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Effects of the Preheating Treatments of Raw Ginseng in the Model System on the Synthesis of the Maillard Type-Browning Reaction Products of Red Ginseng

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Abstract : During our investigations on the relationship between the browning reaction of ginseng root and two compounds (arginyl-fructosyl-glucose and arginyl-fructose) in the model system of steaming and heat-drying processes for the preparation of red ginseng, the preheating treatment of main roots of raw ginseng at 60~70°C prior to the steaming and heat-drying processes was found to bring about the gelatinization of starch granules. The enzymatic hydrolysis of gelatinized starch to maltose, a marked formation of maltose, and the increase of both free arginine and total amino acids, resulting the acceleration of the Maillard type-browning reaction of ginseng root during the steaming and heat-drying processes, and the rise of brown color intensity of red ginseng. These results show that the preheating treatment may be effective for the decrease of inside white of red ginseng.

Key words : Preheating treatment, brown color intensity, browning reaction, formation of maltose, gelatinization of starch, red ginseng, inside white

INTRODUCTION

The brown color intensity of inside or outside part of red ginseng is very important for its quality. Red ginseng of good quality has the same brown color inside and outside of that. On the contrary, red ginseng of the worse or worst quality is classified according to the size and state of white constitution inside (inside white), yellow constitution outside of epidermis, and hole constitution inside of red ginseng. It is thought that the brown color intensity of ginseng root based on the non-enzymatic Maillard reaction in the heating process (enzymes are denatured) for making red ginseng is mainly related with the contents and compositions of reducing sugars and nitrogenous compounds with amino group or imino group in free amino acids, peptides, and proteins. Starch is considered to be the most noticeable component related to inside white, among many kinds of constituents of ginseng root.[1] In order to increase the brown color intensity and to decrease inside white of red ginseng, it will be necessary to promote the production of reducing sugars and free amino acids from carbohydrates and proteins in ginseng root, respectively, during the steaming and heat-drying processes for making red ginseng. In the previous paper,[2] we reported that a marked decrease of starch and a considerable formation of maltose occurred in main roots of ginseng during the steaming process. However, about one third of the initial starch content of raw main roots remained after the steaming process was finished. These results suggest that α-amylase of Korean ginseng root having about 50% of its activity even after heat stability treatment at 80°C for 30 min[3] was almost inactivated before the middle stage of the steaming process. This study deals with the effects of preheating treatments of raw ginseng root in the model system prior to the steaming and heat-drying processes, on the degradation of starch granules, the formation of reducing sugars and free amino acids, and the Maillard type-browning reaction of ginseng root during the steaming and heat-drying processes.

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MATERIALS AND METHODS

1. Materials, chemical reagents, and enzymes

Raw ginseng roots (*Panax ginseng* C. A. Meyer, Araliaceae) were harvested on September, after cultivation for 4 and 6 years in the Suwon experimental farm of KT & G Central Research Institute in Daejon, Korea. L-Arginine and glucose

(Sigma Chemical Co., St. Louis, MO, USA), ABTS [2,2'-azino-di-(3-ethylbenzthiazoline sulfonate)], glucose oxidase (grade III) from *Aspergillus niger*, and peroxidase (grade II) from horse-radish (Boehringer Mannheim Yamanouchi Co., Ltd., Tokyo, Japan) were obtained from commercial sources. Maltose (HHH) was kindly supplied by Hayashibara Biochemical Laboratory, Inc., Okayama, Japan. Other chemicals were of analytical reagent grade and commercially available.

2. Preheating treatments of main roots of raw ginseng

Preheating treatments of raw ginseng roots were done as follows. An autoclave (model KMC-1221, Vision Scientific Co., Bucheon, Korea) was filled up with water to the height of the top surface of lower (bottom) shelf. Three main roots of raw ginseng were put like ginseng root-sandwiches covered with four sheets of wet cheese cloth above and below on the upper shelf in the autoclave. Two of four thermometers wrapped with paper towel were set between the upper four sheets of wet cheese cloth and ginseng roots, and the other two thermometers were set between ginseng roots and the lower four sheets of wet cheese cloth. When the temperature in a display of the autoclave was set at 57, 66, 71, or 81, and then preheating treatment was begun under opened valve, all of four thermometers in the autoclave showed 42, 50, 60, or 70°C, respectively, in the absence of ginseng roots after a 20 min-preheating treatment, and in the presence of three ginseng roots after about 1 hr preheating treatment. Thereafter, there was no temperature change in all of four thermometers during preheating treatments. After preheating treatments, each of three disk sections with about 3 mm in thickness (total wet weight, 6-7 g), each of three cylindrical sections with about 8 mm in thickness (total wet weight, 15-18 g), and each of three cylindrical sections with about 3cm in thickness (total wet weight, 40-80 g), respectively, were cut in 3-7cm away from each top part of three main roots (diameter, 30-37 mm) for the assays of water content, sugar content, and starch granule content.

3. Assay of sugars

Three cylindrical sections (total wet weight, 15-18 g) cut from three main roots of raw or preheated ginseng were homogenized with 80% ethanol using an electric mixer, and the homogenate was boiled for 10 min, evaporated to dryness, allowed to keep in the disiccator containing diphosphorus pentoxide for one week, and pulverized in a mortar with a pestle. The powdered preparation of ginseng root was extracted 3 times with 80% ethanol for 1 hr at 85°C under reflux, and the combined extract was centrifuged. The supernatant solution (80% hot ethanol-soluble fraction) was evaporated to dryness, and the residue was 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, and the residue was dissolved in water. The solution was filtered through a 0.45 μm filter membrane. Glucose and maltose 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. Glucose was also determined by glucose oxidase method using ABTS.[4)]

4. Assay of free amino acids

The powdered preparation of raw or preheated main roots was extracted with 10 volumes of water under stirring for 12 hr at 4°C, and centrifuged. The supernatant extract was freeze-dried, and the resulting powder was dissolved in water, filtered through the filter membrane, and the filtrate was subjected to an amino acid analyzer. Analytical conditions were as follows: system, Waters 1500 HPLC pump and Waters 717 automatic sampler; column, Waters Pico-tag column (3.9×300 mm, 4 μm); detector, Waters 2487 dual λ absorbance detector, 254 nm; data analysis, Waters breeze software.

5. Preparation of starch granules from raw and preheated ginseng roots

Three cylindrical sections (total wet weight, 40-80 g) from three raw or preheated main roots were homogenized 4 times for 30 seconds with 150 ml of cold 0.1% sodium hydroxide aqueous solution using an electric mixer. After having been allowed to stand overnight at 4°C with 150 ml of the additional 0.1% sodium hydroxide aqueous solution under occasional stirring, the slurry was filled up to 2 liters with water, successively passed through two sieves, 60- and 140-mesh bolting cloths, and centrifuged at 6,000 rpm for 10 min. The precipitate was suspended with 350 ml of water, and the suspension was neutralized with 0.5 N hydrochloric acid aqueous solution. To the neutralized suspension was added one eighth volume of toluene, and the mixture was vigorously stirred for 15 min to remove protein, and centrifuged. The precipitate was re-suspended in water, vigorously re-stirred with new toluene, and the mixture was centrifuged. This process was repeated several times until the intermediate water layer was perfectly clear and there was no visible protein in the upper toluene layer. The precipitated starch was washed several times with ethanol using a centrifuge,

air-dried at 30°C, and allowed to keep in a desiccator. While, to the combined toluene layers were added 8 volumes of water, and the mixture was vigorously stirred, and centrifuged. Brown colored contaminant in the precipitate was removed, then the recovered starch in the precipitate was washed with ethanol, air-dried, and allowed to keep in desiccator.

6. Instrumental analyses

Scanning electron micrographs of starch granules were taken on Karl Zeiss DSM 960A scanning electron microscope. Brown color intensity was measured by the absorbance at 420 nm with Hewlett Packard 8452A spectrophotometer.

RESULTS AND DISCUSSION

1. Amounts and forms of starch granules obtained from main roots that were preheated for 6 hr at various temperatures

When main roots of raw ginseng were preheated in the autoclave at 42, 50, 60, or 70°C for 6 hr, starch granules

Table 1. Contents of starch granules, glucose, and maltose of main roots preheated for 6 hr at various temperatures[a]

Preheating temperature (°C)	Preheating time (hr)	Starch granules[c]	Glucose	Maltose
		(mg/g of main root, dry weight)		
[b]	0	247	0.7	0
42	6	234	0.7	0
50	6	121	8.7	33.3
60	6	14	17.8	71.5
70	6	3	11.8	60.4

[a] This experiment was done with raw ginseng roots that were cultivated for 4 years.
[b] main roots of raw ginseng with no preheating treatment
[c] the amounts of starch granules obtained from raw or preheated main roots

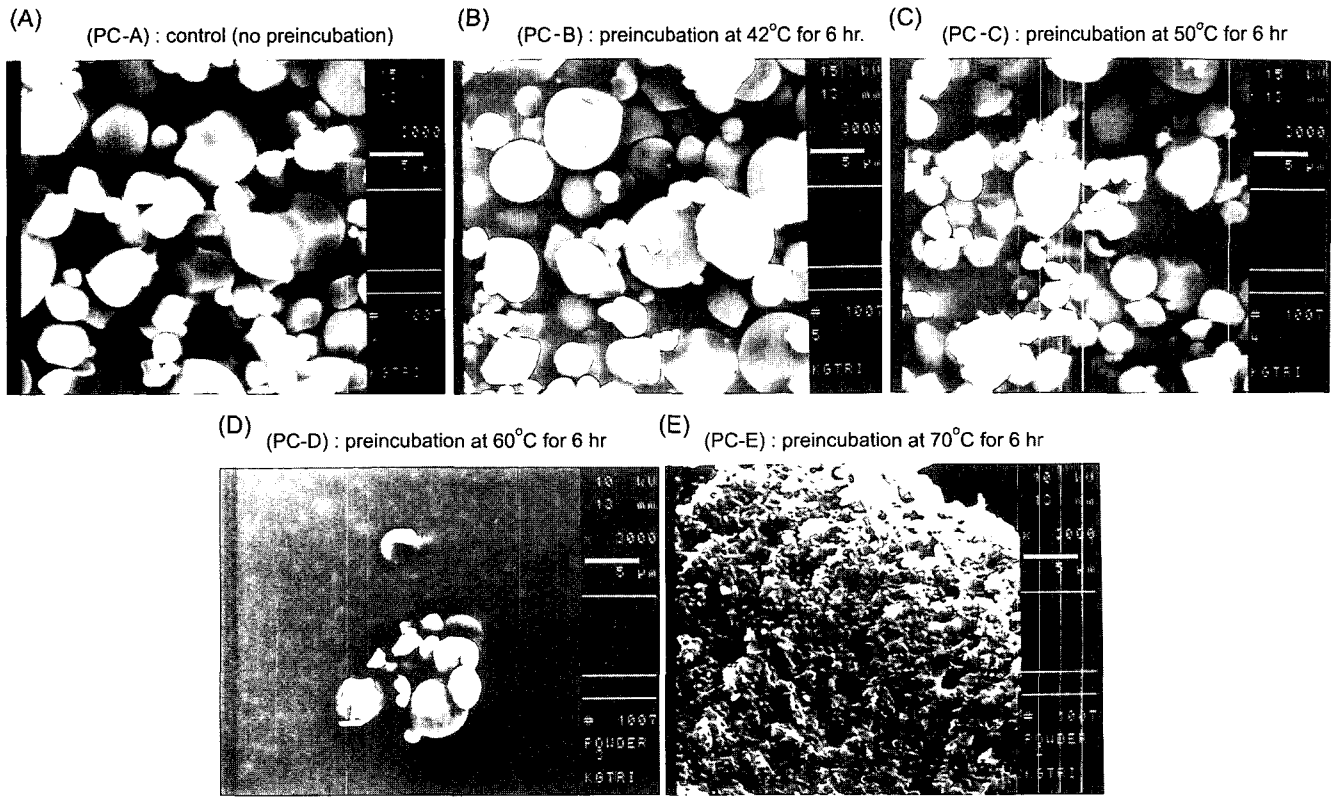

Fig. 1. Scanning electron micrographs of starch granules obtained from ginseng main roots that were preheated for 6 hr at various temperatures. A: control (starch granules obtained from raw ginseng main roots); B: starch granules obtained from main roots that were preheated for 6 hr at 42°C; C, starch granules obtained from main roots that were preheated for 6 hr at 50°C; D; starch granules obtained from main roots that were preheated for 6 hr at 60°C; E; starch granules obtained from main roots that were preheated for 6 hr at 70°C.

obtained from main roots markedly decreased at 50°C, and almost disappeared at 60 or 70°C (Table 1). Fig. 1 shows scanning electron micrographs of starch granules that were obtained from preheated main roots, after a 6 hr-preheating treatment at various temperatures. There was no change in the shape of starch granule, after the preheating treatment at 42°C for 6 hr. After the preheating treatment at 50°C for 6 hr, starch granules of large size decreased and those of smaller size considerably increased. After the preheating treatment at 70°C for 6 hr, no starch granules but only gelatinized starch were observed.

H. J. Kim and J. S. Jo reported that the gelatinization of starch granules of Korean ginseng root began at 50-55 °Cand completed at 65-70.[5] Crude α-amylase preparation from Korean ginseng root was reported to be very stable at temperature below 50°C and have 50% of its activity even after the heat stability treatment at 80°C for 30 min.[3] While, over 91% of ginseng β-amylase activity remained at temperature below 50°C, but most of the enzyme was inactivated at 65°C[6] Both our experimental results and these three reports suggest that gelatinized starch is mainly hydrolyzed by α-amylase of ginseng root during the preheating treatment for 6 hr at 60 or 70°C.

During the preheating process, the starch contents of main roots of raw ginseng were remarkably hydrolyzed to give considerable amount of maltose and glucose. But total amount of maltose and glucose were lower than starch content of raw ginseng. These means that hydrolyzed free sugars were used to form arginine-fructose-glucose with arginine and other browning reaction products by Maillard type browning reaction.[1,2] And also the consumptions of the sugars on the early stage Maillard type browning reaction and the remaining of oligomers of hydrolyzed sugars from starch granules were regarded on the preheating process of raw ginseng.

2. Contents of glucose and maltose of main roots preheated at various temperatures

As shown in Table 1, the formation of glucose and maltose was observed with main roots that were preheated for 6 hr at 50, 60, or 70°C, especially a considerable amount of maltose was formed by the preheating treatment at 60 or 70°C for 6 hr.

3. Browning reaction of the powdered preparation of main roots preheated at various temperatures, during the steaming and heat-drying processes

After the steaming and heat-drying processes, the brown color intensity of the powdered preparation of main roots preheated at 60 or 70°C for 6 hr was about 3 times higher than that of the powdered preparation of raw main roots. Moreover, to the powdered preparation of preheated main roots was added L-arginine being the most abundant free amino acid in raw ginseng root, and then the mixture was treated with the steaming and heat-drying processes. After these processes, the brown color intensity of the mixture containing the powdered preparation of preheated main roots and arginine was about 5 times higher than that of the mixture containing the powdered preparation of raw main roots and arginine, and also 9 to 10 times higher than that of only the powdered preparation of raw main roots (Fig. 2). These results and our previous report[2] suggest that browning reaction products were non-enzymatically synthesized from reducing sugars

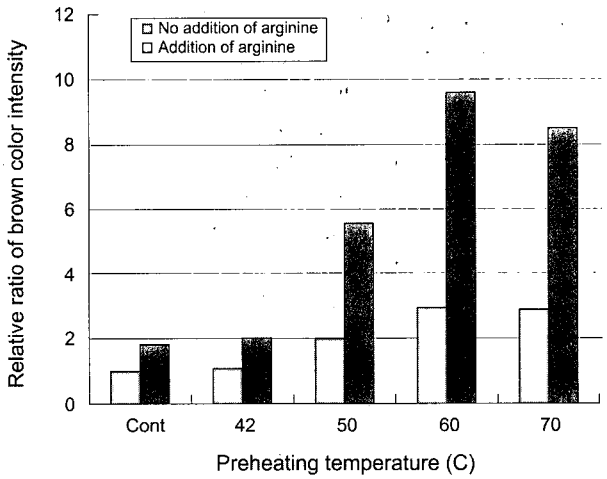

Fig. 2. Browning reaction of the powdered preparations of main roots preheated for 6 hr at various temperatures, during steaming and heat-drying processes.
Reaction mixture contained 250 mg of powdered preparation of raw or preheated main roots and 5 ml of 0.05 M sodium phosphate buffer (pH 6.3) with or without the addition of 44 mg of L-arginine. The mixture was treated with the steaming process at 98°C for 2.5 hr in an autoclave under closed valve, then treated with the heat-drying process at 70°C for 24 hr. After the heat-drying process, the dried mixture was suspended in water to make a final volume of 10 ml, gently stirred for 5 hr at room temperature, and centrifuged. The supernatant was filtered through a 0.45μm-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 of the absorbance (OD 420 nm, 0.338) of the control filtrate of only the powdered preparation of raw main roots.
Cont.: main roots of raw ginseng with no preheating treatment.

(such as maltose and glucose) and free amino acids during the heat-drying process. These sugars and free amino acids were enzymatically formed from gelatinized starch and protein, respectively, during both the preheating treatment at 60 or 70°C for 6 hr and the early stage of the steaming process. Also, they show that the amounts of free amino acids formed from protein (and peptide) during preheating treatment were lower, when compared to those of reducing sugars formed from gelatinized starch.

4. Contents of starch granules, glucose, maltose, free arginine, and total free amino acid of main roots preheated at 70°C for 0.5-6 hr

The preheating treatment of raw main roots at 60 or 70°C for 6 hr was effective for the gelatinization of starch granules, the enzymatic hydrolysis of gelatinized starch to maltose, and the Maillard type-browning reaction of powdered preparation of preheated main roots during the steaming and heat-drying processes. We examined the time-course of the contents of starch granule, glucose, maltose, free arginine, and total free amino acid in main roots during the preheating treatments at 70°C for 0.5-6 hr. As shown in Fig. 3, a rapid decrease of starch granules obtained from main roots and a marked formation of maltose occurred after a 45 min preheating treatment. Thereafter, maltose attained a maximum accumulation with a successive increase after a 3 hr-preheating treatment, and then gradually decreased. A small formation of glucose was observed during the preheating treatment at 70°C for 0.5-6 hr. Free arginine content gradually increased in the early stage of the preheating treatment at 70°C, attained the highest value (about 40% higher than that of raw main root) after a 90 min preheating treatment, and thereafter decreased. Total free amino acid content also attained a maximun after the 90 min-preheating treatment (Table 2).

Therefore, free amino acids hydrolyzed by proteases and peptides of time course preheating were used to form

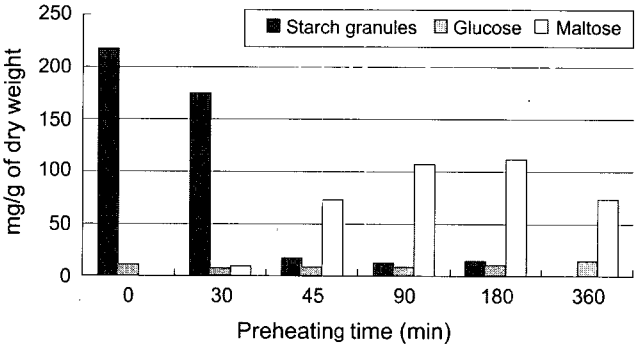

Fig. 3. Time-course of starch granules, glucose, and maltose contents of main roots preheated at 70°C for 0.5-6 hr. This experiment was done with raw ginseng main roots that were cultivated for 6 years.

Table 2. Time-course of free amino acid content of main roots preheated at 70°C for 0.5-6 hr (unit : mg/g of dry weight)

Free amino acid \ Preheating time(min)	0[a)]	30	45	90	180	360
Cysteine	0.08	0.10	0.09	0.10	0.09	0.14
Aspartic acid	4.33	2.90	2.82	3.04	2.43	2.10
Glutamic acid	2.32	3.55	1.17	1.48	1.26	1.24
Asparagine	2.03	0.91	1.18	0.67	0.97	0.88
Serine	0.49	0.33	0.26	0.25	0.22	0.33
Glutamine	3.41	2.30	3.21	2.88	1.90	2.31
Glycine	–	–	–	–	0.08	0.11
Histidine	0.70	0.55	0.68	0.60	0.57	0.57
Arginine	19.61	19.73	23.72	27.16	22.06	23.81
Threonine	0.31	0.22	0.17	0.17	0.18	0.24
Alanine	0.54	0.96	1.36	1.21	1.38	1.66
Proline	0.18	0.31	0.29	0.20	0.15	0.24
Tyrosine	0.50	0.16	0.20	0.18	0.28	0.36
Valine	0.20	0.15	0.15	0.16	0.15	0.24
Methionine	0.05	0.05	0.06	0.08	0.07	0.10
Cystine	0.01	0.01	0.01	0.01	0.01	0.01
Isoleucine	0.12	0.08	0.08	0.11	0.10	0.14
Leucine	0.23	0.25	0.23	0.32	0.27	0.43
Phenylalanine	0.24	0.18	0.01	0.24	0.23	0.30
Tryptophan	0.39	0.19	0.10	0.11	0.20	0.10
Lysine	0.28	0.22	0.23	0.32	0.34	0.29
Total	36.00	33.14	36.00	39.29	32.94	35.60

[a)] main root of raw ginseng with no preheating treatment

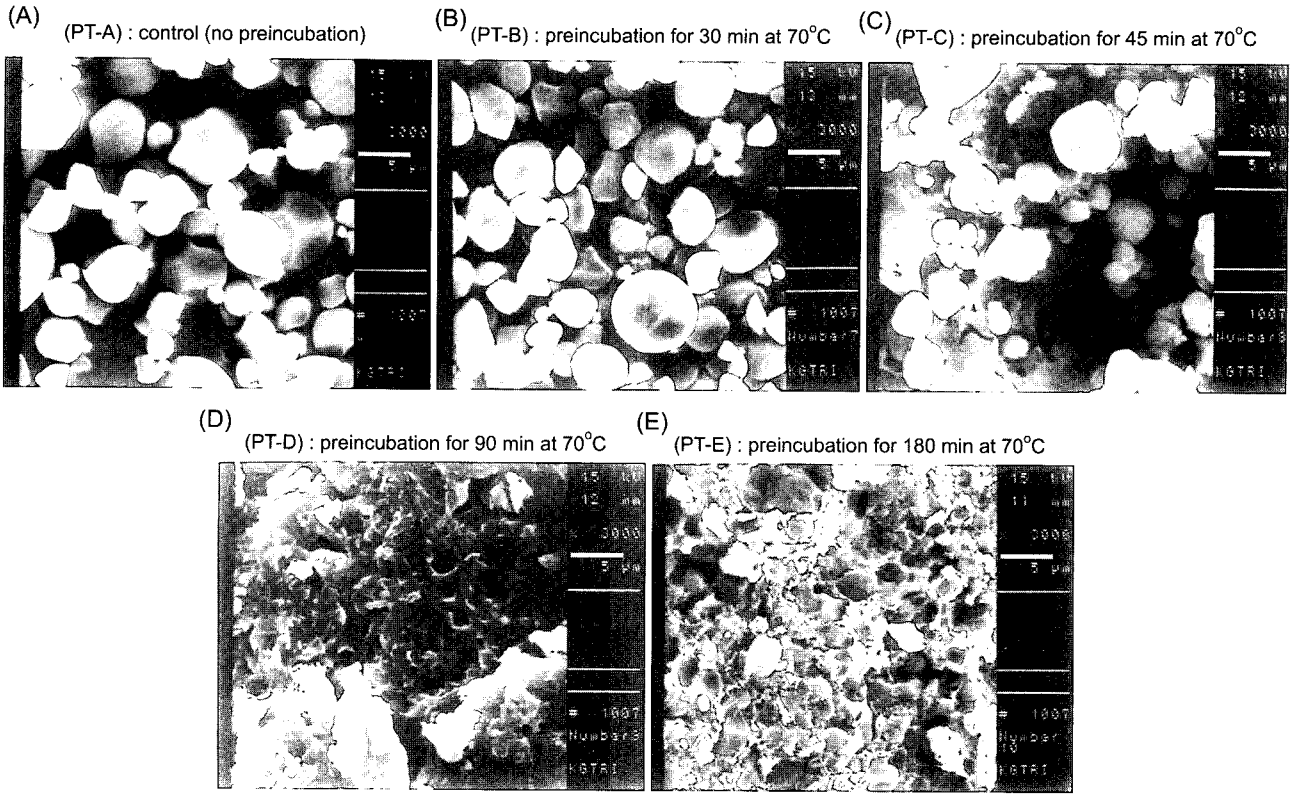

Fig. 4. Scanning electron micrographs of starch granules obtained from main roots that were preheated for 0.5-6 hr at 70°C.
A: control (starch granules obtained from raw ginseng main roots); B: starch granules obtained from main roots that were preheated for 30 min at 70°C; C, starch granules obtained from main roots that were preheated for 45 min at 70°C; D, starch granules obtained from main roots that were preheated for 90 min at 70°C; E, starch granules obtained from main roots that were preheated for 180 min at 70°C; and F, starch granules obtained from main roots that were preheated for 360 min at 70°C.

Maillard type browning reaction products.[2] These browning reactions were regarded to take place on the early stage of Maillard type browning reaction. The browning reaction ginseng by the preheating course were different on the kinds and amount of free amino acids, and the consumption of these amino acids might be regarded to maintain a living in the early stage of preheating stage of raw ginseng.

5. Forms of starch granules obtained from main roots that were preheated at 70°C for 0.5-6 hr

Scanning electron micrographs show that the gelatinization of starch granules proceeded to a considerable extent after the 45 min preheating treatment, and almost completed after the 90 min preheating treatment (Fig. 4).

6. Browning reaction of the powdered preparations of main roots preheated at 70°C for 0.5-6 hr, during the steaming and heat-drying processes

After the steaming and heat-drying processes, the brown color intensity of the powdered preparations of main roots preheated at 70°C for over 45 min was about 1.3 to 1.6 times higher than that of the powdered preparation of raw main roots (Fig. 5). After the steaming and heat-drying processes with the addition of arginine, the brown color intensity of the powdered preparation of main roots preheated at 70°C for over 45 min was 3 to 4-times higher than that of the powdered preparation of raw main roots, and also, 6 to 7 times higher than that of only the powdered preparation of raw main roots.

These results reveal that the preheating treatment of raw main roots at 70°C for over about 1 hr brought about the gelatinization of starch granules, the enzymatic hydrolysis of gelatinized starch to maltose, the enzymatic formation of free amino acids, and accelerated the Maillard type-browning reaction during the steaming and heat-drying processes. Also, these results show that preheating treatment prior to the steaming and heat-drying processes may be a useful method to decrease the inside white of red ginseng. Furthermore, instead of a rapid rise of temperature

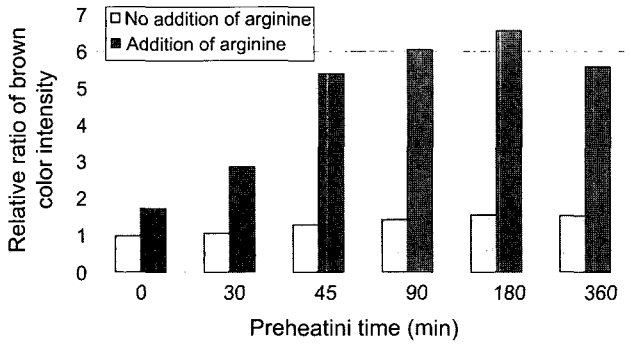

Fig. 5. Browning reaction of the powdered preparations of main roots preheated at 70°C for 0.5-6 hr, during steaming and heat-drying processes.
Reaction mixture contained 250 mg of powdered preparation of raw or preheated main roots, and 5 ml of 0.05 M sodium phosphate buffer (pH 6.3) with or without the addition of 44 mg of L-arginine. The mixture was treated with the steaming process at 98°C for 2.5 hr in an autoclave under closed valve, and the heat-drying process at 70°C for 24 hr. After the heat-drying process, the dried mixture was suspended in water to make a final volume of 10 ml, gently stirred for 5 hr at room temperature, and centrifuged. The supernatant was filtered through the 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, 0.539) of the control filtrate of only the powdered preparation of raw main roots.

to 97-98°C in an autoclave during the steaming process, not only the gradual or gently-sloping rise of temperature to 97-98°C during the steaming process but also the step-wise rise of temperature (the step-wise preheating treatment) which keeps for each several hours at 50, 60, and 70°C prior to the steaming process at 97-98°C may be useful procedures for the acceleration of gelatinization of starch granules, the enzymatic formation of reducing sugars and free amino acids, and the synthesis of browning reaction products, resulting the decrease of inside white in red ginseng. Also, when main roots of raw ginseng were put like ginseng root-sandwiches covered with four sheets of wet cheese cloth, and then treated with preheating and steaming process in an autoclave, we often observed the decrease of yellow epidermis of ginseng root. It may be useful to avoid the yellow constitution outside of epidermis of red ginseng that each main root of raw ginseng is wrapped up with wetted thick cloth, and then successively treated with preheating, steaming, and the step-wise heat-drying at 50, 60, and 70°C These useful procedures are in a future study.

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