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Arginyl-fructosyl-glucose and Arginyl-fructose, Compounds Related to Browning Reaction in the Model System of Steaming and Heat-drying Processes for the Preparation of Red Ginseng

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Abstract : Brown color intensity has been a major factor to estimate the quality of red ginseng and its products. This study deals with the relationship between the browning reaction of ginseng root and two compounds, arginyl-fructosyl-glucose (Arg-fru-glc) and arginyl-fructose (Arg-fru), in the model system of steaming and heat-drying processes for the preparation of red ginseng. During the steaming process, a marked decrease of starch and a considerable formation of maltose occurred in main roots of raw ginseng, but the formation of glucose was scarcely observed. After the heat-drying process, the brown color intensity of the powdered preparation of steamed main roots was 3 to 4 times higher than that of the powdered preparation of raw main roots. Also, when the heat- drying process was done with the addition of L-arginine, brown color intensity of the powdered preparation of steamed main roots was 12 to 13 times higher than that of the powdered preparation of raw main roots. The amount ratios of browning reaction products formed from sugar compounds and amino acids in the model system of steaming and heat-drying treatments *in vitro* were in order of xylose > glucose > fructose > maltose > dextrin (DE 19) > sucrose > dextrin (DE 8) and soluble starch. Each solution of Arg-fru-glc and Arg-fru that were synthesized chemically from maltose plus L-arginine and glucose plus L-arginine, respectively, changed from colorless to brown color during the heat-drying treatment. Amino acids or sugars were effective on the acceleration of each browning reaction of Arg-fru-glc and Arg-fru during the heat-drying treatment.

Key words : Arginyl-fructosyl-glucose, arginyl-fructose, red ginseng, browning reaction, amino acids, sugars.

INTRODUCTION

A new amino acid derivative, arginyl-fructosyl-glucose (Arg-fru-glc), was first isolated in 1994 from Korean red ginseng[1] (Fig. 1). Red ginseng contained a considerable amount of this new compound (42.8 mg/g), while white ginseng possessed only a trace amount. A small amount of another amino acid derivative, arginyl-fructose(Arg-fru), was also detected in Korean red ginseng. These compounds, Arg-fru-glc and Arg-fru, were considered to be formed from L-arginine plus maltose and L-arginine plus glucose, respectively, by the maillard reaction during the processing from raw ginseng to the red one.[1]

Brown color intensity has been a major factor to estimate the quality of red ginseng and its products. This study deals with the relationship between the browning reaction and two compounds, Arg-fru-glc and Arg-fru, in the model system of steaming and heat-drying processes for the preparation of red ginseng.

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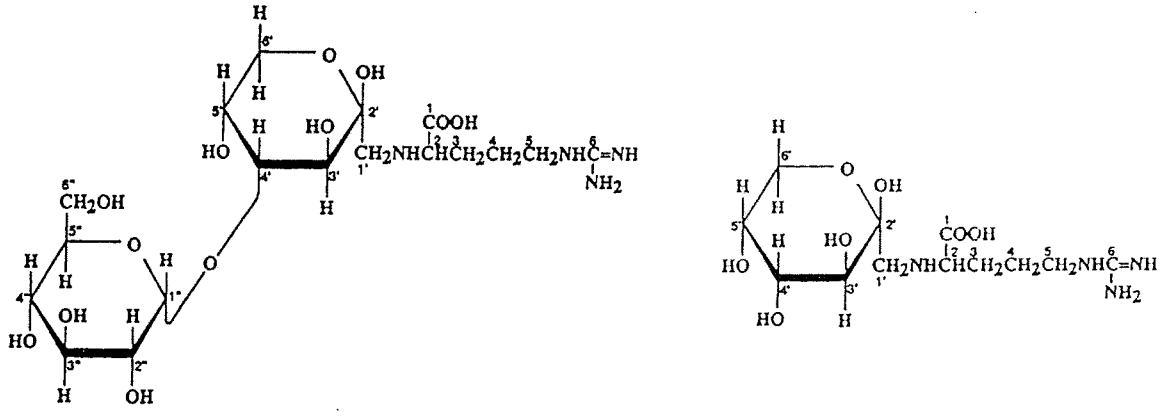
MATERIALS AND METHODS

Materials

Raw ginseng roots (*Panax ginseng* C. A. Meyer, Araliaceae) were harvested on September in 1998, after cultivation for 6 years in the Suwon experimental farm of KT & G Central Research Institute in Daejon, Korea. Amino acids, xylose, glucose, fructose, sucrose, and a-amylase (type II-A) from *Bacillus* sp. (Sigma Chemical Co., St. Louis, MO, USA), soluble starch and silica gel 60 (70-230 mesh)(E. Merck, Darmstadt, Germany), Amberlite IR-120 (Aldrich Chemical Co., Inc., USA), dimethyl sulfoxide (Wako Pure Chemical Industries, Ltd., Osaka, Japan), glucoamylase from *Rhizopus* sp. (Toyobo Co., Ltd., Osaka, Japan), ABTS [2,2'-azino-di-(3-ethyl benz-



(I) Arginyl-fructosyl-glucose (II) Arginyl-glucose

Fig. 1. Chemical structures of arginyl-fructosyl-glucose and arginyl-fructose.

thiazoline sulfonate)], glucose oxidase (grade III) from *Aspergillus niger*, and peroxidase (grade II) from horseradish(Boehringer Mannheim Yamanouchi Co. Ltd., Tokyo, Japan) were obtained from commercial sources. Maltose (HHH) and dextrins [Pine-Dex #1 (DE, 8) and Pine-Dex #4 (DE 19)] were kindly supplied by Hayashibara Biochemical Laboratory, Inc., Okayama, Japan and by Matsutani Chemical Kogyo Co., Ltd, Itami, Japan, respectively. Other chemicals were of analytical reagent grade and commercially available. Arg-fru-glc was synthesized by heating the mixture of maltose and L-arginine dissolved in glacial acetic acid with a reflux condenser in a water bath at 75-80°C under stirring, followed by purification with Amberlite IR-120 (H-type) column chromatography, lyophilization, and duplicate silica gel 60 column chromatography, according to the reported procedure.[2] The FAB-MS, ^{1}H- and ^{13}C-NMR spectral data of the synthesized Arg-fru-glc were identical with those described in the reported paper.[1] Arg-fru was also synthesized by heating the mixture of L-arginine (2.5 g) and glucose (5 g) in glacial acetic acid (50 ml) with a reflux condenser in a water-bath at 70°C for 10 min under stirring, concentrated to dryness, and purified by combined column chromatography of Amberlite IR-120 (H-type) and silica gel 60 according to the previously reported procedures,[2] followed by decolorization with active carbon, to afford Arg-fru as a white powder from the mixture of 85% methanol-*n*-butanol (1 : 1,v/v)(yield, 14.9% on the basis of L-arginine). The synthesized Arg-fru (Fig. 1): a white amorphous powder; FAB-MS (positive): *m/z* 337(M+1)$^{+}$; ^{1}H-NMR (400 MHz, D_2O) δ : 1.51 (1H, ddd-like, H-4), 1.80 (1H, dd-like, H-3), 3.06 (1H, t, H-5), 3.15 (1H, dd, H-2), and 3.50-4.30 (7H, ring protons of sugar); ^{13}C-NMR (100 MHz, D_2O) δ : 24.6 (C-4), 27.3 (C-3), 41.1 (C-5), 53.1 (C-1'), 63.4 (C-2), 64.6 (C-6'), 69.6 (C-3'), 70.0 (C-5'), 70.5 (C-4'), 96.0 (C-2'), 157.5 (C-6), and 173.8 (C-1).

Analyses

The assays of sugars and starch in raw and steamed ginseng roots were done as follows: After the steaming at 97°C for 0-2.5 hr in an autoclave, each of ten disk sections with about 2 mm in thickness (total wet weight, 12-15 g) was cutted in 3cm away from each top part of ten main roots, and the combined ten disk sections were homogenized with 100 ml of 80% ethanol using an electric mixer. The homogenate was boiled for 10 min, evaporated to dryness *in vacuo*, allowed to keep in the desiccator containing diphosphorus pentoxide for one week, and pulverized in a mortar with a pestle. The powdered preparation was extracted 3 times with 80% ethanol for 1 hr at 85°C under reflux, and the combined extract was centrifuged at 3,500 rpm.. The supernatant (80% hot ethanol- soluble fraction) was evaporated to dryness, and suspended in water. The suspension was extracted 3 times with *n*-butanol saturated with water to remove ginseng saponins, and centrifuged. The water layer was evaporated, dissolved in water, and filtered through a 0.45 μm-filter membrane. Sugars in the filtrate were analyzed by HPLC. HPLC conditions were as follows: column, LiChrosorb NH_2 (4×250 mm, 5 μm); solvent, acetonitrile-water (84 : 16, v/v); flow rate, 1.3 ml/min; detector, Water's R-401 differential refractometer. Also, the precipitate (80% hot ethanol-insoluble fraction) was extracted 3 times with dimethyl sulfoxide to solubilize starch by heating in a boiling water bath for 30 min, and then the

amount of starch in the extract was measured in the following two steps: the complete hydrolysis of starch to glucose by the combined actions of glucoamylase and a trace amount of a-amylase, and then the determination of glucose by glucose oxidase method using ABTS.[3,4] Brown color intensity was measured by the absorbance at 420 nm with Hewlett Packerd 8452A spectrophotometer. FAB-mass spectrometry was done on a Finnigan MAT 900 mass spectrometer. NMR spectra were taken on a Burker AMX 400 spectrometer in D_2O.

RESULTS

1. Browning reaction of main roots of raw ginseng in the model system of steaming and heat-drying processes

During the steaming process, the starch content of main roots of raw ginseng markedly decreased and a considerable amount of maltose was formed, but the formation of glucose was very weak (Table 1). The sucrose content decreased a little during the steaming process. To the powdered preparation of steamed main roots was added L-arginine being the most abundant free amino acid in raw ginseng root,[5] and then the mixture was treated with heat-drying. After the heat-drying process, the brown color intensity (dark brown color) of the mixture containing the powdered preparation of steamed main roots and arginine was 12 to 13 times higher than that (light brown color) of the mixture containing the powdered preparation of raw main roots and arginine (Table 2). While, after the heat-drying process without the addition of arginine, the brown color intensity (light brown color) of the powdered preparation of steamed main roots was 3 to 4 times higher than that (light yellow color) of only the powdered preparation of raw main roots. These results show that a considerable amount of reducing sugar such as maltose was formed from starch in main root of fresh raw ginseng during the steaming process, but the formation of free amino acid from protein and peptide was low.

2. Formation of browning reaction products from starch-type carbohydrates and amino acids in the model system of steaming and heat-drying treatments *in vitro*

When the mixture of starch-type carbohydrate and amino acid dissolved in buffer (pH 6.4) was treated with steaming

Table 1. Fluctuation of starch and sugars in main roots of ginseng during steaming process (unit: %, dry weight basis)

Time of steaming at 97°C (min)	Starch[a)]	Maltose[b)]	Sucrose[b)]	Glucose[c)]
0	29.3	+	11.0	0.2
60	12.7	14.5	11.5	0.4
110	10.0	15.2	9.1	0.1
150	10.6	15.0	7.9	0.3

*starch (mg) = glucose (mg)×0.9
[a)] assay by glucoamylase (with a trace amount of α-amylase)-glucose oxidase method
[b)] assay by HPLC
[c)] assay by HPLC and glucose oxidase method

Table 2. Browning reaction of the powdered preparation of steamed main roots with or without the addition of L-arginine during heat-drying process at 70°C

Sample No.	Time of Steaming (min)	L-Arginine Added (mg)	Time of Heat-drying (hr)	Relative ratio of brown color intensity
1	0	0	20	1.00
2	60	0	20	3.33
3	110	0	20	3.16
4	150	0	20	3.89
5	0	44	20	3.40
6	60	44	20	40.77
7	110	44	20	42.87
8	150	44	20	44.67

Reaction mixture contained 250 mg of the powdered preparation of main roots treated with steaming for 0-2.5 hr at 97°C and 5 ml of 0.05 M sodium phosphate buffer (pH 6.4) with or without 44 mg of L-arginine. The mixture was treated with heat-drying for 20 hr at 70°C After the heat-drying treatment, the dried mixture was suspended in water to make a final volume of 10 ml, stirred gently for 3 hr at room temperature, and centrifuged at 3,500 rpm. The supernatant was filtered through a 0.45 μm-filter membrane. Brown color intensity of the filtrate was measured by the absorbance at 420 nm. The relative ratio of brown color intensity of each filtrate was calculated as the ratio to the absorbance ($OD_{420\ nm}$, 0.135) of the control filtrate of only the powdered preparation of raw ginseng main roots.

and heat-drying, the amount ratios of browning reaction products were in order of glucose>maltose> dextrin (DE 19)≫dextrin (DE 8)>soluble starch (Fig. 2). Also, their ratios for sugars were in order of xylose>glucose> fructose>maltose≫sucrose (Fig. 3). The browning reaction

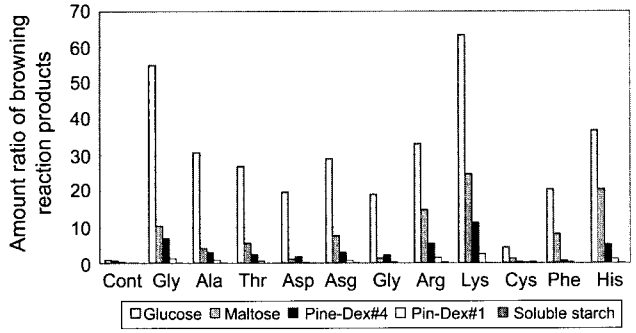


Fig. 2. Formation of browning reaction products from starch-type carbohydrates and amino acids by steaming and heat-drying treatments.
Reaction mixture contained 1 mmole of sugar (or 100 mg of Pine-Dex #1, #4, soluble starch) and 0.2 mmoles of amino acid in 10 ml of 0.2 M sodium phosphate buffer (pH 6.4). The mixture was treated with steaming for 2.5 hr at 100°C in an autoclave, and then with heat-drying 20 hr at 70°C After the heat-drying treatment, the dried mixture was dissolved in water to make a final volume of 10 ml, and filtered through the filter membrane. Brown color intensity of the filtrate was measured by the absorbance at 420 nm. The amount ratio of browning reaction products in each filtrate was calculated as the ratio to the amount (OD 420 nm, 0.374) in the control filtrate of glucose.
Cont, control; Gly, glycine; Ala, alanine; Thr, threonine; Asp, aspartic acid; Asg, asparagines; Glu, glutamic acid; Arg, arginine; Lys, lysine; Cys, cysteine; Phe, phenylalanine; and His, histidine.

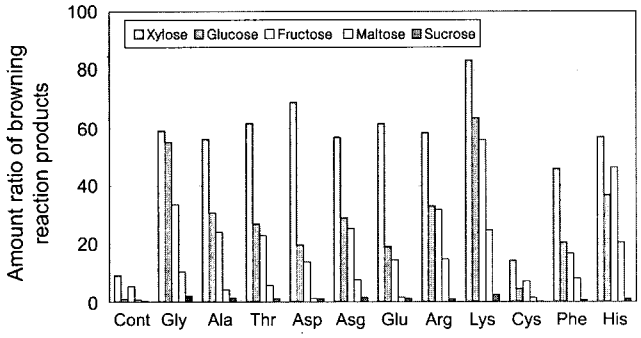


Fig. 3. Formation of browning reaction products from sugars and amino acids by steaming and heat-drying treatments.
Reaction mixture contained 1 mmole of sugar and 0.2 mmoles of amino acid in 10 ml of 0.2 M sodium phosphate buffer (pH 6.4). The other experimental conditions were the same as described in Fig. 2.

of maltose was accelerated in order of lysine>histidine> arginine>other amino acids tested. Browning reaction products were formed from maltose and arginine *in vitro* during the heat-drying treatment.

3. Formation of browning reaction products from Arg-fru-glc or Arg-fru in the model system of steaming and heat-drying treatments *in vitro*

A new conjugate of sugar and amino acid, Arg-fru-glc, was isolated from Korean red ginseng.[1] The conjugate is considered to be an early product formed non-enzymatically from maltose and L-arginine by Maillard reaction. As shown in Fig. 4 and 5, browning reaction products were formed from only Arg-fru-glc *in vitro*, and the solution of Arg-fru-glc dissolved in buffer (pH 6.4) changed from colorless to brown color during the steaming and heat-drying treatments, but did little during only the steaming treatment. The browning reaction of Arg-fru-glc was accelerated by the addition of amino acids (such as lysine, glycine, histidine, alanine, arginine, threonine, and asparagine) or sugars (such as xylose, glucose, and fructose). Also, the similar accelerating effects of sugars and amino acids were observed with the browning reaction of Arg-fru (Fig. 6).

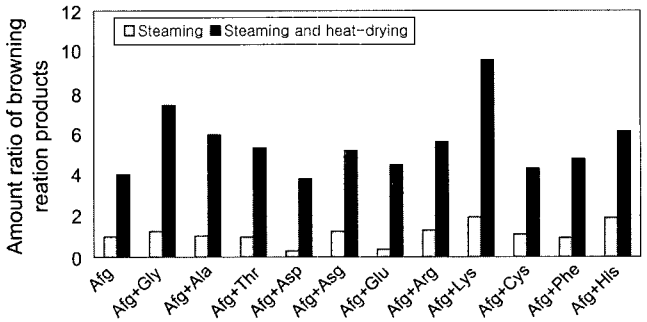


Fig. 4. Addition effects of amino acids on browning reaction of Arg-fru-glc by steaming and heat-drying treatments.
Reaction mixture contained 40 μmoles of Arg-fru-glc and 0 or 40 μmoles of amino acid in 2 ml of 0.2 M sodium phosphate buffer (pH 6.4). The mixture was treated with steaming for 2.5 hr at 100°C in the autoclave, and then with heat-drying for 20 hr at 70°C After the heat-drying treatment, the dried mixture was dissolved in 0.05 M sodium phosphate buffer (pH 6.4) to make a final volume of 10 ml, and filtered through the filter membrane. Brown color intensity of the filtrate was measured by the absorbance at 420 nm. The amount ratio of browning reaction products in each filtrate was calculated as the ratio to the amount (OD 420 nm, 0.106) in the control filtrate of Arg-fru-glc after the steaming treatment. Afg: arginyl-fructosyl-glucose.

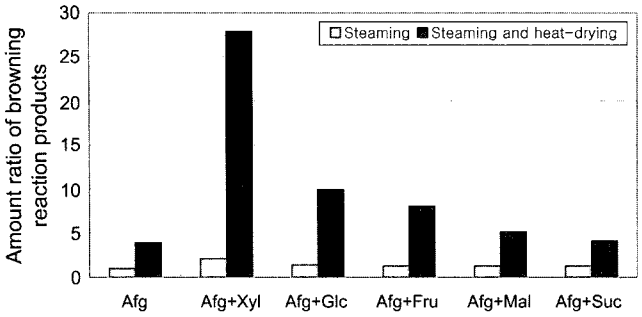


Fig. 5. Addition effects of sugars on browning reaction of Arg-fru-glc by steaming and heat-drying treatments.
Reaction mixture contained 40 μmoles of Arg-fru-glc and 200 μmoles of sugar in 2 ml of 0.2 M sodium phosphate buffer (pH 6.4). The other experimental conditions were the same as described in Fig. 4. Afg : arginyl-fructosyl-glucose, Xyl : xylose, Glc : glucose, Fru : fructose, Mal: maltose, and Suc: sucrose.

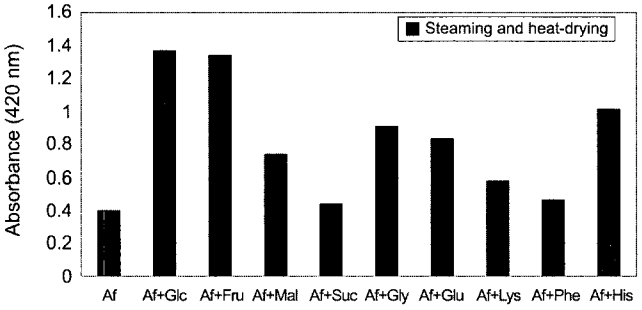


Fig. 6. Addition effects of sugars and amino acids on browning reaction of Arg-fru by steaming and heat-drying treatments.
Reaction mixture contained 8 μmoles of Arg-fru and 40 μmoles of sugar (or 30 μmoles of amino acid) in 0.4 ml of 0.2 M sodium phosphate buffer (pH 6.4). After steaming at 100°C for 2.5 hr in the autoclave and heat-drying at 70°C for 20 hr, browning reaction products were dissolved in 2 ml of 0.05 M sodium phosphate buffer (pH 6.4), and filtered. The brown color intensity of the filtrate was measured by the absorbance at 420 nm. Af: arginyl-fructose.

DISCUSSION

We studied on the relationship between the browning reaction of ginseng root and two compounds, Arg-fru-glc and Arg-fru, in the model system of steaming and heat-drying processes for making red ginseng. The marked decrease of starch and a considerable formation of maltose were observed in main roots of ginseng during the steaming process. When the heat-drying process was done with the addition of arginine, the formation of browning reaction products was observed in quantity with the powdered preparation of steamed main roots, but did a little with the powdered preparation of raw main roots. Also, browning reaction products were formed from maltose and arginine *in vitro*, but did not from soluble starch and arginine during the heat-drying treatment. These results strongly show that these browning reaction products were non-enzymatically synthesized from added arginine and maltose being enzymatically formed from starch in the steamed ginseng root, by maillard reaction during the heat-drying process.

A new conjugate of sugar and amino acid, Arg-fru-glc, was isolated from Korean red ginseng.[1] Arg-fru-glc is considered to be an early product formed non-enzymatically from maltose and arginine by maillard reaction. Browning reaction products were formed from chemically synthesized Arg-fru-glc (or Arg-fru) only *in vitro* by the heat-drying treatment. The browning reaction of Arg-fru-glc (or Arg-fru) was accelerated by the addition of sugar or amino acid during the heat-drying treatment. These above-mentioned results suggest that Arg-fru-glc (and Arg-fru) is an important compound related to browning reaction during the heat- drying process for the preparation of red ginseng.

The content of maltose in Korean red ginseng has been reported to be 9-10% of dry weight.[6,7] While, Table 1 shows that the ginseng powder from main roots treated with steaming for 2.5 hr contained maltose in the concentration of about 15 percent. During the heat-drying process, about one third of maltose in steamed main roots might conjugate with added arginine and other amino acids to produce Arg-fru-glc as a main product and other amino acid-fructosyl-glucose, respectively, followed by the acceleration of browning reaction of Arg-fru-glc in the presence of amino acids or sugar. In order to raise brown color intensity that has been a major factor to estimate the quality of red ginseng, it will be necessary to promote the hydrolysis of starch to maltose (or glucose), the hydrolysis of sucrose, and the hydrolysis of protein and peptide to free amino acid in main root of ginseng during the steaming process, followed by the marked formation of browning reaction products during the heat-drying process.

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