J. Ginseng Res.
Vol. 24, No. 1, 41-45(2000)

Enzymatic Hydrolysis of Korean Ginseng Starch and Characteristics of Produced Maltooligosaccharides

Na-Mi Kim#, Jong-Soo Lee* and Byung H. Lee**

#Korea Ginseng and Tabacco Research Institute, Taejeon 305-345, Korea
**Department of Genetic Engineering, Pai-Chai University, Taejeon 302-735, Korea*
***Department of Food Science and Agricultural Chemistry, Macdonald College of McGill University, 21,111 Lakeshore, Ste-Anne-de Bellevue, QC, H9X 3V9, Canada*

(Received March 6, 2000)

Abstract : Maltooligosaccharides were produced from ginseng starch by hydrolysis of α-amylase. And it was investigated that physicochemical properties and intestinal bacteria growing effect of maltooligosaccharides. The optimum level of the ginseng maltooligosaccharides was produced when 10% ginseng starch was hydrolyzed with 50 unit of Amano A® α-amylase per gram starch at 85°C for 24h. Viscosity and water holding capacity of the ginseng maltooligosaccharides were 37.7 cps at 20°C and 110.1% at 75% relative humidity, respectively. The ginseng maltooligosaccharides enhanced the growth of *Bifidobaterium infantis*.

Key words : Maltooligosaccharide, Korean ginseng starch, α-amylase, intestinal bacteria growth factor.

INTRODUCTION

Korean ginseng (*Panax ginseng C.A. Meyer*) is a medicinal plant used for treatment and prevention of many diseases.[1] From long physiological and biochemical studies on the ginseng effect, saponin was elucidated as main pharmacological substances of ginseng. Furthermore, non-saponin fractional substances such as alkaloids, acidic polysaccharide, phenolic compounds, polyacetylenes, protein, and amino acid were also isolated from Korean ginseng and their pharmacological action were reported.[2]

Recently, ginseng is used in the production of health food such as ginseng drink and tea. However, ginseng drink has a problem of precipitate formation during storage and deterioration of quality. Precipitates formation in ginseng drink is caused by co-relation of ginseng starch and protein[3,4] and Kim et al[4] reported precipitates formation was reduced through elimination of the ginseng starch.

With the rising concern for oligosaccharide as physiologically active ingredients,[5,6] some attempt were made to find a new maltooligosaccharide from starch. In general, functional maltooligosaccharide was produced from starch by liquefying of α-amylase and it has some physiological functionality such as low calorie, anti-calcinogenicity and ability to promote growth of intestinal bacteria.[7,8] Production and characterization of oligosaccharides were intensively studied, but it has not been studied on development of functional maltooligosaccharides from Korean ginseng starch and further prevention of precipitates formation in the ginseng drink.

Therefore, in this study, optimum conditions for production of maltooligosaccharides from ginseng starch were investigated and characterized the ginseng maltooligosaccharides.

To whom correspondence should be addressed.
(Tel) 042-866-5424; (Fax) 042-866-5419
(E-mail) nmkim@gtr.kgtri.re.kr

MATERIALS AND METHODS

1. Materials, strains and culture condition

Korean fresh ginseng (four-years old) was purchased from Kumsan area of Korea and amylases were from Amano Co. (USA). All chemicals were of analytical reagent grade and obtained from Sigma Chemical Co. (St. Louis. Mo. USA). *Lactobacillus casei* LLG, *Lactobacillus helveticus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium infantis* were obtained from biotechnology lab., Macdonald College of McGill University, Canada. *Bifidobacterium bifidum* and *Bifido-*

bacterium logum were also 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. The other intestinal bacteria were cultivated in MRS medium at 37°C in anaerobic chamber.

2. Extraction of starch from Korean ginseng

One hundred grams of fresh ginseng roots were homogenized in distilled water (1:1 w/v) and filtered through gauze, following centrifuged at 6000×g for 10 min. The precipitates were suspended in distilled water and centrifuged at 6000×g for 10 min and then repeated 3 times. The crude ginseng starch was resuspended in 99.9% methanol and removed the impurities by centrifugation at 8000×g for 10 min. The supernatants were lyophilized and obtained the ginseng starch.

3. Production and purification of the ginseng maltooligosaccharides

Ginseng starch slurry (10%) was gelatinized by boiling for 20 min and adjusted to pH 6.0 and then hydrolyzed with Amano-A® α-amylase for 8 h at 85°C. The hydrolysates were heated at 100°C for 20 min for inactivation of the amylase and then filtered. The filtrates were absorbed on active carbon-celite column (active carbon : celite 545 =2 : 1) and washed with distilled water, following 5% ethanol. The maltooligosaccharide was eluted by 20% ethanol and then lyophilized.

4. Assay of ginseng maltooligosaccharides by RP-HPLC

A Waters HPLC system (Millipore, MA, USA) consisted of a 600E system controller, a U6K injector, a RI detector and millenium 2010 chromatography manager, was used to detect maltooligosaccharides. Sample were injected on an TSK Gel 60 column (Waters, Japan) and eluted at a flow rate of 1.0 m*l*/min of water (18 ohm)/acetonitril (HPLC grade)/tris=35/65/0.1 in an isocratically for 60 min. Standards of maltooligosaccharides were maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltohepatose (Wako, Japan).

5. Characteristics of ginseng maltooligosaccharides

Viscosity was determined by using Ostwald viscometer (Sigma, USA) on the 5.0% solution at 20°C, 40°C and 60°C. Water holding capacity was determined as follow; 10g sample was placed in relative humidy 50%, 75% and 90% chamber saturated with sodium dichromate, sodium chlorate and zinc sulfate for 2 weeks and then weighed the increased weight. Sweetness of the ginseng maltooligosaccharide was compared with sucrose by sensory evaluation test on the 5% solution. In order to investigate availability of the ginseng maltooligosaccharide by intestinal bacteria, 0.5% (w/v) of the ginseng maltooligosaccharide were added in optimum medium and 10^7~10^8/m*l* of bacteria were inoculated and incubated at 30°C or 37°C for 48 h. anaerobically. Absorbance was determined at 660 nm and calculated their relative growth (RG) as follow.[9]

$$RG\ (\%)=(OD_{oligo}-OD_{media})/(OD_{glucose}-OD_{media})\times 100$$

(OD_{oligo} : absorbance at 660 nm on growth in the maltooligosaccharide containing media, OD_{media} : absorbance at 660 nm on growth in glucose-free media, $OD_{glucose}$: absorbance at 660 nm on growth in glucose containing media)

RESULTS AND DISCUSSION

1. Optimum hydrolyzing conditions for the ginseng maltooligosaccharides (MOS) production

To optimize conditions for the MOS production from ginseng starch, effect of concentration of ginseng starch and amylase, reaction time and addition of calcium chloride were investigated. 10% of ginseng starch were hydrolyzed with two kinds of commercial α-amylase under the recommended conditions and determined content of the MOS produced (Table 1). Biozyme L® α-amylase produced exclusively maltose and maltotriose, while Amano A® α-amylase produced various maltooligosaccharides such as maltotriose, maltotetraose, maltopentaose and maltohexaose (G3-G6). The differences of main hydrolysis products between Biozyme L® α-amylase and Amano A® α-amylase might be caused by differences of enzyme origins, that is, Biozyme L® α-amylase was produced from *Aspergillus niger* while Amano A® α-amylase was produced from *Bacillus subtilis*. Because Amano A® α-amylase produced more MOS(G3-G6) than Biozyme L® α-amylase, it was decided to use Amano A® α-amylase for production of the MOS from ginseng starch. It was reporeted that α-amylases from different origins produce various types of main hydrolysis products.[10,11] G1, G2. G3 and several branched dextrin were mainly produced by human salivary and pancreatic α-amylase, G2 and G3 by porcine pancreatic α-amylase, G2-G7 by plant α-amylase,

initially maltose and maltooligosaccharides by fungal α-amylase, G3 and G4 by *Clostridium sp.*, G3 by *streptomyces griseus*, G4 by *Pseudomonas stutzeri, Pseudomonas saccharophilia, Bacillus circulans, streptomyces sp.*, G5 by *Bacillus licheniformis, Bacillus cereus NY-14, Pseudomonas KO-8940, Bacillus sp.*, and G6 by *Bacillus circulans, Aerobacter aerogenes, Bacillus subtilis, Bacillus circulans* F-2. Specifically, α-amylase of *Bacillus subtilis* and related bacteria (aerobic, spore forming, mesophiles) is divided into two types from the specificity : starch liquefying α-amylase and starch saccharifying α-amylase. The liquefying α-amylase is one of the industrially important enzyme and the enzyme produce G1-G6 and various branched oligosaccharide from starch.

In order to select optimum concentration of ginseng starch for the MOS production, various concentration of ginseng starch (5~30%) were gelatinized with boiling for 20 min and hydrolyzed with Amano A® α-amylase. As shown in Table 2, the amounts of MOS produced were increased from 375.8 μg to 1044.96 μg per 30 m*l* but yields were decreased from 25.05% to 11.61% as the starch concentration raised from 5% to 30%. So, optimum concentration of ginseng starch was selected to 10%. It is considered that the lower yield of the MOS at above 20% starch concentration is due to the poor solubility of ginseng starch and production of light molecular branched dextrin (>G_6). Generally, solubility of ginseng starch were reported below 20% in heating for 30 min at 90°C.[11]

Because the degree of hydrolysis of starch by α-amylase vary depending on the amount of enzyme added more or less, we investigated the optimum concentration of Amano A® α-amylase for MOS production. 1~200 Units of α-amylase per gram starch were added in 10% ginseng starch solution and digested for 24 h at 85°C.

As the enzyme concentration increased, total and G2, G3 oligosaccharide contents were gradually increased, however G6 and G7 oligosaccharide contents were not increased or slightly increased at above 50 unit of enzyme concentration (Table 3). Therefore, we concluded that 50 unit of Amano A® α-amylase per gram starch is suitable for production the MOS.

Next, the effect of enzyme reaction time on the production of MOS was investigated at various reaction time (2~24h) for mixture of 10% ginseng starch and 50 units of Amano A® α-amylase per gram starch (Table 4). Optimum reaction time for MOS production was 24 h and it was produced approximately 26.95g of MOS from 100g ginseng starch. Total MOS contents were not change as reaction time was longer, however G2, G3 contents were

Table 1. Comparison of maltooligosaccharides production by commercial α-amylases (Unit; g/100g ginseng starch)

Enzyme*	G2	G3	G4	G5	G6	Total
Amano A	13.84	3.40	3.13	3.03	4.81	28.21
Biozyme L	6.82	6.48	0.27	0.96	1.73	16.26

*Substrate: 10% (w/v) ginseng starch
Amano A® Substrate: 10% (w/v) ginseng starch Amano A reaction condition: temp. 85°C, pH 6.0, enzyme unit 100 U/g, reaction time 24 h
Biozyme L® reaction condition: temp. 60°C, pH 5.0, enzyme unit 100 U/g, reaction time 24 h

Table 3. Effect of enzyme concentrations on the production of maltooligosaccharides (Unit; g/100g ginseng starch)

Enzyme conc. (unit/g starch)*	G2	G3	G4	G5	G6	G7	Total
1	0.54	1.13	1.41	0.43	0.58	2.38	6.47
5	1.24	1.77	1.73	0.75	1.96	5.24	12.68
10	2.27	2.53	1.76	1.18	2.75	5.24	15.73
25	3.08	3.01	1.76	1.71	3.53	6.19	19.28
50	3.63	3.93	1.76	1.92	4.97	9.04	25.25
100	5.14	4.62	2.93	2.78	5.37	9.04	30.36
200	7.36	5.00	3.64	3.32	5.50	9.04	33.86

*Substrate was 10% ginseng starch
*Reaction conditions; 85°C, pH 6.0, 24 h, 100 rpm shaking

Table 4. Effect of enzyme reaction time on the production of maltooligosaccharides (Unit; g/100g ginseng starch)

Reaction time(h)*	G2	G3	G4	G5	G6	G7	Total
2	4.65	4.32	2.40	3.85	6.38	5.62	27.22
4	4.54	4.24	2.41	3.85	6.41	5.71	27.16
8	3.68	3.55	2.46	3.74	6.54	6.19	26.16
16	2.71	3.54	2.64	3.74	6.68	6.67	25.98
24	2.65	3.55	2.76	4.17	6.68	7.14	26.95

*10% ginseng starch was digested with 50 unit of α-amylase per gram starch at 85°C for various reaction time.

Table 2. Effect of starch concentrations on the production of maltooligosaccharides (Unit; μg/30 m*l*)

Starch conc.(%)*	G2	G3	G4	G5	G6	Total	Yield(%)
5	63.09	67.60	76.35	62.03	106.73	375.80	25.05
10	115.09	101.86	93.77	90.88	144.23	585.83	19.53
20	200.34	147.23	105.82	134.15	204.80	792.34	13.21
30	215.68	217.61	129.93	158.68	323.06	1044.96	11.61

*Ginseng starch was digested with 100 unit enzyme per 10 m*l* ginseng starch solution of different concentration at 85°C for 24 h.

decreased and G6, G7 contents were increased controversially.

Because activity of α-amylase is activated by calcium ion,[10] effect of calcium ion on the production of ginseng MOS was investigated at various concentration of calcium chloride (2 mM~10 mM). Calcium ion had little effected on the MOS production from ginseng starch by α-amylase (data not shown).

Based on these results, it was concluded that optimum reaction conditions were 50 unit of Amano A® α-amylase per gram ginseng starch, 10% starch concentration and 24 h of reaction time for the production of the ginseng MOS from ginseng starch.

2. Characteristics of the ginseng maltooligosaccharide

Generally, maltooligosacchrides were known that it have some kinds of characteristics such as high viscosity and water holding capacity, low sweetness compared to sucrose and less color formation than corn syrup in drink preparation.[1] The MOS produced from ginseng starch by treatment of α-amylase was purified by active carbon-celite column chromatography described above. We investigated characteristics of the purified MOS such as viscosity, water holding capacity, sweetness and availability by intestinal bacteria. Viscosities of the ginseng MOS (5% soln.) was 37.7 cps at 20°C, 24.5 cp at 40°C and 14.6 cps at 60°C, respectively. It was higher than that of sucrose (Fig. 1). Water holding capacity of the ginseng MOS in relative humidity 50%, 75% and 90% were 5.03%, 10.12% and 55.17% respectively, which were higher than that of sucrose (Table 5). We inferred that high water holding capacity of the MOS was caused by high contents of maltotriose which has high water holding capacity. In addition, sweetness of the ginseng MOS (5% soln) was evaluated to 25.8% that of sucrose.

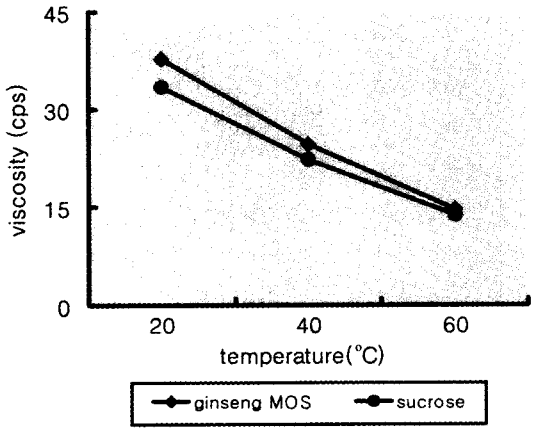

Fig. 1. Viscosities of the ginseng maltooligosaccharides.

Table 5. Water holding capacity of the ginseng maltooligosaccharides unit : % (g water/100g)

Components	Relative humidity(%)		
	50	75	90
Maltoolgosccharide	5.03	10.12	55.17
Sucrose	-0.13	3.01	21.04

One of important functionality of maltooligosaccharides is growth promotion effect of useful intestinal bacteria. We investigated the effect of the ginseng MOS on growth of some useful intestinal bacteria (Table 6). The growth of *B. infantis* was enhanced by ginseng MOS rather than glucose. *L. helveticus, L. casei, L. rhamnosus* and *B. longum* utilized the ginseng MOS. However, *B. bifidum* and *L. acidophilus* had little ability to utilize it.

요 약

인삼전분으로부터 기능성 말토올리고당을 생산하기 위하여 인삼전분에 대한 말토 올리고당 생산 최적조건을 검토하고 이들을 정제 한 후 물리화학적 특성과 장내 유용 세균에 대한 생육효과를 조사하였다. Amano-A® amylase를 사용하였을 때 glucose가 4개 이상 결합된 말토 올리고당이 많이 생성되어 최적 효소로 선정하였고, 이 효소를 이용한 말토 올리고당 생산 최적조건은 인삼전분 10%, 효소 첨가 농도 50 unit/g 전분 과 반응시간 24시간이었다. 인삼전분을 효소분해하여 생산하고 carbon-celite로 정제한 말토올리고당의 점도와 보수력은 각각 37.7 cps(20°C)와 110%(75% 상대습도)로 설탕에 비하여 높았으며, 감미도는 설탕의 25.6% 이었다. 또한 생산된 말토올리고당은 장내 유용세균인 *Bifidobacterium infantis* 의 생육을 촉진시켰다.

Table 6. Availability of the ginseng maltooligosaccharides by intestinal bacteria

Components	*B. bifidum*	*B. infantis*	*B. longum*	*L. casei*	*L. acidophilus*	*L. rhamnosus*	*L. helveticus*
Glucose	+++	+++	+++	+++	++	+++	+++
Maltooligo saccharides	+ (18.6)**	+++ (122.5)	++ (51.6)	+ (47.9)	+ (7.1)	++ (50.6)	++ (96.3)

*Intestinal bacteria were incubated in each optimal media containing glucose or ginseng MOS.
**( ); relative growth (%, Material and Methods)

REFERENCES

1. The Soc. for Korean Ginseng : Understanding of Korean Ginseng. p35, Hanrimwon, Seoul, Korea (1995).
2. Han B. H. : Chemical and biochemical studies on the non-saponin substances of ginseng. The 3rd symposium of life science. Korean Ginseng Res. Inst. p113 (1992).
3. Kim N. M., Yang J. W., Kwak Y. S. and Sung H. S. : *Korea J. Ginseng Sci.* **18**, 122 (1994).
4. Kim N. M., Lee J. T. and Yang J. W. : *Korea J. Ginseng Sci.* **20**, 54 (1996).
5. Berger, J. L., Lee B. H. and Lacroix C. : *Biotechol. Letter* **17**, 1077 (1995).
6. Blenford D. : Functional foods : prescribing dietary medicine. Food Ingredients and Analysis. 17, 21 (1995).
7. Modler, H. W., McKeller R. C. and Yaguchi M. : Bifidobacteria and bifidogenic factor. Can. Inst. Food Sci. Technol (1992).
8. Park K. H. and Kim J. W. : Modulation of B. amylolytic enzyme and production of branched oligosaccharides. Proc. '94 Arg. Biotechnol. Symposium. Suwon. Korea. p.51 (1994).
9. Kim J. H. : Production and characteristics of β-galactosidase and galactooligosaccharide from *L. bulgaricus*. Ph D. Thesis of Chungnam Natl. University. Graduate school (1992).
10. The Amylase Res. Soc. Japan (1988). Handbook of Amylases and Related Enzymes. p.18, Pergamon Press, New York, U.S.A.
11. Park, K. H. : Development of functional oligosacharide-producing enzymes symposium on biologically active compounds. Research group of biologically active compounds p.28 (1995).
12. Ahn Y. J., Kim M. J., Kawamura, T., Yamamoto, T., Fujisawa, T. and Mitsuoka, T. : *Korea J. Ginseng Sci.* **14**, 253 (1990).