of antioxidant compounds in Ginseng extract that prevent fat oxidation and creation of undesirable flavor in the product.

Overall, it can be concluded that Ginseng extract has no negative impact on the sensory attributes of probiotic Doogh enriched with mint powder. But, with increasing the percentage of the extract, the utility decreases slightly, mainly due to the presence of saponins compounds, and bitter, sweet, and earthy tastes in it [9], which are a bit like licorice flavors and are unfamiliar to Iranian consumers. In general, some plant-based additives containing desirable properties for survival of probiotics can be added to fermented milk products as a prebiotic. Despite having some unfavorable sensory characteristics, these additives should be used in such amounts that the final probiotic product remains acceptable to the consumers.

## CONCLUSIONS

Today, because of the prevalence of chronic diseases such as CardioVascular Diseases (CVDs), diabetes and cancer that have high medical costs, production and consumption of functional foods can play an

important role in preventing the occurrence of such diseases as well as reducing the cost of treatment. Since Doogh is a popular fermented beverage in Iran, adding Ginseng extract and powdered mint can help to promote community health. In this study, Ginseng herb, with confirmed valuable medicinal properties, was used for the production of probiotic Doogh containing mint powder, and some properties of the produced Doogh samples during storage at 4°C were evaluated. Increasing the level of Ginseng extract in the produced samples of Doogh did not lead to significant changes in pH, but decreased acidity and increased *L\**, *a\** and *b\** values. Adding Ginseng extract increased the survival rate of probiotic bacteria in the probiotic Doogh containing mint powder. In general, the sample containing 1 g L<sup>-1</sup> Ginseng extract that showed the highest score in sensory evaluation after the control sample was selected as the best treatment.

## REFERENCES

1. Aluko, R. 2012. *Functional Foods and Nutraceuticals*. Springer, New York (NY).
2. Attele, A. S., Wu, J. A. and Yuan, C. S. 1999. Ginseng Pharmacology: Multiple Constituents and Multiple Actions. *Biochem. Pharma.*, **58**: 1685-1693.
3. AOAC. 2005. *Official Methods of Analysis*. Association of Official Analytical Chemists, Arlington, VA, USA.
4. Bakirci, I. and Kavaz, A. 2008. An Investigation of Some Properties of Banana Yogurts Made with Commercial ABT-2



- Starter Culture during Storage. *Int. J. Dairy Technol.*, **61**: 270-276.
5. Beheshtipour, H., Mortazavian, A. M., Haratian, P. and Khosravi-darani, K. 2012. Effect of *Chlorella vulgaris* and *Arthrospira platensis* Addition on Viability of Probiotic Bacteria in Yogurt and Its Biochemical Properties. *J. Eur. Food Res. Technol.*, **235**: 719-728.
  6. Blumenthal, M. 2003. *The ABC Clinical Guide to Herbs*. American Botanical Council, Austin, Texas.
  7. Bonczar, G., Wszolek, M. and Siuta, A. 2002. The Effects of Certain Factors on the Properties of Yogurt Made from Ewe's Milk. *J. Food Chem.*, **79**: 85-91.
  8. Chen, J., Zhao, R., Zeng, Y. M., Meng, H., Zuo, W. J., Li, X. and Wang, J. H. 2009. Three New Triterpenoid Saponins from the Leaves and Stems of *Panax quinquefolium*. *J. Asian Natur. Product Res.*, **11**: 195-201.
  9. Chung, H. K., Lee, Y. C., Rhee, Y. K. and Lee, S. Y. 2011. Consumer Acceptance of Ginseng Food Products. *J. Food Sci.*, **76(9)**: 516-522.
  10. Cimo, A., Soltani, M., Lui, E. and Hekmat, S. 2013. Fortification of Probiotic Yogurt with Ginseng (*Panax quinquefolius*) Extract. *J Food Nut Disorder.*, **2(2)**: 1-5.
  11. Codex Alimentarius Commission. 2010. *Project Document for a Regional Standard for Doogh*. Prepared by the Islamic Republic of Iran. CX/NEA 11/6/7.
  12. Corthout, J., Naessens, T., Apers, S. and Vlietinck, A. J. 1999. Quantitative Determination of Ginsenosides from *Panax Ginseng* Roots and Ginseng Preparations by Thin Layer Chromatography-Densitometry. *J. Pharma. Biomed. Analysis*, **21**: 187-192.
  13. Duda, R. B., Taback, B., Kessel, B., Dooley, D. D., Yang, H., Marchiori, J., Slomovic, B. M. and Alvarez, J. G. 1996. PS2 Expression Induced by American Ginseng in MCF-7 Breast Cancer Cells. *Annal. Surgical Oncol.*, **3**: 515-520.
  14. Gardiner, P. 2000. *Peppermint (Mentha piperita)*. Longwood Herbal Task Force.
  15. Haddadin, M. S. Y. 2010. Effect of Olive Leaf Extracts on the Growth and Metabolism of Two Probiotic Bacteria of Intestinal Origin. *Pak. J. Nut.*, **9(8)**: 787-79.
  16. Hekmat, S., Cimo, A., Soltani, M., Lui, E. and Reid G. 2013. Microbial Properties of Probiotic Fermented Milk Supplemented with Ginseng Extracts. *J Food & Nut Sci.*, **4**: 392-397.
  17. Kailasapathy, K. and Chin, J. 2000. Survival and Therapeutic Potential of Probiotic Organisms with Reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *J. Immunol. Cell Biol.*, **78(1)**: 80-88.
  18. Kenarova, B., Neychev, H., Hadjiivanova, C. and Petkov, V. D. 1990. Immunomodulating Activity of Ginsenoside Rg1 from *Panax ginseng*. *Japan. J. Pharmacol.*, **54**: 447-454.
  19. Keville, K. 2000. Planting the Future: Saving Our Medicinal Herbs. In: "*Panax quinquefolius*", (Eds.): Gladstar, R. and Hirsch, P. Healing Arts Press, Rochester, VT.
  20. Kim, J. Y., Germolec, D. R. and Luster, M. I. 1990. *Panax ginseng* as a Potential Immunomodulator: Studies in Mice. *J. Immunopharmacol. Immunotoxicol.*, **12**: 257-276.
  21. Kim, S. H. and Park, K. S. 2003. Effects of *Panax ginseng* Extract on Lipid Metabolism in Humans. *J. Pharmacol. Res.*, **48**: 511-513.
  22. Kim, H. G., Yoo, S. R., Park, H. J., Lee, N. H., Shin, J. W., Sathyanath, R., Cho, J. H. and Son, C. G. 2011. Antioxidant Effects of *Panax ginseng* c.a. Meyer in Healthy Subjects: A Randomized, Placebo-Controlled Clinical Trial. *J. Food Chem. Toxicol.*, **49**: 2229-2235.
  23. Kim, H. G., Cho, J. H., Yoo, S. R., Lee, J. S., Han, J. M., Lee, N. H., Ahn, Y. C. and Son, C. G. 2013. Antifatigue Effects of *Panax ginseng* c.a. Meyer: A Randomised, Double-Blind, Placebo-Controlled Trial. *Plos One J.*, **8(4)**: 1-8.
  24. Lee, B. H., Hyung, J. Y., Myeong, S. P., Bin, K. and Geun, E. J. 2006. Transformation of the Glycosides from Food Materials by Probiotics and Food Microorganisms. *J. Microbiol. Biotechnol.*, **16(4)**: 497-504.
  25. Marcia, K. W., Daniel, F. F., Vicki, L. and Lisbeth, M. G. 2006. Fruit Yogurt Processed with High Pressure. *Int. J. Food Sci. Technol.*, **41**: 464-467.
  26. Parada, J. L, de Ceire, G. Z., de Mule, M. C. Z. and de Cano, M. M. S. 1998. Lactic Acid Bacteria Growth Promoters from



- Spirulina platensis*. *Int. J. Food Microbiol.*, **45**: 225-228.
27. Park, K. S., Hwang, D. S., Lee, J. M., Jang, J. K., Lee, K. B. and Lee, C. H. 2013. Inhibitory Effect of *Panax ginseng* c. a. Meyer on Gynecological Cancer. *J. Oriental Pharma. Exp. Medicine*, **13**: 217-223.
  28. Saito, H., Tsuchiya, M., Naka, S. and Takagi, K. 1977. Effects of *Panax ginseng* Root on Conditioned Avoidance Response in Rats. *Japan. J. Pharmacol.*, **27**: 509-516.
  29. Schlag, E. M. and Mcintosh, M. S. 2006. Ginsenoside Content and Variation among and within American Ginseng (*Panax quinquefolius* L.). *J. Pop. Phytochem.*, **67**: 1510-1519.
  30. Scholey, A., Ossoukhova, A., Owen, L., Ibarra, A., Pipingas, A., He, K., Roller, M. and Stough, C. 2010. Effects of American Ginseng (*Panax quinquefolius*) on Neurocognitive Function: An Acute, Randomised, Double-Blind, Placebo-Controlled, Crossover Study. *J. Psychopharm.*, **212**: 345-356.
  31. Shah, P. and Mello, P. M. D. 2004. A Review of Medicinal and Pharmacological Effects of *Mentha piperita*. *Natur. Prod. Radance*, **3(4)**: 214-221.
  32. Shrivastava, S., Goyal, S. and Goyal, G. K. 2013. Effect of Modified Atmosphere Packaging on Colour Profile of Paneer Stored at 3±1°C. *J. Agri. Sustain.*, **3(2)**: 195-204.
  33. Soccol, C. R., Vandenberghe, L. P. S. , Spier, M. R., Medeiros, A. B. P., Yamaguishi, C. T., De Dea Lindner, J., Pandey, A. and Thomaz-Soccol, V. 2010. The Potential of Probiotics. *Food Technol. Biotechnol.*, **48(4)**: 413-434.
  34. Sohrabvandi, S., Mortazavian, A. M., Dolatkahnejad, M. R. and Bahadori Monfared, A. 2012. Suitability of MRS-Bile Agar for the Selective Enumeration of Mixed Probiotic Bacteria in Presence of Mesophilic Lactic Acid Cultures and Yoghurt Bacteria. *Iran. J. Biotechnol.*, **10(1)**: 16-21.
  35. Tárrega, A., Salvador, A., Meyer, M., Feuillère, N., Ibarra, A., Roller, M., Terroba, D., Madera, C., Iglesias, J. R., Echevarría, J. and Fiszman, S. 2012. Active Compounds and Distinctive Sensory Features Provided by American Ginseng (*Panax quinquefolius* L.) Extract in a New Functional Beverage. *J. Dairy Sci.*, **95**: 4246-4255.
  36. Wan, D., Jiao, L., Yang, H. and Liu, S. 2012. Structural Characterization and Immunological Activities of the Water-Soluble Oligosaccharides Isolated from the *Panax ginseng* Roots. *J. Planta.*, **235(6)**: 1289-1297.
  37. Xie, J. T., Mehendale, S. and Yuan, C. S. 2005. Ginseng and Diabetes. *Am. J. Chinese Medicine*, **33**: 397-404.
  38. Yarnell, E., Abascal, K. and Hooper, G. C. 2003. *Clinical Botanical Medicine*. Mary Ann Liebert, New York (NY).
  39. Yousefi, M., Nateghil, L. and Azadi, E. 2013. Effect of Different Concentration of Fruit Additives on Some Physicochemical Properties of Yoghurt during Storage. *Scholar. Res. Library*, **4(4)**: 244-249.
  40. Yun, T. K. and Choi, S. Y. 1995. Preventive Effect of Ginseng Intake against Various Human Cancers: A Case-Control Study on 1987 Pairs. *J. Cancer Epidemiol. Biomarker. Prevent.*, **4**: 401-408.

## تولید دوغ پروبیوتیک غنی شده با عصاره جینسینگ قرمز

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### چکیده

امروزه، جینسینگ به دلیل اثرات مفید آن در پیشگیری و درمان سرطان ها، دیابت، بیماری های سیستم اعصاب مرکزی و ... در تولید مواد غذایی سلامتی بخش به کار برده می شود. عمده ترین و شاخص ترین ترکیبات موجود در جینسینگ، جینسینوساید ها هستند که مسئول بیشتر اثرات سلامت بخش شناخته شده این گیاه می باشند. اهداف مطالعه حاضر، تولید نوشیدنی سلامتی بخش و ارزیابی ویژگی های کیفی آن می باشد. اثرات افزودن عصاره جینسینگ قرمز در سطوح مختلف (0، 0/5، 1 و 2 گرم بر لیتر) بر pH، اسیدیته قابل تیتر، شاخص های رنگی، قابلیت زنده مانگی لاکتوباسیلوس اسیدوفیلوس و بیفیدوباکتریوم لاکتیس و پذیرش کلی دوغ پروبیوتیک حاوی پودر نعنای (1gr/1) طی 21 روز نگهداری در 4°C مورد بررسی قرار گرفت. نتایج نشان داد با افزایش عصاره جینسینگ قرمز تا 2 گرم در لیتر، در pH و اسیدیته قابل تیتر نمونه های دوغ پروبیوتیک تفاوت معنی داری مشاهده نشد ( $p > 0/05$ )؛ شاخص های رنگی نمونه های دوغ به طور معنی داری تغییر یافتند ( $p < 0/05$ )؛ قابلیت زنده مانگی باکتری های پروبیوتیک به طور معناداری افزایش یافت ( $p < 0/05$ )؛ و پذیرش کلی به طور معناداری کمتر شد ( $p < 0/05$ ). به طور کلی، نمونه دوغ حاوی 1 گرم بر لیتر عصاره جینسینگ قرمز، بالاترین امتیاز را در قیاس با سایر نمونه ها از نظر پذیرش کلی به خود اختصاص داد.