pISSN 1225-4339 eISSN 2287-4992
Korean J. Food Nutr. Vol. 33. No. 4, 372~380 (2020)
https://doi.org/10.9799/ksfan.2020.33.4.372

THE KOREAN JOURNAL OF
한국식품영양학회지
FOOD AND NUTRITION

Quality Characteristics of Jelly Made from Fermented Red Ginseng Concentrate with Increased Ginsenoside Content by Enzyme Treatment

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Abstract

The purpose of this study is to investigate the physicochemical properties of jelly made from fermented red ginseng concentrate (FRGC) that can be easily absorbed and digested for the health promotion of the elderly. The pH of the jellies tended to decrease with increasing concentration of FRGC. Soluble solid content has significantly higher value when added more than 2%, and the water content of the sample was significantly lower when the FRGC was added 4%. As the amount of FRGC was increased, the total color difference increased, and the hardness of samples decreased significantly. On the other hand, the total ginsenoside contents of the FRGC was 45.50 mg/g. As the concentration of FRGC increased, the content of polyphenol and flavonoids increased. The increasing pattern of polyphenols and flavonoids showed a similar trend. As the content of FRGC increased, ABTS free radical scavenging activity significantly increased (p<0.05), and in the control, the minimum value (62.6 AEAC) and the 4% sample were highest (116.2 AEAC). DPPH radical scavenging activity was like that of ABTS radical scavenging activity. However, there was no significant difference in DPPH radical scavenging activity of 3% and 4% red ginseng jelly.

Key words: fermented red ginseng, jelly, ginsenoside metabolites

Introduction

In recent years, the elderly population has increased greatly due to income improvement and the development of health care technology, but the incidence of lifestyle-related diseases such as diabetes, circulatory system disease, and hypertension is increasing due to dietary changes and lack of exercise. Due to this change in life patterns, many people are very interested in health functional food. In particular, various researches are being conducted to extract bioactive substances and apply them to foods.

Jelly is a semi-solid food that is molded and solidified by mixing sugar and gelling agent in fruit juice and is made according to various gelling agents such as pectin, agar, and gelatin. It should contain minimum 65 percent of total soluble solids and minimum 45 percent of fruit portion (Saha & Bhattacharya 2010). It is a food that is attracting attention as a food for infants and the elderly because it has a good feeling in the mouth, high preference, easy to chew and swallow. Jelly is not only easy to adjust the utritional ingredients but also has the advantage that it can give a variety of physiological functions by adding functional food materials.

The efficacy of Panax ginseng CA. Meyer is highly recognized and trusted based on the clinical records of traditional medicine (Wu et al. 1992; Sato et al. 1994; Mochizuki et al. 1995). The ginsenosides content, known as ginseng's active ingredient, is reported to be 4~10%. These ginsenosides are an important class of physiologically active compounds that are found in many herbs, which possess anti-inflammatory activity and antitumor activity such as the inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis. Ginsenosides, known as the main active ingredient, have a polar structure with glycosides combined with sugar components, so it is difficult to digest in the gastrointestinal or duodenum and is

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mainly absorbed in the form of low polar metabolites through the metabolism of intestinal microorganisms in the small intestine. After ingestion of red ginseng ginsenosides, the absorption rate is increased only when the ginsenoside-Rb1, -Rb2 and -Rc are metabolized into metabolites such as 20-O-β-glucopyranosyl-20(S)-protopanadiol or ginsenoside-Rg3 by human intestinal bacteria (Kanaoka M 1994; Mochizuki et al. 1995; Wakabayashi et al. 1997; Bae et al. 2002). In addition, physiological are also expressed in the body. In order to overcome the difference in the distribution of intestinal microorganisms, it would be desirable to use fermented red ginseng that undergoes a pre-fermentation process using intestinal microorganisms.

Aging is a biological process that is irreversible, predictable, and inevitably progresses to death and is universal to all people. In particular, elderly people are weakened teeth, dentures cannot eat hard (Kim et al., 2010). To improve the health of the elderly, there is an urgent need for the development of chewable and easy-to-swallow foods with good body absorption and enhanced immunity. Therefore, in this study, fermented red ginseng jelly was prepared with fermented red ginseng concentrate containing ginsenosides that was easily digested and absorbed for the health promotion of the elderly.

Materials and Methods

1. Materials

The FRG and NFRG were gifted from BTC Co. (FRG, BTC Co., Ltd, Ansan, Korea). Standard ginsenosides, including the compounds Rg1, Re, Rf, Rh1, Rg2, Rb1, Rc, Rb2, Rd, Rg3, F2, CK, Rk1, Rg5, and Rh2, were purchased from Embo Laboratory (Ginsenosides, Embo Laboratory, Daejeon, Korea). Gelatin was purchased from Gel-tech (Gelatin, Gel-Tech Co., Ltd, Busan, Korea). The other chemicals were of reagent grade and obtained from local suppliers.

2. HPLC analysis of ginsenosides

Each FRG and NFRG formulation (approx. 10 mg) was accurately weighed and dissolved in 3 mL of methanol. After extraction in an ultrasonic bath for 15 min, the samples were transferred to centrifuge tubes and centrifuged at 4,000 rpm for 10 min. The supernatants were collected and the residues were extracted two more times by the same procedure. The supernatants obtained from these three extractions were combined in a vial and evaporated slowly to dryness under a flow of pure nitrogen gas. The residue was reconstituted with 2 mL of water and applied to an SPE C18 cartridge for sample clean-up. The levels of 16 major ginsenosides were analyzed using an HPLC-based technique developed by Lee et al. (Lou et al. 2005; Lee et al. 2009). A Varian Prostar 200 HPLC system (Varian Prostar 200 HPLC system, Varian Inc., Palo Alto, CA, USA) equipped with a quaternary solvent delivery system, an autosampler, and UV detector was used. The column configuration consisted of an IMtakt Cadenza CD-C18 (4.6×75 mm, Imtakt Corporation, Kyoto, Japan). UV absorption was measured at 203 nm. Gradient elution was employed using solvent A (10% acetonitrile) and solvent B (90% acetonitrile) at 40℃; the gradient program was as follows: 0→11 min, 11% B (isocratic); 11→15 min, 11→16% B; 15→16 min, 16→20% B; 16→18 min, 20→21%; 18→24 min, 21% B (isocratic); 24→25 min, 21→22% B; 25→35 min, 22%B (isocratic); 35→36 min, 22→23% B; 36→40 min, 23% B (isocratic); 40→41 min, 23→24%; 41→45 min, 24% B (isocratic); 45→53 min, 24→37% B; 53→61 min, 37→45% B; 61→66 min, 45→46%; 66→73 min, 46→48% B; 73→75 min, 48% B (isocratic); 75→77 min, 48→11%; 77→85 min, 11% B (isocratic). The flow rate was kept at 1.3 mL/min and the sample injection volume was 5 μL. The level of total ginsenosides was determined by the sum of the 15 ginsenosides. Fig. 1 shows the HPLC chromatograms of the 15 standard ginsenosides.

3. Jelly formulation and preparation

Fermented red ginseng concentrate used in this experiment was purchased from BTC Co., Ltd. (FRG, BTC Co., Ltd, Ansan, Korea). The formulation of the fermented red ginseng jelly was determined as shown in Table 1 after preliminary experiment with reference to the preparation of purple sweet potato jelly (Choi & Lee 2013), and the control was not added fermented red ginseng concentrate. After dissolving sucrose in 200 mL of distilled water, put it in a pot and heat it to reach 80℃. Then, add gelatin and fermented red ginseng concentrate dissolved in 100 mL of

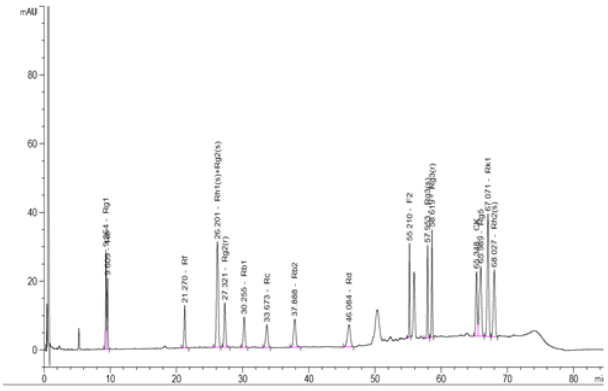

Fig. 1. Chromatogram of standard ginsenosides by HPLC assay. The column configuration consisted of an IMtakt Cadenza CD-C18 (4.6×75 mm). UV absorption was measured at 203 nm. Gradient elution was employed using solvent A (10% acetonitrile) and solvent B (90% acetonitrile) at 40℃.

Table 1. Formulation of jelly prepared with fermented red ginseng concentrate (FRGC)

Ingredient	Fermented red ginseng concentrate (FRGC)				
	0%	1%	2%	3%	4%
FRGC (g)	0	3	6	9	12
Gelatin (g)	11.25	11.25	11.25	11.25	11.25
Sugar (g)	37.5	37.5	37.5	37.5	37.5
Water (mL)	300	297	294	291	288

distilled water and boil it for about 2 minutes to dissolve sucrose, gelatin and fermented red ginseng concentrate. They were placed in a mold of constant size. After cooling at room temperature for 30 minutes, the mixture was allowed to stand for 3 hours in a 4℃ refrigerator and used as an experimental sample.

4. Determination of pH, water content, and soluble solids content

Fermented red ginseng concentrate (10 g) were mixed with 90 mL of water and vortexed for 1 min. The mixture was kept at room temperature for 1 h to separate solid and liquid phases. After carefully removing the supernatant layer, the pH was measured as 5 times using a pH meter (HI 2215, Hanna Instruments Inc., RI, USA) and then expressed as an average value. Also, the soluble solids content (°Brix) was measured using a refractometer (PR-101, ATAGO Co., Tokyo, Japan). The water content of fermented red ginseng jelly was determined by 5 times each gravimetrically by drying at 105℃ for 4 h.

5. Color and appearance of the jelly

The surface color of the jellies was measured using a Minolta model CR-400 chromameter (CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The chromameter was positioned in the center of the jelly top surface, and brightness value (L), red to green value (a), and yellow to blue value (b) were determined. Standard white tile was used to calibrate the color measurement instrument. Measurements were conducted in 5 times, and the considered results were mean values. Digital camera (Fine Pix 2500Z, Fujifilm, Tokyo, Japan) were used to compare the appearance chromaticity. In the same place and lighting, the height between the sample and the camera were kept constant and the flash was not operated.

6. Hardness of jelly

The hardness of jelly was determined by force required to compress the jellies to deformation rate of 25% and the measurement was measured 15 times at room temperature. The force obtained was defined as the hardness of jellies. This was done using the Rheometer (Compac-100, Sun Scientific Co., Ltd, Tokyo, Japan) fitted with a load cell of 2 kg, probe of diameter 40.0 mm, test speed 120.0 mm/min, and deformation rate of 25% for the sample of 3×3×3 cm^3.

7. Assay of polyphenols and flavonoids in jelly

The jelly (10 g) prepared by varying the amount of fermented red ginseng concentrate was mixed with 50 mL of 70% ethanol, homogenized, and extracted at room temperature for 1 h. After extraction, the supernatant obtained by centrifugation at 8,000 rpm for 10 minutes using a centrifuge (VS-21SMT, Vision Scientific Co., Ltd, Gyeonggi, Korea) was used for the assay of polyphenols and flavonoids, and radical scavenging activity.

The total phenolic compound (TP) and total flavonoid (TF) contents of samples were determined by employing Folin-Ciocalteu and p-dimethylaminocinnamaldehyde reagents, respectively, using protocols reported elsewhere (Arnous et al. 2002). The results for TP and TF were expressed as gallic acid equivalents and catechin equivalents, respectively.

8. DPPH and ABTS radical scavenging activity

The radical scavenging ability for 2,2-diphenyl-1-picryl-hydrazyl (DPPH; Wako Pure Chemical Industries, Ltd, Osaka, Japan) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS; Sigma-Aldrich Co., LLC., St. Louis, MO, USA) were measured by the method of Blois MS (1958) and Re et al. (1999), respectively. Radical scavenging capacity was expressed as AEAC (L-ascorbic acid equivalent antioxidant capacity, mg AA eq/g).

9. Statistical analysis

Analysis of the data was performed using SPSS program (Statistics Package for Social Science, Ver. 20.0). To analyze the difference in the averages of samples, one-way ANOVA was conducted, and significant difference was determined by Duncan's multiple range tests at the level of $p<0.05$.

Result and Discussion

1. Ginsenoside in fermented red ginseng concentrate (FRGC)

The ginsenoside composition of FRGC was assayed using HPLC (Table 2). The total ginsenoside contents of the FRGC was 45.50 mg/g. The major ginsenosides of red ginseng, Rb1, Rg1and RG3, were 5.48, 1.00 and 6.54 mg/g in FRGC, respectively. The sum of Rg1, Rb1 and Rg3 should satisfy 2.5 to 80 mg/g for functional foods. Ginsenosides in FRGC was found to meet this condition. Ginsenosides are classified into the following categories according to their chemical constitutions: protopanaxadiols (PPD), protopanaxatriols (PPT), and oleanolic acids, and more than 40 ginsenoside variants have been reported. Among them, six major ginsenosides, including Rb1, Rb2, Rc, Rd, Re, and Rg1, account for 90% (w/w) of the total ginsenosides in white and red ginseng (Kanaoka M 1994; Park J 2004; Sonavane et al. 2008).

Table 2. Ginsenosides of fermented red ginseng concentrate (FRGC)

Ginsenoside (mg/g)	FRGC	Ginsenoside (mg/g)	FRGC
Rg1	1.00±0.12	Rb2	4.57±0.54
Re	2.51±0.30	Rd	4.16±0.31
Rf	1.50±0.17	Rg3	6.54±0.78
Rh+Rg2(s)	2.79±0.27	F2	0.063±0.003
Rg2®	1.33±0.10	CK	0.066±0.02
Rb1	5.48±0.72	Rk1	5.39±0.64
Rc	5.08±0.55	Rg5	5.03±0.61
Rg1+Rb1+Rg3		13.02±1.62	
Total		45.50±5.13	

With the development of new methods for ginsenoside isolation along with better ginseng processing technologies, various minor active ginsenosides have been discovered. Recently, several investigators have reported that Rb1, Rb2, and Rc are metabolized by intestinal bacteria in rats and humans after oral administration, and that the main metabolite of PPD-type ginsenoside is CK. The metabolic pathways of conversion for these three ginsenosides by intestinal bacteria are as follows: Rb1→Rd→F2→CK; Rb2→CO→CY→CK; and Rc→Mc1→Mc→CK. The resulting CK has been shown to inhibit lung metastasis of melanoma cells and *in vitro* tumor cell invasion and migration at nontoxic or marginally toxic concentrations.

2. pH, water content, and soluble solid content

The pH, soluble solids content, and water content of jelly added with fermented red ginseng concentrate are shown in Table 3. The pH of the jellies tended to decrease with increasing concentration of fermented red ginseng concentrate (Table 3). The highest value was 5.82 in the control and the lowest value was 5.12 in the sample containing 4% fermented red ginseng concentrate. Soluble solid content has significantly higher value when added more than 2% and showed maximum value (1.30°Brix) in samples added 3% and 4%. On the other hand, the water content of the sample was significantly lower than the other samples when the fermented red ginseng concentrate was added 4%. The water content of individual jellies ranged from 82.60 to 83.97%.

pH decreased with increasing amount of red ginseng extract (Table 3). This may be due to the presence of organic acids such as citric acid, malonic acid, succinic acid, oxalic acid and malic acid in the extracts of red ginseng and ginseng (Kim et al. 1998; Bae & Nam 2006). In addition, the characteristics of mixed fermented milk added with red ginseng extract were reported to decrease in pH as the amount of red ginseng extract was increased (Bae & Nam 2006).

Table 3. pH, soluble solids content, and water content of jelly incorporated with FRGC

	Fermented red ginseng concentrate (FRGC) (%)				
	0	1	2	3	4
pH	5.82±0.03^{a}	5.51±0.01^{b}	5.32±0.01^{c}	5.21±0.00^{d}	5.12±0.01^{e}
Soluble solids content (°Brix)	0.80±0.00^{b}	0.84±0.05^{b}	1.26±0.05^{a}	1.30±0.00^{a}	1.30±0.00^{a}
Water content (%)	83.87±0.25^{ab}	83.97±0.32^{a}	83.10±0.44^{ab}	82.87±0.85^{ab}	82.60±1.10^{b}

Values are the means ± standard deviation (SD) for each group. Different letters indicate significant differences at $p<0.05$ by Duncan's multiple range tests.

3. Color and appearance of jellies

The L, a, and b color values of the samples are shown in Table 4. The color of the jellies is one of the characteristics that is perceived by the consumers and affects their preference and acceptability. The top surface color of the jellies was influenced by fermented red ginseng concentrate (FRGC) (Table 4). For the top surface, the control (0% FRGC) had the lightest color, and the mean L value was 26.42, which was significantly higher ($p<0.05$) than the mean values (15.21~17.86) of jellies produced with fermented red ginseng concentrate. The lowest value of 15.21 was showed in the sample added with 4%. Lower L values mean darker surface color. In the case of redness (a value), the concentration of fermented red ginseng concentrate, except the control, showed a tendency to decrease significantly as the addition concentration was increased. Total color difference (△E) was 8.90 with 1% red ginseng extract, 10.56 with 2%, 11.32 with 3% and 11.80 with 4% compared to control. There was no significant difference in total color difference between 3% and 4% red ginseng extracts. However, as the amount of red ginseng added increased, the total color difference increased (Table 4). On the other hand, the appearance of jelly prepared by adding fermented red ginseng concentrate is shown in Fig. 2. As the addition concentration of fermented red ginseng concentrate increased, it was found that the color became distinctly dark.

This is similar to the result of the increase in color with red ginseng as shown in the study on the preparation of Dasik using red ginseng powder (Yun & Kim 2006; Ku & Choi 2009). In addition, the total color difference also

Table 4. Color L, a, and b values of jellies incorporated with fermented red ginseng concentrate

Property	Fermented red ginseng concentrate (FRGC) (%)				
	0	1	2	3	4
L value	26.42±0.83^{a}	17.86±0.72^{b}	16.25±0.57^{c}	15.60±0.35^{cd}	15.21±0.36^{d}
a value	1.00±0.15^{c}	3.13±0.09^{a}	1.75±0.29^{b}	0.85±0.13^{c}	0.44±0.04^{d}
b value	5.63±0.30^{a}	4.57±0.17^{b}	2.92±0.16^{c}	2.33±0.06^{d}	2.00±0.01^{e}
△E	-	8.90±1.09^{c}	10.56±0.70^{b}	11.32±0.80^{ab}	11.80±0.62^{a}

$\Delta E=\sqrt{(\Delta L)^2+(\Delta a)^2+(\Delta b)^2}$, Values are the means ± standard deviation (SD) for each group. Different letters indicate significant differences at $p<0.05$ by Duncan's multiple range tests.

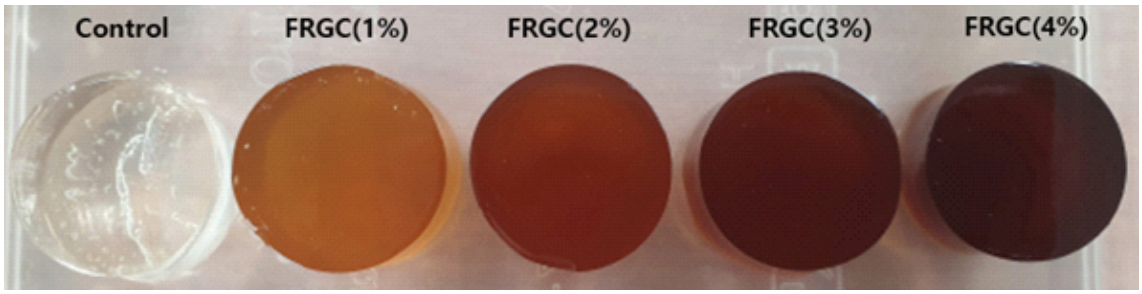

Fig. 2. Photographs of jellies prepared with various contents of fermented red ginseng concentrate (FRGC).

increased with the increase of red ginseng extract during sweet jelly (Yanggaeng) preparation (Ku & Choi 2009) also reported that the increase in color difference due to the addition of red ginseng was due to the increase in lightness difference (△L) and redness difference (△a) but decrease in yellowness difference (△b). However, unlike the Yanggaeng, the total color difference (△E) due to the increase of red ginseng was increased due to the increase in lightness difference (△L), redness difference (△a) and yellowness difference (△b).

4. Hardness of jellies

Hardness of the jellies obtained using the fermented red ginseng concentrate was measured using a rheometer, and the results are shown in Fig. 3. Hardness is the force required to compress up to a defined deformation rate of sample on the first compression. In general, as the fermented red ginseng concentrate increased, the hardness decreased significantly. The maximum value (110.88g-force/cm^2) was shown in the control, and the lowest value (67.85 g-force/cm^2) in the sample added 4%. These results are thought to be due to the weakening of the structure of the jelly as the fermented red ginseng concentrate is added.

The hardness of red ginseng Yanggaeng with red ginseng extract increased as compared to the control. This was because the water content of the red ginseng extract was increased compared to the control. The increase in hardness was judged to be due to the low water content with increasing red ginseng extract concentration (Ku & Choi 2009). However, the hardness of the jelly was decreased as the amount of red ginseng extract increased, unlike that of Yanggaeng. This may be due to the difference between the gel forming agent used in Yanggaeng and jelly manufacturing.

In preparation jelly, the side-chain of gelatin molecules could adhere with the main ingredient in the gels by covalent bonds. The higher solids/sucrose content in the gels afford faster network development during cooling because of sufficient hydrophobic interaction shift the adjacent gelatin portion (Tau & Gunasekaran 2016) which could explain their higher intensity of toughness, stickiness and hardness. Therefore, the addition of red ginseng extract seems to reduce the hardness by preventing the hydrophobic binding of gelatin.

5. Polyphenol and flavonoid contents of jelly added with red ginseng extract

Padayatty et al. (2003) reported that the total phenolic content of flavonoids, phenolic acids, and anthocyanins act as a major factor with free radical scavenging ability. Therefore, the total phenolic and flavonoid contents of jelly prepared with red ginseng extract were measured and the results are shown in Fig. 4. As the concentration of red ginseng extract increased, the content of polyphenol and flavonoids increased. The increasing pattern of polyphenols

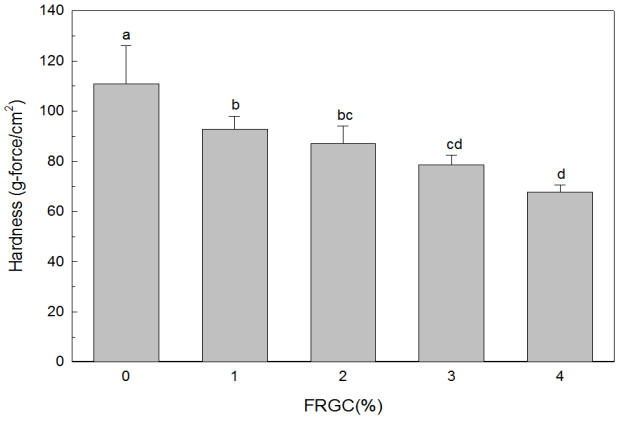

Fig. 3. Hardness of the jellies produced with various contents of fermented red ginseng concentrate (FRGC). Values are the means±standard deviation (SD) for each group. Different letters indicate significant differences at $p<0.05$ by Duncan's multiple range tests.

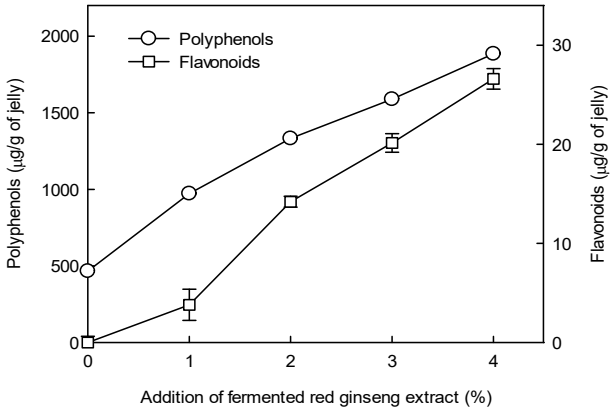

Fig. 4. Contents of polyphenols and flavonoids of the jellies produced with various contents of fermented red ginseng concentrate (FRGC). Values are the means±standard deviation (SD) for each group.

and flavonoids showed a similar trend.

Phenolic compound is a secondary metabolite widely distributed in the plant system (Im & Suh 2009). Because of the phenolic hydroxyl group, phenolic compound has a property of binding to macromolecules such as proteins and enzyme proteins, binding ability to divalent metal ions, and high antioxidant effect (Kwak et al. 2010). Flavonoids are a large group of natural phenolic compounds, consisting mainly of flavonols, flavanols and anthocyanidins (Van Acker et al. 1996). Red ginseng extracts containing flavonoids and polyphenols are important antioxidants. Therefore, it is considered that the bioactive function can be improved by addition of red ginseng extract in the preparation of jelly.

6. Radical scavenging activities of jelly added with red ginseng extract

The radical scavenging activities of jelly prepared with red ginseng extract. ABTS free radical scavenging activity of control was 62.6 AEAC, 1% red ginseng jelly was 70.0 AEAC, 2% red ginseng jelly was 73.0 AEAC, 3% red ginseng jelly was 97.6 AEAC and 4% red ginseng jelly was 116.2 AEAC. ABTS radical scavenging activity increased significantly as the content of red ginseng extract increased ($p<0.05$). The 4% red ginseng jelly with the highest AEAC value was expressed as 116.2 μg ascorbic acid equivalent per g jelly (AEAC), which can be interpreted as having the same antioxidant power as 116.2 μg of ascorbic acid per g of jelly. DPPH radical scavenging activity was similar to that of ABTS radical scavenging activity. However, there was no significant difference in DPPH radical scavenging activity of 3% and 4% red ginseng jelly.

The phenolic compound is a representative compound having a radical scavenging activity, and it is thought that the radical scavenging activity was increased due to the increase of the phenolic compound due to the heat treatment (Dewanto et al. 2002; Turkmen et al. 2005; Choi et al. 2006). In addition, it is thought that the antioxidative effect was increased by forming a substance having antioxidant activity by the Maillard reaction during the heat treatment for the preparation of red ginseng or jelly (Manzocco et al. 2000). Radical scavenging activity and phenolic compounds (polyphenols and flavonoids) content are said to have a positive relationship (Gheldof & Engeseth 2002).

According to the above results, the addition of 3% red ginseng is most suitable as it shows excellent activity not only in jelly properties and sensory evaluation but also in radical scavenging ability. The addition of red ginseng to impart functionality to the jelly will enable the development of new products that can impart physiological activity as well as organoleptic.

Conclusion

Red ginseng was found to have various effects on pH, color, appearance and hardness depending on the concentration. The pH of the jellies tended to decrease with increasing concentration of fermented red ginseng concentrate (FRGC). As the amount of FRGC was increased, the color of samples became distinctly dark and the total color difference increased, and the hardness of samples decreased significantly. The polyphenols and flavonoids of the prepared jelly showed high concentrations in proportion to the concentration of red ginseng. The commercial product of jelly made using fermented red ginseng concentrate was found to be most suitable for the preparation of jelly using fermented red ginseng concentrate at 3% concentration.

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Received 21 March, 2020
Revised 13 June, 2020
Accepted 24 July, 2020