A New Functional Yogurt Supplemented With Ginseng

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ABSTRACT

Yogurt supplemented with beneficial ingredient is mounting to corroborate the consumer perception of health. Water extract panax ginseng was used in yogurt processing to achieve different final concentrations of ginseng. The quality attributes of ginseng yogurt were determined. Total phenolic contents, $DPPH^{\bullet -}$ and $ABTS^{\bullet +}$ scavenging radicals activity and the reducing ability were measured using a spectrophotometric decolorization assay and $OH^{\bullet}$ scavenging activity test using ESR was an excellent target to investigate antioxidants in the fields of food and nutrition. The results showed that the optimal concentration of ginseng extract in yogurt was approximately (0.5 mg ginseng/ml yogurt) which had recorded the best scores of acceptability and quality characteristics. The $DPPH^{\bullet -}$ and $ABTS^{\bullet +}$ free radicals scavenging and the Fe^{3+}– Fe^{2+} transformation were found to increase significantly, while the $OH^{\bullet}$ scavenging activity decreased with the yogurt containing 0.5 mg ginseng/ml yogurt. A strong positive correlation between the antioxidant activity and ginseng concentration was observed.

Key words: antioxidant activity, ginseng, yogurt, phenolic compounds

INTRODUCTION

Today, consumers prefer foods that promote good health and could reduce risk of diseases. Functional foods will be hopeful to good health in the future because it have been classified either as preventive or therapeutic purpose and used alone or mixed together for prevention some of certain diseases (Huggett & Schliter, 1996). Dairy products are excellent media to generate an array of products that fit into the current consumer demand for functional foods (Chandan, 1999). In general, worldwide consumption of fermented milk products, including yogurt, is increasing. Scientific and clinical evidence is mounting to corroborate the consumer perception of health from yogurt (Chandan & Shahani, 1993, Salminen *et al.*, 1998).

Supplementation innovative ingredient is an important development in the market as consumers became increasingly conscious of what they are putting in and on their bodies. Panax ginseng is an indigenous perennial herb helps maintain balance among the various systems of the body and helps optimize health and well being. Clinical studies confirm that ginseng can help enhance endurance, reduce fatigue (Hartz, *et al.* 2004), depression (Hartley, *et al.* 2004), heart failure (You, *et al.* 2005), arteriosclerosis (Zhou, *et al.* 2005), anemia (Lim & Lu 1998), diabetes (Lee, *et al.* 2006), ulcers (Jeong, *et al.* 2003), and improve coordination and reaction time.

Panax ginseng has antioxidant effects and increases immune system activity. The main ingredients of ginseng are ginsenosides, glycosides containing an aglycone. Ginsenosides present in ginseng (Wang, *et al.* 2006) contributes directly to antioxidant activity which has also been shown to protect against superoxide radical induced damage of cultured cardiac myocytes (Zhong, *et al.* 1993). Panax ginseng water extract has also been shown to exhibit a dose-dependent protection against free radical induced injury in pulmonary lungs (Rimar, *et al.* 1996).

The aim of the present work was using of panax ginseng extract at different concentrations to formulate functional skimmed yogurt and evaluating its acceptability, quality characteristics and the antioxidant activity to choose the optimal concentration of ginseng to be used in yogurt

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MATERIALS AND METHODS

Materials

Ginseng (*Panax ginseng*) used in the present work was Korean crude red ginseng extract supplied by the Korean society of ginseng, Seoul, Korea and it was used as ginseng water extract. Reconstituted skim milk powder (low heat, USA origin) was prepared in distilled water and left overnight at 4°C to allow full hydration.

Chemicals and reagents

Folin–Ciocalteu reagent and 2,4,6-Tri 2- pyridyl-s-triazine (TPTZ) from Fluka Chem. Co (Buchs, Swizerland), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical and the reagents, (S)-(-)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX), 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) $ABTS^{\bullet+}$ radical, potassium ferricyanide from (Sigma, St. Louis, Mo. USA), and gallic acid from (MP Biomedicals. Inc. (Eschwege, Germany). 5,5-Dimethyl-1-pyrroline Noxide (DMPO), hydrogen peroxide (H_2O_2), and ferrous sulfate ($FeSO_4 \cdot 7H_2O$) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Starter: Freeze dried culture of *Lactobacillus delbrueckii supsp bulgaricus* and *Streptococcus.thermophilus* (1:1) were obtained from Chr. Hansens laboratories Denmark.

Methods

Yogurt manufacturing

Appropriate amounts of Korean crude ginseng extract were diluted in distilled water which used in reconstituted skim milk (14% total solids) to achieve final concentrations (0.25, 0.50 and 1.0 mg ginseng/ml). Reconstituted milk categorized was heat treated at 90°C for 10 min, then cooled to 43°C, starter was added at the rate of 3% and incubated at 43°C until coagulation occur within 3-4 hr and to approximately pH 4.3, then refrigerated at 5°C. Plain skimmed yogurt without ginseng was prepared by the same procedure and saved as a control.

Evaluation the quality characteristics of ginseng yogurt

This evaluation included pH value, titratable acidity, sensory evaluation (colour, flavour and overall acceptance) using a 9-point hedonic scale (Larmond, 1980), and apparent viscosity, (cP.s) which measures the resistance to a rotating spindle (Brookfield Model DV III, Programmable rheometer) depending on time of shearing and the tested samples were subjected to shear rate a spindle speed of 50 rpm and spindle rotating velocities, at constant temperature of 25°C.

Determination the antioxidant properties of ginseng yogurt

Determination of total phenolic content

Total phenolic (TP) contents in the ginseng yogurt were analyzed by the Folin-Ciocalteu method (Wua & Ng, 2008). Briefly, samples of the yogurt were extracted by centrifuging twice at 10,000xg at 5°C for 10 min, to remove non-hydrolyzed casein and fat. The samples were transferred to test-tube and made to a volume of 0.5 ml with 95% ethanol. Then, 0.5 ml of the Folin-Ciocalteu reagent (0.25 mol/L) and 1.0 ml of Na_2CO_3 reagent (150 g/L) were added, and the mixture was incubated at room temperature for 30 min. The absorbance rate was measured at 725 nm. and total phenolics were expressed in microgram equivalents of gallic acid per millilitre of the sample. Standard curves were established using various concentrations of gallic acid in water.

DPPH radical-scavenging activity

The DPPH radical-scavenging activity of ginseng yogurt was determined as described by Brand-Williams, *et al.* (1995). Briefly, one ml of $DPPH^{\bullet-}$ solution (0.1 mmol/L) was mixed with yogurt extracts (0.5 ml) dissolved in suitable solvents (absolute or 95% ethanol). The mixture was kept at room temperature for 30 min. and then the absorbance was measured at 517 nm by a SP-2000 UV/V Spectrophotometer using 95% ethanol as a blank. Ginseng extract was used as the positive control and the percentage DPPH· inhibition of the test samples was calculated as:

$$\text{Inhibition } \% = (1 - A/A0) \times 100$$

where A0 is the absorbance at 519 nm of DPPH without sample, and A is the absorbance at 519 nm of the reaction mixture containing DPPH and the sample.

$ABTS^{\bullet+}$ radical cation scavenging activity

The scavenging activity of $ABTS^{\bullet+}$ of ginseng yogurt was measured as described by Re, *et al.* (1999) and Thaiponga, *et al.* (2006) with some modifications. The $ABTS^{\bullet+}$ was produced by mixing 0.35 ml of ABTS diammonium salt (7.4 mmol/L) with 0.35 ml of potassium persulfate (2.6

mmol/L). The mixture was kept in the dark at room temperature for 12 hr to allow completion of radical generation, and then diluted with 95% ethanol (about 1:50) then its absorbance was measured at 734 nm by a spectrophotometer (SP–2000 UV). To determine the scavenging activity, $ABTS^{\bullet+}$ reagent (1.2 ml) was mixed with ginseng yogurt sample or negative control (95% ethanol) (0.3 ml) and the absorbance was measured at 734 nm after 6 min. using 95% ethanol as a blank. The percentage inhibition of the samples was calculated as:

$$\text{Inhibition \%} = (1 - A/A0) \times 100$$

where A0 is the absorbance at 734 nm of the negative control, A is the absorbance at 734 nm of the ginseng yogurt sample. TROLOX, with a final concentration range of 0.002–0.022mg/ml, was used as a standard.

Reducing power assay

The Fe^{3+} reducing power of the ginseng yogurt was measured by incubating the reaction mixture (1 ml) containing the yogurt extract (0.75 ml) in 0.75 ml of phosphate buffer (0.2 mol/L, pH 6.6) and 0.75 ml of potassium ferricyanide (1 g/100 ml water) followed by incubating at 50 °C for 20 min. The reaction was terminated by adding 0.75 ml of TCA solution (10 g/100 ml water) and then centrifuged at 3000xg for 10 min and 1.5 ml of the supernatant was mixed with 0.1 ml ferric chloride (0.1 g/100 ml water), the absorbance was measured at 700 nm (Yen & Chen, 1995).. Higher absorbance of the reaction mixture indicated greater reducing power. The experiment was performed in triplicate.

$OH^{\bullet}$ Radical scavenging activity test using Electron Spins Resonance (ESR) Spectrometry

The ESR spectra were recorded by Electron Spins Resonance (ESR) Spectrometry Bruker-Alex-Sys E-5000, in National Research Centre. The conditions of ESR spectrometry were as follows: Center field, 345.4 (5.0 mT); microwave power, 0.00202148 (W); microwave frequency (9.773 Hz); modulation amplitude, (5 G); modulation frequency, (100 KHz); time constant, (0.5 s); scan time, (1.5 min).. The 5,5′-dimethylpyrroline 1-N-oxide (DMPO) was used as a spin-trapping reagent for $OH^{\bullet}$. The Mn^{2+} was used as an external standard to calculate the relative amounts from ESR signal intensity. Twenty microliters of DMPO (1/10 diluted with distilled water (v/v) was mixed with 38 ml of 0.2mM $FeSO_4 \cdot 7H_2O$ and 37 µl of 1mM diethylene triamine penta acetic acid (DTPA). The mixture was stirred with 30 µl of ginseng yogurt solution (dissolved with distilled water or acetone) and 75 ml of 1mM H_2O_2. The solutions were transferred to a capillary tube and placed in the cavity of the ESR spectrometer for measurement. After 5 min, the ESR signal was taken to measure the inhibition of $OH^{\bullet}$ radicals by samples. The inhibition of $OH^{\bullet}$ radical was calculated from the ratio of the peak height of the second signal from the DMPO-OH spin adduct to the signal of Mn2+ and compared to the control ratio. In addition, (--)-epigallocatechin 3-O-gallate (EGCg) was used as an $OH^{\bullet}$-scavenging positive control (Yoshioka, *et al.* 2001). The ginseng yogurt samples used in this test were (0.0, 0.5 and 1.0 mg ginseng/ml yogurt). The resultant spectra were recorded on an ESR electron spin Bruker-Alex-Sys E-5000 operated at x-band frequency.

Statistical Analysis

Data of antioxidant capacity and quality characteristics were statistically analyzed using analysis of variance (SAS, 1988). Standard errors of the means of 3 replicates were derived from the error mean square term of the ANOVA. The least significant difference test (LSD) was used to test differences between means ($P \leq 0.05$).

RESULTS AND DISCUSSION

Evaluating the quality characteristics of ginseng yogurt

Table (1) shows that ginseng had no significant effect ($P \leq 0.05$) on the pH value and acidity at all ginseng concentrations used. Apparent viscosity significantly increased ($P \leq 0.05$) with elevating the ginseng extract concentration in yogurt. In addition, the colour of ginseng yogurt was more surprising to the participants in sensory evaluation that it started from light yellowish with low ginseng concentration to redness with higher concentration but finally evaluation had recorded significant acceptability ($P < 0.05$) to the yogurt (0.5 mg ginseng/ml yogurt) as compared to plain yogurt (control).

Antioxidant activity

Phenolic compounds of ginseng are maltol, salicylic acid, vanillic acid, and *p*-coumaric acid, known as principal antioxidant components of ginseng (Kang, *et al.* 2006a). Total phenolic content of ginseng yogurt was found to increase significantly

Table 1: Quality characteristics of ginseng yogurt containing different ginseng concentrations

Yogurt	pH	Titratable acidity	Apparent viscosity	Colour	Flavour	Overall acceptability
Plain Yog. without gin. (control)	4.45± 0.01^{a}	0.72±0.01^{a}	42±0.66^{d}	8.3±0.52^{a}	8.0±0.09^{a}	7.9±0.27^{a}
Gin.Yog 0.25 mg G/ml Y	4.46±0.01^{a}	0.72±0.01^{a}	44±0.36^{c}	7.9±0.39^{a}	7.9±0.15^{a}	7.8±0.05^{ab}
Gin.Yog 0.50 mg G/ml Y	4.45±0.01^{a}	0.72±0.01^{a}	46±0.39^{b}	8.1±0.44^{a}	7.9±0.01^{a}	8.0±0.03^{a}
Gin.Yog 1.0 mg G/ml Y	4.46±0.01^{a}	0.72±0.0^{a}	50±0.44^{a}	7.2±0.65^{a}	7.4±0.11^{b}	7.7±0.01^{b}

1-9-point hedonic scale was used with (1) = dislike extremely and (9) = like extremely. Means within a column not followed by a dissimilar letter are significantly different (P≤0.05).
All values are mean ± SD of three replications

with elevation the concentration of ginseng in yogurt as shown in Table (2). Yogurt containing ginseng 1% had the highest total phenolic contents.

DPPH$^{\bullet-}$ and ABTS$^{\bullet+}$ scavenging radicals activity of ginseng yogurt significantly (P≤0.05) increased with increasing the concentration of ginseng extract (Table 2). The radical scavenging activities of ginseng are related to maltol, salicylic acid, vanillic acid and *p*-coumaric acid, these 4 phenolic compounds in ginseng (Kang, *et al.* 2006-a). Phenolic acids in ginseng have been suggested as active free radical scavenging components (Kang, *et al.* 2006-b; Kang, *et al.* 2007). Data obtained showed a strong positive correlation between the scavenging activity for both DPPH and ABTS values and phenolic content as r2 = 0.932 and 0.913, respectively. Concerning the reducing power assay, the presence of ginseng in the yogurt samples would result in the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form Fe^{2+}. The amount of Fe^{2+} complex was detected by measuring the formation of Perl's prussian blue at 700 nm. Significant variation in the reducing power of ginseng yogurt was found and the variation might be due to sample ginseng concentrations. The results indicated that increasing ginseng concentration delayed the decline of reducing power as confirmed by Kim, *et al.* (2002).

The ESR-spin trapping technique

Hydroxyl radical is known as an active oxygen species which reacts immediately with DNA (Halliwell & Aruoma, 1991). Therefore, great effort has been made to search for safe and effective natural antioxidants which can scavenge these radicals. In the present study, the hydroxyl radical (OH$^{\bullet}$) scavenging activity changes of ginsengs were investigated. The ESR is thought to be a versatile tool to detect free radicals even with slightly insoluble ginsenosides in suspension. Hydroxyl radical scavenging capacity estimation for lipophilic antioxidants is a challenge due to their poor solubility in aqueous radical generating and measuring systems. In addition, the OH$^{\bullet}$ scavenging activity test using ESR is thought to be the most appropriate to test the antioxidant activities of ginsenosides for the following reasons: OH$^{\bullet}$ has been considered to play

Table 2: Total phenolic content and antioxidant activity of ginseng yogurt containing different ginseng concentrations

Antioxidant activity	Ginseng Yogurt			
	Plain Yog. without gin. (control)	Gin.Yog. 0.25 mg G/ ml Y	Gin.Yog. 0.50 mg G/ ml Y	Gin.Yog. 1.0 mg G/ ml
Total phenolic content (mg GAE/100 ml)	306±0.78^{d}	402±0.54^{c}	431±0.84^{b}	444±0.47^{a}
DPPH radical scavenging activity %	45.07±0.91^{d}	73.18±0.68^{c}	78.24±0.08^{b}	84.46±1.57^{a}
ABTS +. Radical scavenging activity %	14.85±0.60^{d}	24.55±0.40^{c}	27.95±1.00^{b}	37.55±0.95^{a}
Reducing Power (O.D)	0.116±0.004^{d}	0.202±0.003^{c}	0.274±0.002^{b}	0.308±0.003^{a}

All values are mean ± SD of three replications. Means within a row not followed by a dissimilar (P≤0.05).
Gin : Ginseng, Yog: Yogurt.

a direct or indirect role in several diseases, therefore, $OH^{\bullet}$ was suggested to be an excellent target to investigate antioxidants in the fields of food, nutrition, health, and medicines (Cheng, *et al.* 2002; Moore, *et al.* 2006).

The radical scavenging abilities of ginseng in ginseng yogurt (0.5 and 1.0% ginseng) was calculated via double integration area, as the degree of quenching of radical anion (I ESR) and results indicated that radical scavenging activity for both were I ESR = 45% and 38.8%, respectively. Antioxidan activity was determined as an inhibition percentage (%) of the intensity of the hydroxyl dependent ESR signal. It was obvious that as the antioxidant activity increased as the ESR signals of stable radicals decreased or disappeared as shown in Fig. (1). The $OH^{\bullet}$ scavenging activities of ginseng extracts were the highest at 0.05% ginseng. Although the increase in ginseng concentration was highly correlated to radical scavenging ability towards PDDH and ABTS, obtained data showed an opposite trend with OH scavenging activities.

CONCLUSIONS

Ginseng skimmed yogurt at the concentration of 0.5 mg ginseng/ml yogurt was the most acceptable concentration. It exhibited the most suitable scavenging activities towards DPPH, ABTS, hydroxyl radical scavenging and ferrous ion chelating activity. This product also had the most suitable physical quality. Ginseng yogurt (0.5 mg ginseng/ml yogurt), is a new functional yogurt supplemented with ginseng can be considered as a dietary supplement which is rich in antioxidants that prevent illness but not intended to diagnose, treat, cure or prevent any disease.

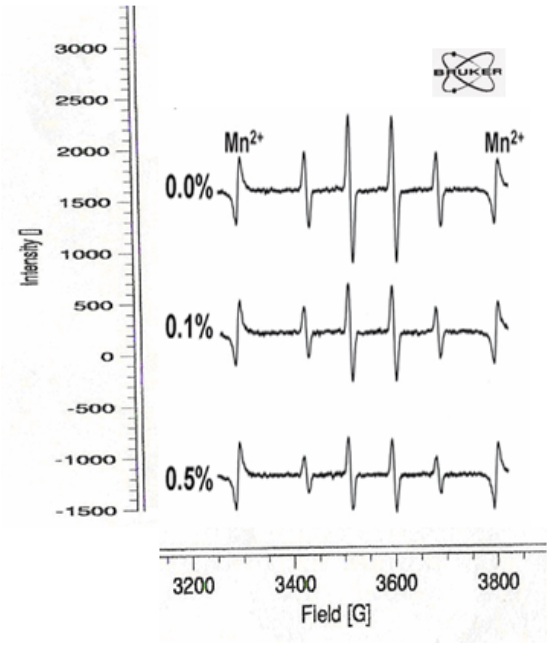

Fig. 1: ESR Spectra of the $OH^{\bullet}$ scavenging activities of ginseng yogurt containing 0.0, 0.5 and 1.0% mg ginseng/ml yogurt

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زبادي وظيفى جديد مدعم بالجينسنج

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أصبح انتاج زبادى مبتكر مدعم بأحد المكونات المفيدة فى مجال الغذاء هدفا يستحوذ على اهتمام المستهلك من منظور الصحة. و فى هذه الدراسة تم استخدم المستخلص المائى للجنسنج فى صناعة الزبادى بدرجات تركيز مختلفة (٠,٢٥، ٠,٥٠، ١,٠ ميلليجرام جينسينج لكل ١ مل زبادى) بهدف الوصول لأفضل تركيز من خلال عدة اختبارات والتىا شتملت على الخواص الطبيعية المميزة للزبادى الناتج،كذلك تقييم المنتج المحتوى على الجينسنج كمصدر طبيعى لمضادات الاكسدة باستخدام اختبارات متعددة٠أوضحت النتائج أن الزبادى المدعم بالجنسنج بنسبة ٥ , ملليجرام جنسنج لكل مل زبادى هو الذى حقق أفضل تقييم ومن ثم فإنه يوصى باستخدامه لما للجينسنج من فائدة لاحتوائه على مضادات أكسدة طبيعية تقوى المناعة الطبيعية للجسم كما أنه يمد الجسم بالنشاط عند قيام الإنسان بمجهود شاق.