Manufacture and characterization of optimized red ginseng drinks containing herbal medicine extracts

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Abstract The optimum mixing ratio of red ginseng, herbal medicine extracts, and oligosaccharides for diabetic improvement was investigated and verified through animal experiments. Using the optimum mixing technique, 2% red ginseng extract, 15% oligosaccharides, and 83% herbal medicine extracts were determined to be optimal for red ginseng beverages. The taste was improved as the amount of red ginseng decreased and oligosaccharides increased, whereas antioxidative activity was increased with increased red ginseng. In the animal model in which streptozotocin was administered to BALB-c mice, the red ginseng administration group exhibited lower blood glucose than the diabetic control group, and the blood glucose level was lower in the herbal medicine and red ginseng extract administration group. Insulin concentration was slightly increased by the red ginseng beverage, and

hepatotoxicity due to excessive intake of red ginseng beverages was not detected.

Keywords Red ginseng beverage · Optimization · Antioxidative activity · Blood glucose

Introduction

The current diabetes population has increased to 10% of the total population, and diabetes is fourth overall in mortality after cancer, cerebrovascular disease, and heart disease (Statistics Korea, 2008). Insulin administration, which is the main treatment for diabetes, causes various side effects. Therefore, studies on the biological activity of natural plants are in process. Some natural vegetable materials that have been reported to improve diabetes include soy protein (Yang, 2015), red ginseng (Kim et al., 2008) and medicinal plants (Lee and Yoon, 2015). Red ginseng contains physiologically active ingredients such as ginseng saponin, polyacetylene, phenolic compound, and acidic peptide (Attele et al., 1999), which are well known to inhibit cancer, protect the nervous system, and provide antioxidant activity (Keum et al., 2000).

Studies on functional drinks containing red ginseng have been actively investigated. Kim et al. (2011a; 2011b) presented data on the content and safety of red ginseng beverages. The anti-allergy and anti-inflammatory effects of red ginseng beverages have been reported (Park et al., 2010). Despite the medical efficacy of red ginseng sports drinks such as fatigue recovery and prevention of muscle wounds, the preference for red ginseng beverages is low due to their unappealing flavor (Kim et al., 2009). The functionality of the beverages by adding 0.5% red ginseng extract to soymilk has greatly improved (Lee, 2012), and

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the addition of red ginseng to noni drinks has resulted in more nutritional and functional properties (Kim, 2011). Yang et al. (1980) also manufactured soft red ginseng beverages containing other natural resource extracts. Studies on these red ginseng beverages suggest that it may be possible to increase the functionality of red ginseng beverages by adding various complex extracts to the red ginseng extract.

Therefore, in this study, the optimum ratio of red ginseng beverage and herbal medicine extracts was determined using the mixture design and optimizing technique, and its characteristics for improving effect on blood glucose through animal models was investigated.

Materials and methods

Materials

Red ginseng concentrate was purchased from the Korean Ginseng Herbal Union (Kumsan, Korea), and 10 g of each red ginseng concentrate was packaged with test certificates from Geumgang Pharmaceutical Co., Ltd. (Changwon, Korea). White atractylis, poria cocos, Chinese yam, and citron concentrates were purchased from Hansung Food Union (Koheung, Korea). Oligosaccharides were provided by Samyang (Buchon, Korea).

Preparation of the oriental medicine extracts

To prepare the oriental medicine extracts, four medicinal herbs (white atractylis, poria cocos, Chinese yam, and citron), which are effective for the treatment of diabetes mellitus, were selected based on advice from the Department of Oriental Medicine Biotechnology in Kyunghee University (Yongin, Korea). Each medicinal herb was washed, chopped to a proper small size, and extracted at 100 °C for 6 h in an extractor (Jung Sung Hascom, Seoul, Korea). The herbal extracts and dry citron powder were mixed at the traditional mixing ratio of 3:3:5:2 (white atractylis: poria cocos: Chinese yam: dry citron).

Statistical modeling, optimization, and trace plot

To optimize the ratio of components, red ginseng beverage was prepared with red ginseng concentrate, oriental medicine extract, and oligosaccharides at variable amounts. The optimization experiment, data analysis, and optimization were accomplished using the Design-expert 7 Program (Stat-Ease, Inc., Minneapolis, MN, USA). On the basis of the modified distance design, the ranges of ingredients were set to 1–2% (w/w) red ginseng concentrate, 5–15% (w/w) oligosaccharides, and 83–94% (w/w) oriental

medicine extract. The response variables included taste, flavor, and antioxidant properties. Experimental mixtures were designed by modified distance design, and 16 experimental points with 5 replications were set according to each blending ratio (Table 1). Optimization for selecting the optimum component ratio was predicted through numerical optimization of the canonical model and contour map. The F-test of significance was performed for each linear, quadratic, and cubic model, and the values of taste, and DPPH were adapted to a linear model ($p < 0.05$) with a canonical form obtained by the modified least regression. Coefficients were used to develop regression models based on Scheffes polynomial form, and trace plots were drawn to determine the effect of each mixture on the constraint values. In this study, a trace plot representing taste and DPPH are shown, and the slope of the trace plot for each red ginseng beverage displays the degree of influence of taste and DPPH value.

Measurement of antioxidative activities of red ginseng beverage using DPPH

To determine the antioxidative activity of red ginseng beverage, 7.9 mg of DPPH (1,1-diphenyl-2-picrylhydrazyl) was added to 200 mL of 80% methanol and stirred for 20 min in a brown glass bottle. Fifty μ L of reaction mixture was mixed with 2.95 mL of DPPH solution (Blois, 1958), and absorbance was then measured at 517 nm after 30 min. The measured value was calculated in milligrams vitamin C equivalent/L (mg VCE/L) in comparison with the DPPH radical scavenging activity value of vitamin C.

Sensory evaluation and statistical analysis of red ginseng beverage

Sensory evaluation of taste and flavor was carried out with 15 skilled panelists using a 9-point scale (1 (worst) to 9 (best)). A 30 mL of the beverage previously stored at 4 °C was evaluated. Data were evaluated by the SPSS statistical program (Statistical Package for the Social Science v17.0, Chicago, IL, USA). Significance test was performed using Duncan's multiple range test ($p < 0.05$).

Diabetes induction and determination of the test group

To investigate the diabetic improvement effect in animal experiments, streptozotocin was administered to BALB-c mice to induce diabetes. Eight-week-old BALB-c mice were fed a general diet and maintained at a temperature of 23 ± 2 °C and a humidity of $50 \pm 5\%$. Streptozotocin was injected at a concentration of 80 mg/kg. After 24 h, fasting glucose was measured in the tail artery, and only the

Table 1 Experimental mixture design and responses of ginseng beverage

Run	Factor			Response		
	Red ginseng concentrate (g)	Oligosaccharide (g)	herbal medicine mix (g)	Taste	Flavor	DPPH (mg VCE/L)
1	2	15	83	6.4	6.7	71.2
2	1	11.7	87.3	6.5	6.3	32.9
3	2	10	88	4.8	5.9	58.8
4	2	8.3	89.7	4.9	6.1	65.4
5	1.2	12.5	86.3	6.1	6.5	44.1
6	1	15	84	6.5	5.9	39.4
7	1	15	84	6.3	5.8	39.4
8	1.5	15	83.5	5.2	5.5	48.9
9	2	5	93	5.9	6.5	67.4
10	2	5	93	5.3	5.5	67.4
11	1	5	94	6.3	6.6	37.1
12	1.5	15	83.5	6.5	6.4	48.9
13	1	10	89	5.5	5.4	70.8
14	1	5	94	4.1	5.6	37.1
15	1.5	7.5	91	2.9	4.7	42.6
16	2	15	83	6.3	6.5	71.2

streptozotocin-induced diabetic mice who exhibited more than 300 mg/dL of blood glucose were selected. The test was divided into 4 groups of a normal group, diabetic control group, diabetic control group + red ginseng group, and diabetic control group + red ginseng + herbal extract group ($n = 10$).

Determination of blood insulin concentration and hepatotoxicity

Insulin levels in the blood were measured as described in a previous report (Lee et al., 2008). After 4 weeks of experimentation, blood was collected from the heart and centrifuged at 3000 rpm to separate plasma. Insulin concentration was measured using a mouse insulin ELISA kit (Crystal Chem., Elk Grove Village, IL., USA). GOT (Glutamic Oxalacetic Transaminase)/GPT (Glutamic-Pyruvic Transaminase) was measured to verify the safety of the developed products. GOT/GPT is a ratio of enzymes that degrade amino acids in the body and can reveal the degree of hepatocyte damage by reflecting hepatic synthesis ability. Therefore, to verify the safety of the product, GOT/GPT value in blood was measured using an enzymatic method, Asan Medical kit (103-K AM kit, (Asan, Korea), to measure hepatotoxicity after diluting 1/3 of the products and administering it to normal mice for 4 weeks.

Results and discussion

Manufacture and optimization of red ginseng beverage with linear programming

The actual experimental points were set by the modified distance design, which was established with the constraint of the mixing ratio of red ginseng extract, oligosaccharides, and herbal medicine extract. Table 1 exhibits 16 experimental points including 5 replicates and shows the results of the response according to composition ratio. Taste components ranged from 2.9 to 6.5, flavor components ranged from 4.5 to 6.7, and DPPH values ranged from 32.9 to 71.2 depending on mixing ratio. To investigate the effect of each component in the mixture, modeling was constructed by the set response, and the significance test was performed using an F-test (Table 2). As a result, a linear model was selected for taste (probability 0.0312) and DPPH (probability 0.0031). The flavor had a probability greater than 0.3, so the effect of the ingredients on flavor was excluded.

The trace plot showing the effect of each ingredient on taste and antioxidant properties is shown in Fig. 1. The trace plot is based on the fact that, when the amount of a particular ingredient is increased, the amounts of the other ingredients decrease and their proportion remains constant. This can be used to deduce the most influential components of the reaction and their tendencies, as well as the effect of the mixed substance (Cornell, 1990). As the amount of red

Table 2 Analysis of selected models and regression at polynomial equations for two response

Response	Model	Prob > F	Equation in terms of Pseudo component
Taste	Linear	0.0312	$2.63A + 6.36B + 5C$
DPPH	Linear	0.0031	$308.94A + 41.34B + 40.3C$

Fig. 1 Trace plot describing the effect of red ginseng, oligosaccharide and herbal medicine mix component on taste (A) and DPPH (B). A–A: Red ginseng concentrate, B–B: Oligosaccharide, C–C: Herbal extracts)

ginseng (A–A) was increased, it produced a negative effect on taste. When the content of herbal medicine extract (C–C) was increased, the taste also slightly worsened. However, as the content of oligosaccharides (B–B) increased, the taste of the beverage improved (Fig. 1A).

The DPPH values applied to the trace plot using the linear model (Fig. 1B) showed that the DPPH value increased remarkably as the amount of red ginseng increased (A–A), but the oligosaccharides had little effect (B–B). When the amount of herbal extract increased (C–C), the DPPH value decreased. Conclusively, red ginseng tremendously enhanced antioxidative activity even though taste was worsened.

Optimum contents of red ginseng beverage

The optimal mixing ratio of the red ginseng beverage was performed as described in previous methods by Moon et al.

(2011). The target components were red ginseng extract, oligosaccharides, and herbal medicine extract, and the response variables were determined based on taste and DPPH value. These optimum values of each mixing component are shown in Table 3. As a result of prediction of the optimum values, the optimum ratio of the red ginseng beverage was 2% (w/w) red ginseng extract, 15% (w/w) oligosaccharides, and 83% (w/w) herbal medicine extracts. With these optimum values, the taste score was 6.02, and the DPPH value was 65.67 (mg VCE/L). The optimization method shows the overlapping range when desirability was overlaid on the contour map. The maximum desirability was calculated during the iterative operation, and the desirability was measured to be 0.830 (Data didn't show). Numerous studies have been performed using the mixture design and optimization technique to determine optimal mixing. Optimization of the processing conditions of *Jeonbokjang* (Moon et al., 2011), optimization of the selection and manufacturing of the microbial strains to produce high quality *chungkukjang* (Hwang et al., 2008), optimization of wine with an optimum blending ratio of various domestic grapes (Koak et al., 2010), and optimization of the black ginger manufacturing process (Ban et al., 2010) have been carried out using an experimental design and optimization techniques. In this study, the optimization technique was applied not only to improve the functionality of a ginseng beverage, but also to enhance its taste and flavor. The functionality of the ginseng beverage created at the optimized ingredient mixing ratio was investigated through animal experiment values of blood glucose, glucose tolerance, insulin secretion, and hepatotoxicity.

Effects on blood glucose and glucose tolerance

The effect of 4-week animal testing on blood glucose level is shown in Fig. 2. Normal rats exhibited almost no change in blood glucose level during the 4-week period. In the diabetic control rats, blood glucose increased continuously during the 4 weeks. In the diabetic control group, rats treated with only red ginseng extract exhibited a significant decrease in blood sugar from 425 to 343 mg/dL during storage for 4 weeks. In the diabetic control group, the blood glucose level decreased from 416 to 332 mg/dL after 4 weeks in rats treated with the mixture of red ginseng and herbal medicine extracts. The blood glucose level of the diabetic group was higher than that of the normal group.

Table 3 Optimum constraint values using the numerical optimization method

Constraint name	Goal	Numerical optimization solution
Red ginseng concentrate (g)	In range	2%
Oligosaccharide (g)	In range	15%
Herbal medicine mix (g)	In range	83%
Taste	Max	6.02
DPPH	Max	65.67

Fig. 2 Effects of red ginseng and herbal extracts on blood glucose levels in streptozotocin-induced diabetic mice

Red ginseng products lowered blood glucose compared to the diabetic control group, and there was greater blood glucose reduction through the addition of herbal medicine.

The effect of 4-week animal testing on glucose tolerance is shown in Table 4. In normal rats, there was little change in glucose tolerance level, from 141 to 138 mg/dL, after 4 weeks. In the diabetic control rats, the glucose tolerance level increased from 411 to 453 mg/dL. In the diabetic control group, the rats treated with red ginseng extract decreased glucose tolerance level from 425 to 343 mg/dL. The glucose tolerance level also decreased from 416 to 332 mg/dL in the diabetic rats treated with the mixture of red ginseng and herbal medicine extracts. Glucose tolerance improved in the herbal medicine supplement group (Table 4). As described in previous studies, red ginseng

components significantly contributed to the hypoglycemic effect, as seen in the results from the present study (Kim et al., 2011a; 2011b; Lee et al., 1998).

Effects on insulin secretion and hepatotoxicity

The results of insulin concentration measurements in the blood to investigate the effects of red ginseng and red ginseng + herbal medicine intake through the 4-week animal experiment are shown in Table 5. The insulin concentration in the normal rats was 20.9 μ U/mL after 4 weeks, whereas the insulin concentration was 9.7 μ U/mL in the diabetic control rats over the same period. The insulin concentration in the diabetic rats treated with the red ginseng extract was 11.9 μ U/mL, and that in the diabetic rats administered a mixture of red ginseng extract and herbal medicine was 13.4 μ U/mL. Insulin concentration was significantly lower in the diabetic group compared to the normal group. However, the intake of red ginseng drink slightly increased the insulin concentration, while the addition of herbal medicines increased the insulin concentration even more in the diabetic group. The effect of decreasing insulin secretion results in many changes in glucose metabolism, such as the conversion of the main energy source of skeletal muscle to fat. Red ginseng caused a decrease in insulin content in our study that was similar to results described in a previous report (Kim et al., 2011a; 2011b).

To verify the safety of the product, the effects of GOT/GPT on hepatotoxicity were measured through a 4-week animal experiment (Table 5). A higher GOT/GPT value indicated increasing hepatocyte damage. GPT in normal rats was 18.9 IU/mL, and that in diabetic rats treated with

Table 4 Effects of red ginseng and herbal extracts on glucose tolerance in streptozotocin-induced diabetic mice

	Initial glucose tolerance (mg/dL)	Final glucose tolerance (mg/dL) after 4 weeks
Control	141 ^a	138 ^a
STZ-Control	411 ^b	453 ^b
STZ + Red Ginseng	425 ^c	343 ^c
STZ + Red Ginseng + Herbal extract	416 ^b	332 ^c

Columns with different letters of a, b, and c indicate significant statistical differences ($p < 0.05$)

Table 5 Effects of red ginseng and herbal extracts on blood insulin level, GPT, and GOT in streptozotocin-induced diabetic mice

	Insulin (μ U/mL)	GPT (IU/mL)	GOT (IU/mL)
Control	20.9 ^a	18.9 ^a	31.5 ^b
STZ-control	9.7 ^b		
STZ-red ginseng	11.9 ^{bc}		
STZ-red ginseng-herbal extracts	13.4 ^c	16.4 ^a	29.8 ^b

Columns with different letters of a, b, and c indicate significant statistical differences ($p < 0.05$)

GPT and GOT were measured only in control and STZ-red ginseng-herbal extracts for the safety

the mixture of red ginseng extract and herbal medicines was 16.4 IU/mL after 4 weeks.

The change in GOT, which is an indicator of liver disease with GPT in plasma, was 31.5 IU/mL in the normal control group and 29.8 IU/mL in the red ginseng + herbal extract group. There was, however, no significant difference between the two groups. Therefore, it seems that the consumption of red ginseng and medicinal herb extract did not affect blood liver disease or toxicity.

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Compliance with ethical standards

Conflicts of interest The authors have declared no conflicts of interest.

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