Food ©2009 Global Science Books

Changes in Concentrations of Ginsenosides and Free Amino Acids in Ginseng and Ginseng Solution during the *Jung Kwa* Process

Ka-Soon Lee[1*] • Gwan-Hou Kim[1] • Hyun-Ho Kim[1] • Mi-Ran Song[2] • Mee-Ree Kim[2]

[1] Geumsan Ginseng and Medicinal Crop Experimental Station, Chuncheongnam-do Agricultural Research and Extension Services, Geumsan 312-804, Korea
[2] Department of Food and Nutrition, Chungnam National University, Daejeon 305-764, Korea

Corresponding author: * **lkasn@korea.kr**

ABSTRACT

This study was carried out to investigate the quality characteristics of *Jung Kwa* ginseng (JKG) and *Jung Kwa* ginseng solution (JKGS) prepared according to the boiling frequency of the *Jung Kwa* Process. JKG was made as follows: 8 kg of washed ginseng was boiled for 5 min with 16 L water from which 8 L boiling water was removed. 10 kg sucrose was added to the remaining boiled ginseng soaking in hot water. JKG was boiled for 60 min in sugar syrup and soaked for 24 hrs repeating the boiling process 5 times. After the final process the crude saponin content of Jung Kwa (JKG 6) increased 4-fold more, while the ginsenoside Rf and Rd contents increased 77- and 16-fold more than raw ginseng, respectively. The content of crude saponin in the second last JKG solution (JKGS 5) was 61.88 mg/g. Others ginsenosides in JKG decreased. As increasing the number of times boiled, crude protein and total free amino acid contents decreased 84.9 and 94.7% of raw ginseng in JKG but increased 98.2 and 78.9% in JKGS, respectively. Especially, the arginine content of JKG 6 decreased 49.18-fold more than raw ginseng while γ-aminobutyric acid and two unknown compounds, not present in fresh, untreated ginseng, were formed as intermediate products during *Jung Kwa* processing. The γ-aminobutyric acid content was 34.13 mg/100 g in JKG 6. In addition, unknown compound 1 was formed more than unknown compound 2 in JKG while unknown compound 2 was formed more than unknown compound 1 in JKGS.

Keywords: crude protein, crude saponin, free amino acid derivatives, ginsenoside-Rd and -Rf, *Jung Kwa* ginseng, Korean ginseng, *Panax* ginseng
Abbreviations: DAD, diode array detector; **ELSD**, evaporative light scattering detector; **JKG**, *Jung Kwa* ginseng; **JKGS**, *Jung Kwa* ginseng solution; **WG**, washed ginseng

INTRODUCTION

The root of ginseng (*Panax ginseng* C.A. Meyer) is commonly used for traditional medicine in Asian regions, particularly in Korea, especially, but mainly as a food-stuff at present. The most commonly consumed ginseng is fresh (50%), white (30%), or red ginseng and its products. Ginseng roots and their extracts have become increasingly popular around the world as health supplements and additives to foods and beverages. A number of researchers have studied the components of ginseng since 1854, initially with the research of Garriques (1854), whose research group identified the chemical structures of ginsenosides. These ginsenosides have been reported to show the following effects: anti-cancer (Mochizuki *et al.* 1995), anti-diabetic (Yokozawa *et al.* 1985), central nervous system protection (Takagi *et al.* 1972), anti-arteriosclerotic and anti-hypertensive (Jung *et al* 1985; Yoon *et al.* 1993), improvement of liver function and clearing of hangovers (Matsuda *et al.* 1991), anti-fatigue and anti-stress (Saito *et al.* 1974; Wang *et al.* 1983), anti-oxidative (Jeong *et al.* 2002), anti-inflammatory (Matsuda *et al.* 1990), promotion of protein synthesis (Yokozawa *et al.* 1990), and strengthening of immunity (Jie *et al.* 1984). Recently, researchers have been studying ginseng to maximize the benefits of ginseng in food products (Ahn *et al.* 1999; Yoon *et al.* 2005; Paek *et al.* 2006; Lee *et al.* 2008), although processed food using ginseng is very limited (Ryu 2003).

Jung Kwa (JK) is a food made by soaking and boiling in sugar syrup. In particular, *Jung Kwa* ginseng (JKG) is a popular traditional food in Korea since the 1800's (Lee *et al.* 1987). Specifically, JK is made by boiling the root, stem or fruit of plants in a sugar syrup, thus maintaining the plant's original shape. Many studies have been carried out studies on enhancing its textural qualities (Cho *et al*. 1984; Kim *et al.* 1985; Lee *et al*. 2001; Paek *et al.* 2006; Kwon *et al*. 2009), but the chemical properties of JK have not been systematically reported yet. An amino-carbonyl reaction takes place in the process of boiling ginseng in sugar syrup. Ginseng has a high content of a free amino acid, arginine (Lee *et al.* 1978; Rhee *et al.* 1983; Lee *et al.* 2000), thus JK products have many newly emerging components. JKG is one of the ginseng products that is consistently consumed in Korea. In Korea, ginseng production exceeded 21,820 tons in 2007 and ~3000 tons of ginseng and its products were exported, with the amount of traded ginseng products in the Korea being $US 92 million, with JK accounting for 3% of the market in 2007 (Korea Customs Service 2007). To date, only one report of research on JK ginseng's physiological characters has been reported (Kim *et al*. 1990). These authors reported that the rat group treated with JK ginseng showed good nutritional efficiency in the body. So, we analyzed the general composition of JK ginseng (Lee *et al*. 2009) and are studying various fields of JK ginseng and its solution (JKGS).

The chemical composition on JKG and JKGS was previously reported (Lee *et al*. 2009), and this study mainly describes the changes of ginsenosides, free amino acid and its derivatives in JKG and JKGS prepared according to different boiling frequencies in the JK process.

Received: 20 June, 2009. Accepted: 22 August, 2009.

Original Research Paper

MATERIALS AND METHODS

Materials

The roots of 4-year cultivated Korean ginseng (*Panax ginseng* C.A. Meyer) were collected at Geumsan in Korea on October 15, 2008 and were stored at 0 ± 1°C in a refrigerator until the JKG was processed. Standard ginsenosides (99% purity), including Rd (a ginsenoside of protopanaxadiol saponin-type in ginseng, attached -glucose-glucoside bond at C_3 and -glucoside at C_{21} of protopanaxadiol) and Rf (a ginsenoside of protopanaxatriol saponin-type in ginseng, attached -glucose-glucoside bond at C_6 of protopanaxadiol), used in this experiment were purchased from Eugene Science, Daejeon, Korea (Wang *et al*. 1999). Eighteen major, standard amino acids were purchased at 0.25 μmol/mL (Amino Acid Protein Hydrolysate Standard, Pickering Laboratories Inc., USA).

Sample preparation

Intact ginseng roots of good quality were selected, 50 ± 5 g per root (2.5 cm thick and 15 cm long) and thoroughly washed. Fine pinholes to a depth of 0.5 cm were made in washed roots by a bamboo needle at 20 pinholes/root, and were equally spaced.

Preparation of JKG and JKGS

To investigate the quality characteristics of JKG and JKGS prepared according to the boiling frequency of the JKG process (Lee *et al*. 2009). JKG was made as follows: 8 kg of washed ginseng (WG) was boiled for 5 min with 16 L water from which 8 L boiling water was removed. To the remaining boiled ginseng soaking in hot water 10 kg of sucrose was added. JKG was boiled for 60 min in sugar syrup and soaked for 24 hrs. Using this process, JKG was boiled another 4 times and finally was boiled for 60 min in sugar syrup with 350 g fructo-oligo liquid (Samyang Co., Korea, components: solidity >75%, oligosaccharides >50%, sweetness 60% at sucrose level, made from sugar cane) (**Fig. 1**). **Fig. 2** provides a visual image of various products prepared by increasing the number of times JKG was boiled in sugar syrup, in which 10 kg of sucrose was dissolved in 8 L of hot-water.

Wash ginseng (**WG**, 8 kg)

↓ ←Add 16 L water

Boil (5 min)

↓

1st boiled ginseng (**JKG 1**)

↓→ Remove water (8 kg Ginseng *Jung Kwa* solution: **JKGS 1**)

↓← Add sucrose (10 kg)

↓← Boil for 60 min and soak for 24 hr

2nd boiled ginseng (**JKG 2**)

↓ → (**JKGS 2**)

↓← Boil for 60 min and soak for 24 hr

3rd boiled ginseng (**JKG 3**)

↓ → (**JKGS 3**)

↓← Boil for 60 min and soak 24 hr

4th boiled ginseng (**JKG 4**)

↓→ (**JKGS 4**)

↓← Boil for 60 min and soak for 24 hr

5th boiled ginseng (**JKG 5**)

↓← Add fructo-oligo liquid (350 g)

↓← Boil for 60 min and soak for 24 hr

↓→ (**JKGS 5**)

Last boiled ginseng (**JKG 6**)

Fig. 1 Preparation procedure for *Jung Kwa* ginseng.

Determination of crude saponin

At first, JKG was dried and powdered to assess crude saponin content. According to Ando *et al.*'s method (1971), the procedure was

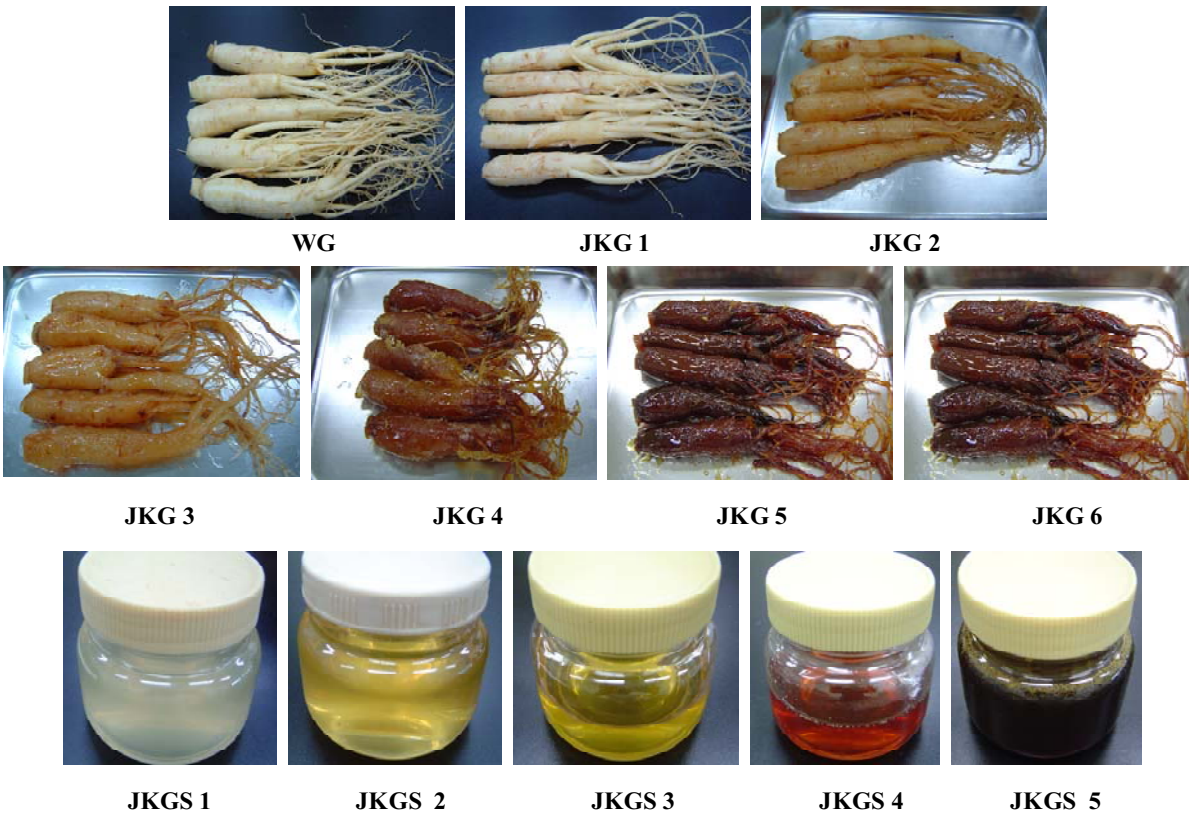

Fig. 2 Picture of *Jung Kwa* ginseng and *Jung Kwa* ginseng solution prepared with increasing the number of times boiled in sugar syrup.

as follows: powdered JKG and JKGS were prepared according to the boiling frequency of the JK process extracted in 70% methanol and then methanol solvent was removed by evaporation. The extract was separated with ethyl ether 3 times, followed by removal of lipid-soluble materials with an ethyl ether phase. The water phase was treated 3 times with water-saturated-*n*-buthanol. The *n*-butanol fraction, which was obtained in a separating funnel, was concentrated to dry stasis below 40°C by a vacuum evaporator (LABORATA 4000, Heidolph, Germany). All processes were performed quantitatively, and the amount of concentrate was equivalent to that of crude saponin.

Analysis of ginsenosides

The level of 12 major ginsenosides (Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rh1 and Rh2) of the concentrate was analyzed by high performance liquid chromatography (HPLC) according to the method of Ko *et al.* (2005): the crude saponin of each sample was dissolved in 50 mL methanol and assessed by HPLC using an Agilent 1200 binary HPLC system (Agilent, USA) with an ELSD (evaporative light scattering detector) detector (Altec 3300, USA). A Prevail Carbohydrate ES column (5 μm, 4.6 × 250 mm, Altech, USA) was also used. A gradient elution system consisting of A (acetonitrile: water: isopropyl alcohol = 80: 5: 15) and B (acetonitrile: water: isopropyl alcohol = 80: 25: 15) was used [25% B (0 min); 85% B (28 min); 100% B (35 min); 25% B (50 min)]. The column temperature was fixed at 35°C using a column oven. The running fluid speed was set at 0.8 mL/min. The chromatogram was generated using the ELSD detector. Peak identifications were based on retention times and comparisons with injected standard ginsenosides. All solutions were filtered through 0.45 μm membrane syringe filters (ADVETEC, Toyo Roshi Kaisha, Ltd, Japan) before analysis.

Determination of crude protein

The crude protein content of JKG and JKGS was obtained by the micro-Kjeldahl method (AOAC 1995). The percent nitrogen was determined and converted to protein using a 6.25 factor using an Automatic Nitrogen Analyzer (VAPODEST 45TL, TT125, C. Gerhart, Germany).

Analysis of free amino acid composition and amino acid derivatives

Each sample (5 g) was extracted with 100 mL of 50% ethanol and centrifuged at 3,000 × *g* for 30 min. 10 mL aliquots of supernatant were condensed using a rotary evaporator at 35°C under reduced pressure. After condensing, 10 mL of 0.2 N-sodium citrate buffer solution (pH 2.2) was added to each evaporated sample. These buffer solutions, which included free amino acid, were filtered by a 0.2 μm membrane filter (Whatman Co., UK). Free amino acids were analyzed by HPLC (Agilent 1200, USA) equipped with a Pinnacle PCX post-column derivatizer (Pickering Laboratories; column temp. 48°C and reactor temp. 130°C) and with a sodium ion-exchange column (3.0 × 250 nm, Pickering Laboratories) and a DAD (diode array detector) detector (Agilent 1200, USA) at 570 nm. A gradient elution system consisting of 0.2 N sodium citrate buffer solution A (pH 3.28) and B (pH 7.40) was used [0% B (0 min); 100% B (32 min); 0% B (58 min)] (Application manual 2003, Pickering Laboratories, Inc). The flow rate was 0.3 mL/min. Amino acids and related compounds were detected by the ninhydrin reaction.

RESULTS AND DISCUSSION

Crude saponin content and ginsenosides of JKG and JKGS: effect of boiling times in sugar syrup

The crude saponin content of JKG and JKGS increased as the number of times boiled in sugar syrup increased (**Fig. 3**). The crude saponin content (233.60 mg/g) of the final JK (JKG 6) was 4-fold higher than crude saponin (54.02 mg/g) of raw ginseng. Rf (33.37 mg/g) and Rd (6.61 mg/g), components of JKG ginsenosides, increased 77- and 16-fold,

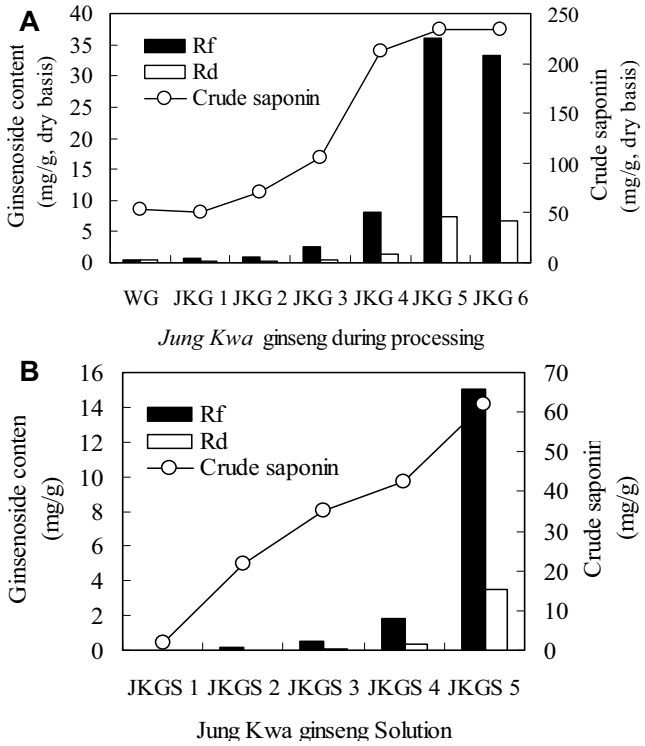

Fig. 3 Concentration of crude saponin, ginsenoside-Rf and -Rd in *Jung Kwa* ginseng (A) and solution (B) prepared by increasing the number of times boiled in sugar syrup.

respectively more than Rf (0.43 mg/g) and Rd (0.41 mg/g) of raw ginseng. Rf and Rd of JKGS were 15.02 and 3.54 mg/g, respectively. The other ginsenosides in JKG decreased (Lee *et al*. 2009). It appears as if the decrease of some ginsenosides is reason for their thermolability. An HPLC chromatogram of standard ginsenosides, JKG 6 and JKGS 5 is shown in **Fig. 4**. Furthermore, unknown peaks appeared in the JKG spectra when ginsenosides were analyzed by HPLC chromatography. These compounds will be studied, including their molecular structure and functionality.

In the crude saponin content of JKG, Kim *et al.* (1985) reported the effect of honey concentration on the quality of honeyed ginseng in its manufacturing process. In that study, the crude saponin content of honeyed ginseng was ~1% of the undried sample. This differs considerably from our result. This difference may be due to the extraction method of saponin. Zhang *et al.* (2006) reported the effect of ethanol concentration on the extraction yield of ginsenoside from *P. quinquefolium* L. roots (American ginseng), in which the extraction yield of ginsenoside was improved by increasing the ethanol concentration by 10-70%. When the ethanol concentration was higher than 70%, the extraction yield of ginsenoside decreased slowly as ethanol concentration increased. Based on these findings, we decided that the alcohol concentration used on ginsenoside extraction should be 70%. Despite the great demand for JKG, there is only one report on JKG' functional characteristics (Kim *et al*, 1990) in which the rat group treated with JK ginseng showed good nutritional efficiency in the body, especially, by decreasing the β–lipoprotein but increasing the HDL-cholesterol levels in plasma. We thus feel that it is worthwhile to research JKG's other functional characteristics.

Crude protein content of JKG and JKGS prepared with the number of boiling times in sugar syrup

The crude protein content of JKG and JKGS as a function of the number of times they were boiled in sugar syrup is shown in **Fig. 5**. In JKG, the crude protein content decreased as the number of times it was boiled in sugar syrup increased. The contents decreased sharply at JKG 2 (4.6%)

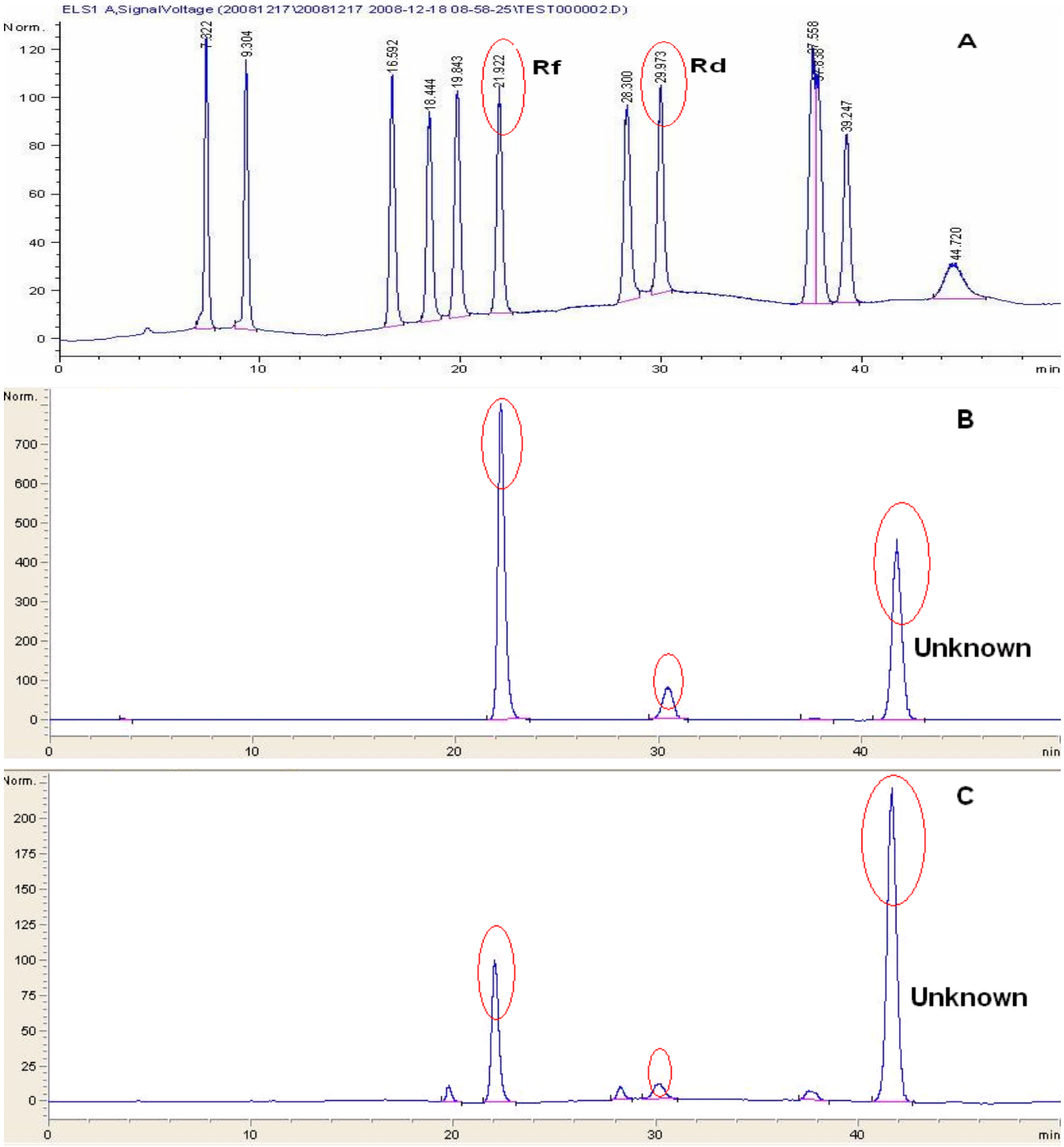

Fig. 4 HPLC chromatogram on standard of ginsenosides, JKG 6 and JKGS 5. (**A**) standard of ginsenosides, (**B**) JKG 6, (**C**) JKGS 5.

and decreased slightly after JKG 2 to 2.6% at JKG 6. In JKGS, the crude protein contents increased as the number of times boiled in sugar syrup increased, even if their content was little, i.e. 0.6% at JKGS 5. In particular, the crude protein content of JKGS 5 was lower than that of JKGS 4, and this may be the reason for adding 350 g fructo-oligo liquid after the 5th boiling step.

Free amino acid and amino acid derivatives JKG and JKGS prepared with the number of boiling times in sugar syrup

The free amino acid content of WG and JKG prepared with increasing the number of times boiled in sugar syrup is shown in **Table 1**. The total free amino acid content in dried WG was 4,770.42 mg/100 g. The main free amino acid was arginine, which accounted for 69.20% of total free amino acids. This result was similar to existing reports in which the arginine ratio to total free amino acid was 51.4-77.8% (Lee *et al.* 2000), 45.2% (Choi *et al.* 1985) and 58.3% (Yukinaga *et al.* 1994). Total free amino acid and arginine contents of JKG decreased as increasing the number of times boiled in sugar syrup, 49.18- and 18.93-fold that of raw ginseng, respectively. γ-Aminobutyric acid (GABA) was not present in fresh, untreated ginseng, but it was formed as an intermediate product during JK processing and at 34.13 mg/100 g in JKG 6. GABA is the chief inhibitory neurotransmitter in the mammalian central nervous system. It plays an important role in regulating neuronal excitability throughout the nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone. Although chemically it is an amino acid, GABA is rarely referred to as such in the scientific or medical communities (Li K *et al*. 2008). In addition, two peaks

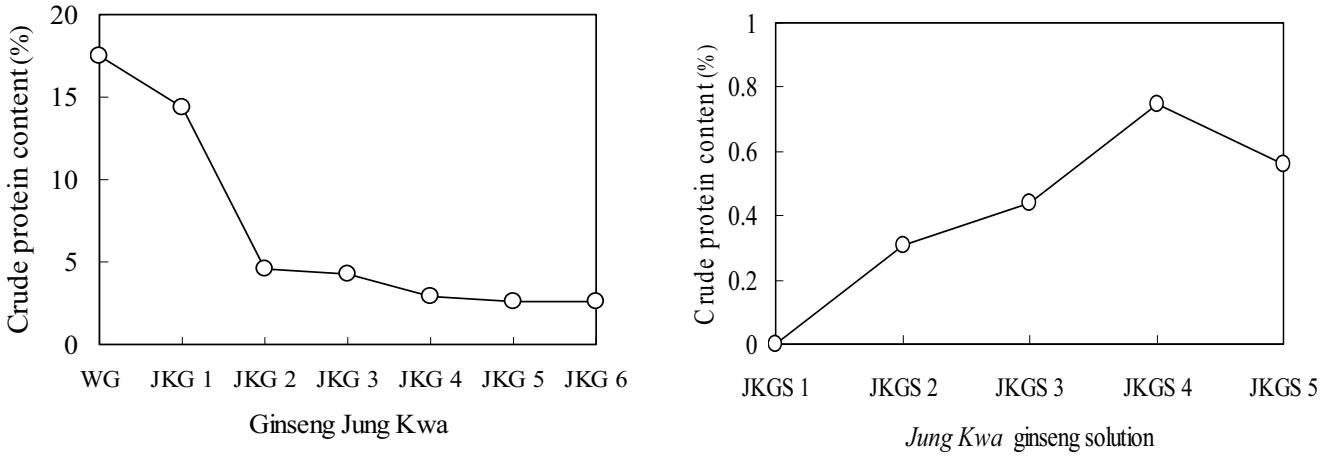

Fig. 5 Crude protein content of *Jung Kwa* ginseng and *Jung Kwa* ginseng solution prepared by increasing the number of times boiled in sugar syrup.

Table 1 Free amino acid and amino acid derivatives content of *Jung Kwa* ginseng prepared with increasing the number of times boiled in sugar syrup.

	WG (mg/100 g)	JKG 1	JKG 2	JKG 3	JKG 4	JKG 5	JKG 6
Aspartic acid	78.05 ± 2.35[1a]	45.99 ± 1.04[b]	18.78 ± 0.56[c]	14.68 ± 0.51[c]	7.56 ± 0.34[d]	6.52 ± 0.33[d]	6.54 ± 0.40[d]
Threonine	52.14 ± 1.41[a]	46.22 ± 1.12[a]	24.31 ± 0.75[b]	21.36 ± 0.66[b]	18.52 ± 0.62[b]	10.27 ± 0.52[c]	9.01 ± 0.36[c]
Serine	244.21 ± 10.42[a]	172.72 ± 8.54[b]	39.63 ± 1.12[c]	32.75 ± 0.89[c]	6.56 ± 0.11[d]	6.44 ± 0.15[d]	6.43 ± 0.20[d]
Glutamic acid	57.20 ± 3.14[a]	55.82 ± 1.76[a]	30.76 ± 0.97[b]	12.75 ± 0.84[c]	6.89 ± 0.24[d]	6.71 ± 0.39[d]	6.66 ± 0.39[d]
Proline	43.63 ± 1.02[a]	36.54 ± 0.98[a]	22.50 ± 0.85[b]	10.47 ± 0.33[c]	5.21 ± 0.09[d]	4.01 ± 0.01[d]	-
Glycine	172.20 ± 14.63[a]	13.00 ± 1.22[b]	5.40 ± 0.44[c]	4.84 ± 0.25[c]	4.76 ± 0.13[c]	-	-
Alanine	58.71 ± 1.72[b]	124.85 ± 8.75[a]	31.84 ± 1.14[c]	27.80 ± 0.79[c]	12.01 ± 0.25[d]	11.41 ± 0.22[d]	11.37 ± 0.30[d]
Valine	27.41 ± 1.08[a]	29.73 ± 1.27[a]	20.72 ± 0.86[b]	15.98 ± 0.54[c]	12.50 ± 0.30[c]	12.07 ± 0.18[c]	12.06 ± 0.21[c]
Methionine	45.00 ± 2.22[a]	16.88 ± 1.04[b]	14.12 ± 0.41	9.82 ± 0.60[c]	8.78 ± 0.52[c]	8.45 ± 0.45[c]	8.50 ± 0.32[c]
Isoleucine	72.21 ± 3.26[a]	34.83 ± 1.53[b]	6.57 ± 0.26[c]	7.32 ± 0.71[c]	4.69 ± 0.31[c]	5.16 ± 0.14[c]	5.11 ± 0.20[c]
Leucine	54.00 ± 2.01[a]	27.77 ± 1.08[b]	22.14 ± 0.74[b]	7.32 ± 0.38[c]	5.41 ± 0.26[c]	6.67 ± 0.51[c]	6.66 ± 0.76[c]
Tyrosine	123.31 ± 9.68[b]	554.90 ± 21.51[a]	84.05 ± 2.23[c]	59.09 ± 1.68	19.44 ± 0.79	17.94 ± 0.59	17.46 ± 0.41
Phenylalanine	158.18 ± 10.35[a]	17.52 ± 0.44[b]	18.01 ± 0.53[b]	18.94 ± 0.25[b]	20.58 ± 0.54[b]	22.34 ± 1.20[b]	22.01 ± 0.91[b]
Unknown 1[b]	-	-	32.68 ± 2.74[b] (0.79)	88.01 ± 3.12[a] (1.02)	89.72 ± 2.06[a] (3.14)	91.06 ± 2.30[a] (3.65)	91.42 ± 2.12[a] (3.74)
β-alanine	113.87 ± 8.76[a]	73.22 ± 1.26[b]	21.30 ± 1.00[c]	17.82 ± 0.84[c]	15.20 ± 0.31[c]	15.07 ± 0.80[c]	15.11 ± 0.72[c]
GABA	-	-	-	19.54 ± 0.54[b]	29.87 ± 1.42[a]	31.22 ± 2.04[a]	34.13 ± 1.75[a]
Unknown 2[b]	-	-	-	24.22 ± 3.01[b] (0.34)	36.61 ± 2.46[a] (1.29)	38.14 ± 2.91[a] (1.54)	37.50 ± 2.77[a] (1.53)
Lysine	138.53 ± 8.44[b]	245.24 ± 9.67[a]	153.00 ± 5.78[b]	170.70 ± 4.77[b]	43.06 ± 0.40[c]	21.47 ± 1.58[d]	19.78 ± 0.86[d]
Histidine	30.09 ± 1.05[a]	30.09 ± 0.88[a]	16.38 ± 0.61[c]	23.49 ± 0.82[b]	8.00 ± 0.09[d]	3.81 ± 0.31[e]	4.02 ± 0.20[e]
Arginine	3301.68 ± 35.66[a]	2000.66 ± 24.74[b]	670.11 ± 10.53[c]	564.89 ± 15.69[c]	76.70 ± 3.67[d]	67.16 ± 5.12[d]	67.14 ± 3.51[d]
Total	4,770.42 ± 18.99[a]	3,525.98 ± 16.28[b]	1,199.62 ± 12.14[c]	1,039.56 ± 12.06[c]	305.74 ± 10.28[d]	256.72 ± 8.24[d]	251.99 ± 2.88[d]

[a] Values are mean ± SD (n=3). Different superscripts indicate significant differences among treatment groups by Duncan's multiple range test ($p<0.05$)
[b] Data expressed as peak area in ginseng *Jung Kwa* solution; figures in parenthesis = area ratio of total peak areas; GABA = γ-aminobutyric acid

of unknown ninhydrin-positive substances formed at RT (retention time) = 32 (relative area at JKG 6= 3.74%) and 39 min (relative area at JKG 6= 1.53%) in the HPLC chromatogram of JKG 2 and increased slightly as the number of times boiled increased. Yukinaga *et al.* (1994) also indicated that three unknown ninhydrin-positive substances (UK-I, II and III) were detected with an amino acid analyzer in the water extract of Korean red ginseng and UK-II and III were identified as Arg-Fru-Glu and Arg-Fru, respectively. In addition, an unknown compound 1 existed between phenylanine and β-alanine and an unknown compound 2 existed between GABA and lysine, exactly what Yukinaga *et al.* (1994) had reported. Generally, the reaction between reducing sugars and amino acids is known as the Maillard reaction or non-enzymic browning reaction (Maillard 1913). The Maillard reaction is a complicated reaction that produces a large number of so-called Maillard products such as aroma compounds, ultra-violet absorbing intermediates, and dark-brown polymeric compounds named melanoidins (Wijewickreme *et al.* 1997). In this study, the color of JKG and JKGS turned dark-brown as the number of times boiled in sugar syrup increased (**Fig. 2**).

The free amino acid content of JKGS prepared by increasing the number of times boiled in sugar syrup increased is shown in **Table 2**. When 8 kg of washed ginseng was boiled for 5 min with 16 L water and then 8 L of boiling water was removed, in JKGS 1, 12 free amino acids were detected in the water that was removed after boiling, even though this amount was trivial. The free amino acid content of JKGS also increased as the number of times boiled in sugar syrup increased. JKGS had a high arginine (main free amino acid in ginseng) content even if its amount was much less than raw ginseng, because it would seem that arginine of raw ginseng was eluted in JKGS as the number of times boiled increased. JKGS also had two peaks of unknown ninhydrin-positive substances at an RT of about 32 min and 39 min from the HPLC chromatogram of JKGS 3. Although their peaks (unknown 1 and 2) were less than those of JKG, they increased by increasing the boiling time (i.e. times boiled) in sugar syrup. Unlike JKG, the peak area of unknown compound 1 was smaller than that of unknown compound 2 while the relative area ratio of both peaks on total free amino acid peak area was 0.51 and 1.54%, respectively at JKGS 5.

Table 2 Free amino acid and amino acid derivatives content of *Jung Kwa* ginseng solution prepared with increasing the number of times boiled in sugar syrup.

	JKGS 1 (mg/100 g)	JKGS 2	JKGS 3	JKGS 4	JKGS 5
Aspartic acid	1.52 ± 0.41[1c]	2.26 ± 0.85[c]	3.44 ± 0.66[b]	5.22 ± 1.10[a]	5.08 ± 1.02[a]
Threonine	-	1.04 ± 0.01[b]	2.57 ± 0.74[a]	2.81 ± 0.80[a]	2.16 ± 0.56
Serine	5.05 ± 1.04[b]	9.02 ± 1.22[a]	9.36 ± 1.07[a]	11.42 ± 1.23[a]	10.03 ± 1.05[a]
Glutamic acid	0.47 ± 0.09[c]	1.20 ± 0.15[b]	1.19 ± 0.25[b]	3.21 ± 0.92[a]	3.14 ± 0.73[a]
Proline	-	1.01 ± 0.04	1.45 ± 0.15	1.53 ± 0.09	1.47 ± 0.12
Glycine	0.36 ± 0.07[b]	0.62 ± 0.12[ab]	0.95 ± 0.14[ab]	1.24 ± 0.22[a]	1.22 ± 0.17[a]
Alanine	4.78 ± 1.01[b]	11.25 ± 1.41[a]	13.80 ± 1.96[a]	14.02 ± 2.00[a]	13.99 ± 1.87[a]
Valine	0.31 ± 0.07[b]	1.70 ± 0.12[ab]	2.30 ± 0.93[a]	2.64 ± 0.82[a]	2.55 ± 1.01[a]
Methionine	-	2.15 ± 0.70[b]	1.53 ± 0.52[b]	3.04 ± 0.46[a]	2.87 ± 0.81[a]
Isoleucine	-	1.91 ± 0.02[b]	2.52 ± 0.79[b]	4.27 ± 1.53[a]	4.22 ± 1.02[a]
Leucine	2.37 ± 0.40[ab]	1.75 ± 0.51[b]	2.59 ± 0.68[a]	2.96 ± 0.14[a]	2.85 ± 0.26[a]
Tyrosine	9.77 ± 1.28[a]	3.89 ± 1.04[c]	6.93 ± 2.03[b]	9.21 ± 1.98[a]	9.16 ± 2.00[a]
Phenylalanine	9.60 ± 2.34[a]	2.33 ± 1.03[c]	3.79 ± 1.14[b]	4.15 ± 1.02[b]	4.02 ± 1.33[b]
Unknown 1[b]	-	110.10 ± 10.14[c] (0.29)	163.08 ± 11.42[b] (0.38)	362.52 ± 15.23[a] (0.47)	368.61 ± 13.27[a] (0.51)
β-alanine	10.43 ± 1.69[b]	8.88 ± 2.08[b]	14.51 ± 1.77[a]	15.92 ± 2.01[a]	15.97 ± 0.96[a]
GABA	-	-	10.22 ± 1.52	12.41 ± 1.44[a]	16.22 ± 2.01[a]
Unknown 2[b]		337.84 ± 20.10[c] (0.71)	1023.75 ± 19.63[b] (1.50)	1684.30 ± 25.21[a] (1.42)	1688.82 ± 21.25[a] (1.54)
Lysine	2.95 ± 0.85[c]	5.76 ± 1.26[b]	17.02 ± 2.54[a]	18.44 ± 2.10[a]	18.24 ± 21.85[a]
Histidine	1.59 ± 0.20[c]	3.32 ± 0.73[b]	5.88 ± 0.40[a]	6.55 ± 0.68[a]	6.48 ± 0.57[a]
Arginine	67.25 ± 3.14[d]	173.06 ± 10.87[c]	226.08 ± 12.58[b]	482.14 ± 22.59[a]	430.57 ± 20.54[a]
Total	116.45 ± 5.96[d]	231.15 ± 8.44[c]	326.13 ± 10.42[b]	601.18 ± 8.57[a]	550.24 ± 9.04[a]

[a] Values are mean ± SD (n=3). Different superscripts indicate significant difference among treatment groups by Duncan's multiple range test ($p<0.1$)
[b] Data expressed as peak area in ginseng *Jung Kwa* solution; figures in parenthesis = area ratio of total peak areas; GABA = γ-aminobutyric acid

CONCLUSIONS

In this study, we observed changes in crude saponin, ginsenosides (Rd and Rf), crude protein, free amino acids and amino acid derivatives contents of JKG and JKGS as the number of times these were boiled in sugar syrup increased during JKG processing.

In JKG 6, the content of Rf and Rd, components of ginsenosides, increased 77- and 16-fold, respectively more than raw ginseng and the crude saponin content was 233.60 and 61.88 mg/g in JKGS. As the boiling time increased, crude protein and total free amino acid contents of JKG decreased 49.18- and 18.93-fold that of raw ginseng, respectively while in JKGS 5, crude protein and total free amino acid contents increased to 0.56% and 7.3 mg/g, respectively. Especially, the arginine content of JKG 6 decreased to 49.18-fold that of raw ginseng and GABA, while unknown compounds 1 and 2 were formed as intermediate products during JK processing and GABA content was 34.13 mg/100 g in JKG 6. In addition, unknown compound 1 was formed more than unknown compound 2 in JKG while unknown compound 2 was formed more than unknown compound 1 in JKGS. As a next phase of our research, Rd, Rf and the unknown compounds identified in JKG and JKGS will be studied in greater detail.

ACKNOWLEDGEMENTS

This research was supported by the Agricultural R & D Promotion Center, Republic of Korea (108009-03-1-HD120).

REFERENCES

Ahn YG, Kim SK, Shin CS (1999) Studies on ginseng vinegar. *Korean Journal of Food and Nutrition* **12**, 447-454

Ando T, Tanaka O, Shibata S (1971) Chemical studies on the oriental plant drugs. Comparative studies on the saponins and sapogenins of ginseng and related crude drugs. *Soyakugaku Zasshi* **25**, 28-33

A.O.A.C. (1995) *Official Methods of Analysis* (16th Edn), Association of Official Analytical Chemists, Washington, DC, pp 69-74

Cho SH, Kang RK, Lee HG (1984) A study on ingredients preparation method of lotus root *Jung Kwa*. *Journal of the Korean Society and Food Nutrition* **13**, 42-50

Choi C, Yoon SH, Bae MJ, An BJ (1985) Proteins and amino acid composition of korea ginseng classified by years. *Journal of Korean Food Science and Technology* **17**, 1-4

Garriques SS (1854) On panaquuilon, a new vegetable substance. *Annalen der Chemie Pharmacie* **90**, 231

Jeong CS, Hyun JE, Kim YS (2002) Anti-oxidative effect of ginsenoside Rb_1 on the HCl ethanol-induced gastric tissue in rats. *Journal of Korean Pharmacology* **33**, 252-256

Jie YH, Cammisuli S, Baggiolini M (1984) Immunomodulatory effects of *Panax ginseng* C.A. Meyer in the mouse. *Agents Actions* **15**, 386-391

Jung IS, Cho YD (1985) Effect of ginseng saponin fraction on absorption of cholesterol and serum lipid components. *Journal of Korean Ginseng Science* **9**, 232-239

Kim ES, Jeong JI, Kim HJ, Kim MJ (1990) Effect of honeyed ginseng on growth and biochemical components of rats. *Thesis of Dankuk National University* **24**, 559-570

Kim HJ, Jung DK, Joo HK (1985) The effect of honey concentration on the quality of honeyed ginseng in the process of manufacturing honeyed ginseng. *Journal of Korean Ginseng Science* **9**, 128-134

Ko SK, Lee KH, Hong JK, Kang SA, Sohn UD, Im BO, Han ST, Yang BW, Chung SH, Lee BY (2005) The change of ginsenoside composition in ginseng extract by the vinegar process. *Food Science and Biotechnology* **14**, 509-513

Korea Customs Service (2007) The annuals of trade statistics. Available online: http://www.customs.go.kr

Kwon HJ, Park CS (2009) Quality characteristics of bellflower and lotus root *Jeonggwa* added *Omija* (*Schizandra chinensis* Baillon) extract. *Korean Journal of Food Preservation* **16**, 53-59

Lee CH, Maeng YS (1987) A literature review on tradition Korean cookies, *Hankwa*. *Korean Journal of Dietary Culture* **2**, 55-69

Lee CH, Nam KY, Choi KJ (1978) Relationship between the age and chemical components of ginseng root's portion. *Korean Journal of Food Science and Technology* **10**, 263-268

Lee HG, Kim HJ (2001) Sensory and mechanical characteristics of wax gourd *Jung Kwa* by different recipes. *Korean Journal of Food Cookery Science* **17**, 412-420

Lee HJ, Yoo BS, Byun SY (2000) Differences in free amino acids between Korean ginsengs and mountain ginseng. *Korean Journal of Biotechnology and Bioengineering* **15**, 323-328

Lee KS, Kim GH Kim HH, Seong BJ, Lee HC, Lee YG (2008) Physicochemical characteristics on main and fine root of ginseng dried by various temperature with far-infrared drier. *Korean Journal of Medicinal Crop Science* **16**, 211-217

Lee KS, Kim GH Kim HH, Song MR, Kin MR (2009) Quality characteristics of ginseng *Jung Kwa* and *Jung Kwa* solution on *Jung Kwa* process. *Journal of the Korean Society of Food Science and Nutrition* **38**, 587-593

Li K, Xu E (2008) The role and the mechanism of gamma-aminobutyric acid during central nervous system development. *Neuroscience Bulletin* **24**, 195-200

Maillard LC (1913) Formation et matières humiques par action de polypeptides sur sucres. *Comptes Rendus de l'Academie des Sciences* **156**, 148-149

Matsuda H, Samukawa K, Kubo M (1990) Anti-inflammatory activity of ginsenoside Ro. *Planta Medica* **56**, 19-23

Matsuda H, Samukawa K, Kubo M (1991) Anti-hepatitic activity of ginsenoside Ro. *Planta Medica* **57**, 523-526

Mochizuki M, Yoo YC, Matsuzawa K, Sato K, Saiki I, Tonooka S, Samukawa K, Azuma I (1995) Inhibitory effect of tumor metastasis in mice by

saponins, ginsenoside-Rb_2, 20(R)-, and (S)-ginsenoside-Rg_3, of red ginseng. *Biological and Pharmaceutical Bulletin* **18**, 1197-1202

Paek JK, Kim JH, Yoon SJ (2006) Quality characteristics of ginseng Jung Kwa after different soaking times in sugar syrup. *Korean Journal of Food Cookery Science* **22**, 792-798

Ryu GH (2003) Present statues of red ginseng products and its manufacturing process. *Korean Journal of Food Industry and Nutrition* **8**, 30-37

Rhee SH, Zong MS (1983) A study on the analysis of amino acids in Korean ginseng. *Korean Journal of Environment Health Society* **9**, 37-53

Saito H, Yoshida Y, Tagaki K (1974) Effects of *Panax ginseng* root on exhaustive exercise in mice. *Japanese Journal of Pharmacology* **24**, 119-126

Takagi K, Saito H, Nabata H (1972) Pharmacological studies of *Panax ginseng* root: Estimation of pharmacological actions of *Panax ginseng* root. *Japanese Journal of Pharmacology* **22**, 245-249

Wang BX, Cui JC, Liu AJ, Wu SK (1983) Studies on the anti-fatigue effect of the saponins of stems and leaves of *Panax ginseng* (SSLG). *Journal of Traditional Chinese Medicine* **3**, 89-94

Wang X, Sakuma T, Asafu-Adjaye E, Shiu GK (1999) Determination of ginsenosides in plant extracts from *Panax ginseng* and *Panax quinquefolius* L. by LC/MS/MS. *Analytical Chemistry* **71**, 1579-1584

Wijewickreme AN, Kitts DD, Durance TD (1997) Reaction conditions influence the elementary composition and metal chelating affinity of nondialyzable model Maillard reaction products. *Journal of Agricultural Food Chemistry* **45**, 4577-4583

Yokozawa T, Oura H (1990) Facilitation of protein biosynthesis by ginsenoside-Rb_2 administration in diabetic rats. *Journal of Natural Products* **53**, 1514-1518

Yokozawa T, Kobayashi T, Oura H, Kawashima Y (1985) Studies on the mechanism of the hypoglycemic activity of ginsenoside-Rb2 in streptozotocin-diabetic rats. *Chemical Pharmaceutical Bulletin* **33**, 869-872

Yoon SH, Joo CN (1993) Study on the preventive effect of ginsenosides against hypercholesterolemia and its mechanism. *Korean Journal of Ginseng Science* **17**, 1-12

Yoon SR, Lee MH, Park JH, Lee IS, Kwon JH, Lee GD (2005) Changes in physicochemical compounds with heating treatment of ginseng. *Journal of Korean Society and Food Nutrition* **34**, 1572-1578

Yukinaga M, Yinan Z, Takeshi T, Kenji K, Hiromichi O (1994) Isolation and physiological activities of a new amino acid derivative from Korean red ginseng. *Korean Journal of Ginseng Science* **18**, 204-211

Zhang S, Chen R, Wu H, Wang C (2006) Ginsenoside extraction from *Panax quinquefolium* L. root (American ginseng) root by using ultrahigh pressure. *Journal of Pharmaceutical and Biomedical Analysis* **41**, 57-63