

## Effect of $\alpha$ -Amylase on the Qualities of Red Ginseng Extract

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**Abstract :** In order to improve the qualities of red ginseng extract and decrease precipitate formation in ginseng drink, red ginseng extract were hydrolyzed with  $\alpha$ -Amylase and characteristics of the hydrolyzed ginseng extract were investigated. 1.08% of isomaltose were produced and glucose content was increased from 2.83% to 11.03% in the hydrolyzed red ginseng extract. Total ginsenoside content of the hydrolyzed ginseng extract were decreased from 1,661 mg/100 g extract to 1,389 mg/100 g extract. The hydrolyzed ginseng extract enhanced the growth of *Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus helveticus*. Bitterness and astringency of the hydrolyzed ginseng extract were lower than those of the ginseng extract. Precipitate formations in ginseng drink prepared with the hydrolyzed ginseng extract were significantly reduced in the storage conditions of 40°C for 4 weeks compared to those of control.

**Key words :**  $\alpha$ -Amylase hydrolysis, red ginseng extract, decrease of precipitate, intestinal bacteria growth factor

### INTRODUCTION

Recently, consumption of ginseng drink as health food is increased according to increase of interest in health.<sup>1)</sup> However, ginseng drink has a problem of precipitate formation during storage and it is led to deteriorate quality of ginseng drink.<sup>2)</sup> It has been reported that precipitation formation in ginseng drink was caused by interaction between starch and protein in ginseng extracts.<sup>3)</sup>

Meanwhile, functional maltooligosaccharides are mainly produced by hydrolysis with some amylase on starch and they have some physiological function such as low calorie, anticarcinogenicity and ability to promote growth of *Bifidobacterium* and some intestinal lactic acid bacteria.<sup>4-6)</sup>

In order to prevent precipitate formation in ginseng drink by ginseng starch and further to develop new functional substance from ginseng starch, we previously reported the effects of  $\beta$ -amylase and transglucosidase on the quality of red ginseng extract<sup>7)</sup> and optimum condition for the production of maltooligosaccharide from ginseng starch and its characteristics.<sup>8)</sup>

In this study,  $\alpha$ -amylase treated on red ginseng extract and characteristics of the hydrolyzed ginseng extract were

investigated.

### MATERIALS AND METHODS

#### 1. Materials, strains and cultivation

Korean red ginseng extract prepared by Korean Ginseng and Tobacco Research Institute was used for research samples. Amano A<sup>®</sup>  $\alpha$ -amylase was purchased from Amano Co.(USA) and all chemicals were of analytical reagent grade and HPLC grade. *Lactobacillus casei* LLG, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium infantis* were obtained from Biotechnology Laboratory, Macdonald College of McGill University, Canada. *Bifidobacterium bifidum* and *Bifidobacterium longum* were from the American Type Collection Culture (ATCC, Rockville, MD, USA). *Lactobacillus acidophilus* and *Bifidobacterium longum* were cultivated in M17 medium and ATCC 1053 medium at 30°C in anaerobic chamber, respectively and the other bacteria were cultivated in MRS medium at 37°C, anaerobically.<sup>8)</sup>

#### 2. Hydrolysis of red ginseng extract

Korean red ginseng extract (20 g) were solved in 50 ml of 50 mM phosphate buffer (pH 6.0) and hydrolyzed with 50 units of  $\alpha$ -amylase (Amano A<sup>®</sup>) at 85°C for 24 h and heated at 100°C for 15 min for inactivation of enzyme.

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Sample was centrifuged in  $8,000 \times g$  at  $5^\circ\text{C}$  for 20 min and the supernatant was used for analysis of sugars, functionalities and qualities.

### 3. Assay of sugars by RP-HPLC

A Waters HPLC System (Millipore, MA, USA) consisted of a 600E system controller, a U6K injector, a RI detector and millennium 2010 chromatography manager was used to detect isomaltose and other sugars. Samples were injected on an TSK Gel 60 column (Waters, Japan) and eluted at a flow rate of 1.0 ml/min. of water (18 ohm)/acetonitril (HPLC grade)/tris=35/65/0.1 in an isocratically for 60 min.<sup>7,8)</sup>

### 4. Assay of ginsenosides

Analysis of ginsenosides of the hydrolyzed red ginseng extract were performed as previous paper.<sup>7)</sup>

### 5. Evaluation of availability by intestinal bacteria

0.5%(w/v) of the extract samples were added in optimum medium and  $10^7 \pm 10^8$ /ml bacteria were inoculated and then incubated at  $37^\circ\text{C}$  or  $30^\circ\text{C}$  for 48 h anaerobically. Absorbance at 660 nm was determined and calculated their relative growth (RG) as follow.<sup>8,9)</sup>

$$RG(\%) = \frac{(OD_{\text{hydro}} - OD_{\text{media}})}{(OD_{\text{glucose}} - OD_{\text{media}})} \times 100$$

( $OD_{\text{hydro}}$ : absorbance at 660nm on growth of the hydrolyzed ginseng extract containing media,  $OD_{\text{media}}$ : absorbance at 660 nm on growth of glucose-free media,  $OD_{\text{glucose}}$ : absorbance at 660 nm on growth of glucose containing media)

### 6. Sensory evaluation

Samples were coded with three-digit random numbers and presented to participants. Sensory attributes of the hydrolyzed ginseng extract were scored on a nine-point hedonic scale (9=very strong to 1=very weak), and performed statistical analysis through valience analysis.

### 7. Precipitate formation

The pH of the hydrolyzed ginseng extract solution were adjusted from 3.0 to 4.5 by citric acid and sterilized at  $85^\circ\text{C}$  for 30 min and then stored for 4 weeks at  $40^\circ\text{C}$  incubator. After storing of each period, the hydrolyzed ginseng extract solution was centrifuged and weighed the precipitates.

## RESULTS AND DISCUSSION

### 1. Sugar contents

Sugar contents of the hydrolyzed ginseng extract were shown in the Table 1. Isomaltose as oligosaccharides was newly produced 1.08 g per 100 g of the hydrolyzed ginseng extract. Generally,  $\alpha$ -amylase hydrolyzes from amylose to glucose, maltose, maltotriose, maltooligosaccharides. But it is reported that on a long incubation, isomaltose is formed from maltose by this enzyme action.<sup>10)</sup> The maltotriose and the other maltooligosaccharides (G4-G7) were not detected and this result was quite different from those of produced maltotriose, maltotetraose, maltopentose and maltohexaose (G3-G6) from ginseng starch by  $\beta$ -amylase.<sup>8)</sup> We guessed that starch in red ginseng extract is dextrinized and more available to enzyme than raw ginseng starch. Total sugar contents of the hydrolyzed ginseng extract were increased from 15.74% to 19.31%. Glucose and fructose contents were also significantly increased, which is considered hydrolysate of maltooligosaccharides, sucrose and maltose.

### 2. Ginsenosides contents

Ginsenosides contents of the hydrolyzed ginseng extract were shown in Table 2. Total ginsenoside contents of the hydrolyzed ginseng extract were decreased about 270 mg per 100 g ginseng extract. Also, individual ginsenoside content were reduced. In previous paper,<sup>8)</sup>  $\beta$ -amylase and transglucosidase decreased ginsenoside- Rb<sub>1</sub> and Rb<sub>2</sub> but

**Table 1.** Sugar contents of the hydrolyzed ginseng extract

(Unit : %, dry weight basis)

Sample	rhamnose	fructose	glucose	sucrose	maltose	isomaltose	Total
Hydrolyzed ginseng extract	1.31	3.13	11.03	0.84	1.92	1.08	19.31
Ginseng extract	1.48	2.53	2.70	2.83	6.20	-	15.74

**Table 2.** Ginsenoside contents of the hydrolyzed ginseng extract

(Unit : mg/100 g ginseng extract)

Sample	Rb <sub>1</sub>	Rb <sub>2</sub>	Rc	Rd	Re	Rf	Rg <sub>1</sub>	Total
Hydrolyzed ginseng extract	39.1	30.9	50.8	83.4	79.1	269.5	836.7	1389.5
Ginseng extract	50.6	40.5	66.7	103.4	111.7	323.4	965.4	1661.7

**Table 3.** Availability of the hydrolyzed ginseng extract on growth of lactic acid bacteria.

Sample	<i>B. bifidum</i>	<i>B. infantis</i>	<i>B. longums</i>	<i>L. casei</i>	<i>L. acidophilus</i>	<i>L. rhamnosus</i>	<i>L. helveticus</i>
Hydrolyzed ginseng extract	+ (11.2)*	+ (20.4)	+ (21.4)	++ (52.3)	+ (37.1)	++ (81.6)	++ (48.9)
Ginseng extract	+ (17.3)	+ (26.9)	+ (21.4)	++ (47.7)	+ (38.5)	+ (17.0)	++ (44.7)

\*( ) ; relative growth for glucose (%)

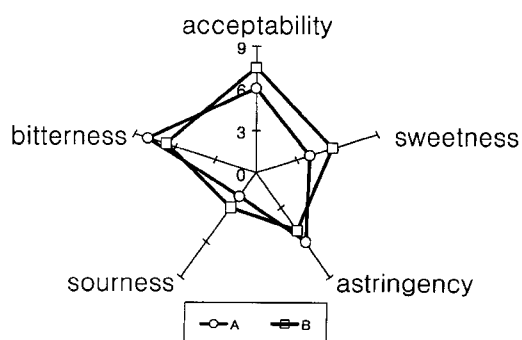
increased ginsenoside-Rd in the hydrolyzed red ginseng extract. In this study, decreasing of overall ginsenosides was resulted in 24h long time hydrolyzing of  $\alpha$ -amylase.

### 3. Availability by intestinal bacteria

Effect of the hydrolyzed ginseng extract on the growth of some intestinal bacteria was investigated (Table 3). The hydrolyzed ginseng extract enhanced the growth of *L. rhamnosus*, *L. casei* and *L. helveticus* and especially, growth of *L. rhamnosus* in the hydrolyzed ginseng extract containing medium was better than that of ginseng extract. However, the growth of bifidobacteria were not enhanced by both of ginseng extract. It is due to low isomaltose contents which was known growth factor of intestinal bacteria. This results were different to those of red ginseng extract hydrolyzed with  $\beta$ -amylase and transglucosidase,<sup>7)</sup> in which 5.2% isomaltose was contained.

### 4. Sensory evaluation

Taste profile of the hydrolyzed ginseng extract was compared to that of ginseng extract using 0.5% solution. As shown in Fig. 1, bitterness and astringency as undesirable taste of ginseng extract were reduced in the hydrolyzed ginseng extract, at that time F-value were 19.22 and 10.36 respectively. Whereas sweetness and sourness were increased. It was reported that bitter taste in ginseng is originated from ginsenosides and reduced in prosapo-

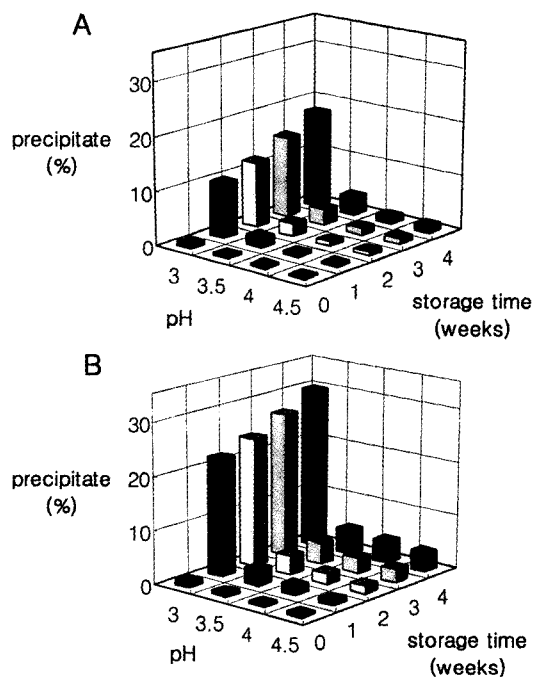


**Fig. 1.** Taste profiles of red ginseng extract (A) and the hydrolyzed red ginseng extract (B).

genins and sapogenins.<sup>11)</sup> In this study bitterness and sweetness are considered decreasing of ginsenosides content and increasing of total sugar content. The sour taste in hydrolyzed ginseng extract are considered as originated from enzyme taste.

### 5. Precipitate formation

Precipitate formation was investigated on 5% solution of the hydrolyzed ginseng extract (pH 3.0~4.5) during 4 weeks storage at 40°C. Precipitate was formed in both of the ginseng extract solution of pH 3.0 for 1 week storing (Table 2). However, precipitate was not formed at pH 4.0 and pH 4.5 in the hydrolyzed ginseng extract solution for 2 weeks storing. After 4 weeks less precipitate formed in hydrolyzed ginseng extract solution than control in all pH range (Fig. 2). From these data,  $\alpha$ -amylase treatment on the ginseng extract is considered as very



**Fig. 2.** Precipitate formation of red ginseng drink (pH 3.0~4.0) prepared with the hydrolyzed red ginseng extract (A) and ginseng extract (B) during storage for 4 weeks at 40°C.

desirable to reduce the precipitate formation in ginseng drink.

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