Enhancing the Organoleptic and Functional Properties of Jujube by a Quick Aging Process

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ABSTRACT: Black jujube was made by aging dried jujube and its physiochemical characteristics, antioxidant activities and α-glucosidase inhibitory activities were evaluated. The moisture and sugar contents were increased depending on the period of aging times and the pH was reduced thereby increasing acidity. The color of black jujube extract was changed from red to black resulting in decreases of Hunter color values L, a and b. As the aging progressed, sucrose was decomposed by increasing glucose and fructose, indicating higher contents of the total reducing sugars. Among the six different types of organic acids extracted from dried jujube, the levels of oxalic acid and citric acid were increased as the aging progressed. The total polyphenol contents in ethanol and water extracts of dried jujube were 7.74 and 8.12 mg/g, respectively. The water extract of black jujube aged for 48 hr contained the highest polyphenol contents at 16.82 mg/g. The 5’-hydroxymethylfurfural (5’-HMF) contents of black jujube extract significantly increased by longer aging times, and contained higher contents in the ethanol extract than water extract. The ethanol extract of black jujube showed the highest 5’-HMF content with 338.89 mg% after aging for 3 days. Also, IC50 values of black jujube aged for 72 hr evaluated by DPPH and ABTS radical assays were 0.54 and 0.59 mg/mL, respectively. α-Glucosidase inhibitory activities of black jujube at the concentration of 3.33 mg/mL (ethanol extract) increased from 65 to 80 % after aging for 72 hr.

Keywords: black jujube, antioxidant, aging, α-glucosidase, organic acid

INTRODUCTION

Jujube (Ziziphus jujuba Miller) belongs to the family Rhamnaceae, which is widely distributed throughout tropical and subtropical climates around the world (1). Jujube has elliptical fruits with thin skin which are initially green but gradually change to reddish brown. Jujube fruits have been recognized as nutritious foods and have important uses in traditional medicine in oriental countries.

The jujube fruit has a light aroma complementing a sweet and slightly sour refreshing taste. In particular, jujube fruits are richer in sugar, vitamin C, bioflavonoids, edible fiber and minerals than other fruits (2). Medicinal ingredients such as sterols, alkaloids, saponins, serotonin, polyphenol, and flavonoids (3) along with triterpenoids (4) and c-GMP (5) are reportedly contained in jujube fruits. The methanol extracts, from the fruit, pharmacologically protect liver (6), suppress cancer cell proliferation (7) and generate antioxidant effects (8). Due to the increasing interest in healthy longevity in an aging society, researches on natural bioactive materials and functional and health foods are heightened.

Fresh jujube is difficult to keep for a long period of time and thus it is usually used after drying. The fruit is sun-dried where jujube is cultivated since its quality is largely affected by the drying method. However, the fruit often decomposes or softens during a rainy season, resulting in microbial contaminations as well as a poor hygienic status. Therefore, researches to improve its shelf-life and functions are required to process and distribute jujube effectively.

The Maillard reaction, which generates non-enzymatic browning in food processes, greatly affects the flavors of foods including melanoidin generated from chemical reactions between reducing sugars and amino acid residues. Melanoidin has attracted interest due to a report on its beneficial effects on human health (9). In South Korea, black garlics have been produced in recent years by the chemical reaction of indigenous ingredients in garlic.
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browning. Therefore, in this study, dried jujube was effectively converted into black jujube with enhanced quality and functional properties for a short period. The organo-leptic and physiochemical properties as well as the antioxidant activity and α-glucosidase inhibitory activity of water and ethanol extracts were evaluated. These results can be used as preliminary data to utilize black jujube as a novel ingredient for new functional and health foods.

Materials and Methods

Materials
The dried jujube was obtained from Dae-Heung Nong San (Gyeongbuk, Korea). All the samples were washed under running water and dried at room temperature for 24 hr. Ethanol was purchased from Duksan Chemical Co. (Ansan, Korea) and the HPLC solvent used was of HPLC grade (J.T. Baker, Philipsburg, NJ, USA). DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), α-glucosidase and p-nitrophenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Production of black jujube
Whole dried jujube with skin was placed in a heat-resistant plastic airtight container (30×20×20 cm) with a stainless steel net. To prevent drying, 100 mL of water was placed into the container and then the jujube was aged in a drying oven at 80°C for 72 hr. Black jujube was freeze-dried, packed in to sealing bags and kept at −20°C.

Measurement of moisture and sugar contents
The moisture contents of jujube and black jujube were measured by an infrared moisture determining balance (FD-720, KETT Electric Laboratory, Tokyo, Japan). The total sugar content was measured by a portable digital refractometer (Refractometer Pocket PAL-3, Atago, Tokyo, Japan).

Measurement of color values
The Hunter color values of drying jujube and black jujube were obtained in terms of L (lightness), a (redness), and b (yellowness) values using a Hunter Lab colorimeter (Color Reader, CR-10, MINOLTA, Osaka, Japan). The 20 mL of distilled water was added to 5 g of sample and extracted for 30 min at room temperature. The solution was centrifuged at 1,000×g for 15 min and 1 mL of supernatant was measured in a screw cap test tube (PYREX, diameter 13 mm). ΔE value was calculated for the L, a, b values in the difference between the standard white plate, with $L=97.37$, $a=0.12$, $b=1.82$, respectively, and the sample.

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\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}
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Measurement of pH and total acidity
The pH of black jujube was determined with a pH meter (model 420A+, Thermo Fisher Scientific, Hudson, NH, USA). The titratable acidity was measured by determining the 0.1 N NaOH content necessary for adjusting to pH 8.3 and then expressed with tartaric acid content (% v/v).

Free sugars and non-volatile organic acid contents
Two grams of freeze-dried black jujube was placed in a 50 mL conical tube and extracted for 30 min with 18 mL of distilled water. The extract was centrifuged at 1,000×g for 15 min and filtered through a 0.45 μm membrane filter. Glucose, sucrose, and fructose were quantified on a Knauer HPLC system (Knauer Co., Berlin, Germany) consisting of a refractive index detector, a column heater set at 35°C, and a Shodex monosaccharide column (Shodex, Tokyo, Japan); the isocratic mobile phase was 75% acetonitrile delivered at 1 mL/min. Non-volatile organic acid contents were quantified using a method adapted from Nakagawa et al. (16). Five grams of freeze-dried black jujube was placed in a 50 mL falcon tube and ex-
tracted for 30 min with 25 mL of distilled water. The extract was centrifuged at 1,000×g for 15 min and filtered through a 0.45 μm membrane filter and then applied to a Sep-pak plus C18 cartridges (55–105 μm, Waters Co., Milford, MA, USA). The Sep-pak column had been previously prepared by washing with 4 mL of methanol followed by 8 mL of boiling deionized-distilled H2O. The non-volatile organic acids were quantified on a Knauer HPLC system and a UV detector (210 nm), a column heater set at 30°C, and a Shodex Hypersil Gold aQ C18 column (4.6×250 mm) (Thermo Co., Waltham, MA, USA); the isocratic mobile phase was 20 mM H3PO4 delivered at 1 mL/min. Non-volatile organic acids were expressed as mg/100 g.

**Preparation of black jujube extracts (BJE)**

Dried jujube and black jujube seeds were removed and then freeze-dried. The powdered jujube was extracted with 10 volumes of 70% ethanol or distilled water for 12 hr 3 times at 25°C using a shaking incubator (SI-900R, JEIO TECH Co., Daejeon, Korea). The filtered extracts were concentrated by evaporation (EYELA, Rikakikai Co., Tokyo, Japan) under reduced pressure. After the extracts were thoroughly dried for complete removal of solvent, the dried extract was then stored in a deep freezer (−80°C).

**5'-Hydroxymethylfurfural content of BJE**

BJE samples (1 g) were dissolved with deionized water in a 10 mL volumetric flask. After thorough mixing, 50 mL acetone was added to the fraction funnel and extracted 3 times. Samples were removed from the solvent completely and added to 2 mL of methanol. Samples were filtered through a 0.45 μm membrane filter (Millipore, Billerica, MA, USA) and analyzed by HPLC. The chromatographic determination was carried out on a Shimadzu LC-20A prominence manufactured by Shimadzu Company, with a SPD-20A UV/VIS detector, using μBondapack C18, 4.6×300 mm, 10 μm columns (Waters Co.). The isocratic HPLC system used a water/acetonitrile mix (80:20) as the mobile phase for analysis. The mobile phase flow rate was 0.6 mL/min, with the sample injection volume of 20 μL and the column temperature at 30°C. The HMF was detected in the UV region at 280 nm.

**DPPH radical scavenging activity**

The DPPH free radical scavenging activity of BJE was evaluated by the Blois method (18). Different concentrations (0.1~1.5 mg/mL) of BJE were prepared and diluted to 3 mL with ethanol. Then, 1 mL of ethanolic DPPH solution (0.1 mM) was added to the samples. These samples were mixed and then incubated in the dark at 30°C for 30 min. The absorbance was measured at 517 nm against blank samples. A decrease in absorbance indicates DPPH free radical scavenging activity. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and vitamin C were used as the positive control group.

**ABTS radical scavenging activity**

The ABTS radical scavenging activity of BJE was evaluated according to the method of Pellegrini et al. (19) with minor modifications. ABTS is blue-green in color with a characteristic absorbance at 734 nm. ABTS radical cation was produced by reacting ABTS (2 mM) in H2O and potassium persulphate (2.45 mM) at room temperature for 12 hr. The ABTS radical solution was diluted with phosphate buffer (0.1 M, pH 7.4) to achieve an absorbance of 0.750±0.025 at 734 nm. Then, 1 mL of ABTS radical solution was added to 3 mL of BJE in methanol at different concentrations (10~30 μg/mL). These samples were mixed, incubated in the dark for 30 min, and the absorbance at 734 nm was measured for each concentration relative to a blank. Decreased absorbance of the samples indicates ABTS radical cation scavenging activity. Vitamin C and trolox were used as a positive control group.

**In vitro α-glucosidase inhibitory assay**

Two grams of freeze-dried black jujube was placed in a 50 mL falcon tube and extracted for 30 min with 18 mL of distilled water or 70% ethanol. The α-glucosidase inhibitory assay of the BJE was determined using a modified procedure reported by Choe et al. (20). α-Glucosidase (EC 3.2.1.20) isolated from *Saccharomyces cerevisiae* and the substrate 4-nitrophenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich. The initial concentrations of the enzyme and substrate solutions were 0.75 unit/mL and 2 mM in 50 mM potassium phosphate buffer (pH 6.5), respectively. The enzyme solution (20 μL/microtritre well plate) was mixed with the samples in
a clear 96-well microplate (flat bottom) and the reaction was started by addition of the substrate solution (50 µL/well). The plates were incubated at 37°C for 30 min after shaking and the reaction was stopped by adding 0.1 M Na₂CO₃ (100 µL/well). Enzyme inhibition was determined by the absorbance of 4-nitrophenyl (product) at 405 nm, as measured with a microplate reader (Spectramax 340 pc 384, Molecular Device, LLC, CA, USA). Background absorbance was determined using a non-enzyme control microplate containing the potassium phosphate buffer (20 µL/well) and was subtracted from the absorbance of sample and controls. Acarbose was used as a positive control group.

Statistical analysis
All experiments were performed in triplicate. The results were expressed as the mean±standard error values (SE). One-way analysis of variance (ANOVA) followed by Duncan’s multiple comparison test was performed. Statistical Package for the Social Science (SPSS, Version 20.0, SPSS Inc., Chicago, IL, USA) was used.

RESULTS AND DISCUSSION

Quality characteristics of black jujube produced from different aging periods
The jujube as a valuable fruit crop can be grown under a variety of environmental conditions, including those in dry areas. The soluble solid content of jujube fruit is about 20–40% (21). The carbohydrate content in Chinese jujubes can reach as high as 80–85%, including higher sugars and even protein contents (22). Therefore, as expected, the physicochemical properties of jujube fruit can be changed by the Maillard reaction. Conclusively, black jujube with higher palatability could be produced by aging at 80°C for 2 days. The moisture in foods greatly affects the appearance, texture, and sensual quality and plays an important role in spoilage of foods microbiologically (23). In the Maillard reaction, the brown pigment formation is also dependent on the moisture content of fruits and maximum browning occurs at 30% moisture, which corresponds to a water activity of 0.6~0.8 (24). Table 1 shows the changes in moisture content of jujube according to the aging period. The moisture content of jujube was about 36.56% and that of black jujube increased to 47.92% with longer aging periods. Thus, for the first stage of the Maillard reaction, dried jujube contained suitable moisture content for the browning reaction. The changes in moisture content during the aging process was also confirmed by a sensorial evaluation performed by visual inspections. In conclusion, the flesh of jujube fruit absorbed moisture while dried jujube was aged in the condition of saturated humidity at a high temperature of 80°C.

The total soluble solid content of dried jujube was about 10.37°Bx and significantly increased by 77.90°Bx after freezing drying. During ripening, total soluble solid content of jujube increased and more than 75% of the pectin in the jujube was water-soluble pectin, supporting that the total soluble solid content of ripen jujube is relatively high. Interestingly, the total soluble solid content of black jujube slightly increased to 81.71°Bx after aging for 72 hr. The estimated increase of soluble solid content was stemmed from the transformation of insoluble carbohydrate into dextrin or monosaccharide during thermal processing (25).

The pH was reduced and the acidity increased when prolonging the aging process. The initial acidity of 1.08% increased to 3.33% after aging for 72 hr (Table 1). The pH of dried jujube was mildly acid with pH 4.96 and decreased to pH 3.86 as the aging period became longer (72 hr). In the Maillard reaction, nonvolatile components generated by sugars and peptides were largely affected by the reaction temperature. The pH was rapidly reduced as the reaction temperature increased, which was reported caused by organic acids created during the browning process (9). Black jujube has a strong sourness and some bitterness after aging for 72 hr, giving it a low palatability. During the aging at a high temperature, the sourness of jujube is largely increased and the texture becomes softer. However, the soluble solid content of aged jujube is partially increased. These results imply that aged jujube may have significantly different acids and sugar compositions. Therefore, the balance of sweetness and sourness should be considered to produce the

| Sample          | Aging time (hr) | Water content (%) | Total soluble solids (°Bx) | pH     | Titratable acidity (%) |
|-----------------|-----------------|-------------------|---------------------------|--------|------------------------|
| Non drying      |                 |                   |                           |        |                        |
| Dry jujube      | 0               | 36.56±0.66a       | 77.90±0.99a               | 4.65±0.4a | 1.08±0.03a              |
| Black jujube    | 24              | 45.73±1.51b       | 79.80±1.10ab              | 4.30±0.02a | 1.86±0.11b              |
|                 | 48              | 47.01±0.69b       | 80.75±0.59b               | 4.20±0.05a | 2.82±0.14c              |
|                 | 72              | 47.92±1.27b       | 81.71±0.97b               | 3.86±0.01a | 3.33±0.10c              |
| Freeze drying powder |            |                   |                           |        |                        |
| Dry jujube      | 0               | 36.56±0.66a       | 77.90±0.99a               | 4.65±0.4a | 1.08±0.03a              |
| Black jujube    | 24              | 45.73±1.51b       | 79.80±1.10ab              | 4.30±0.02a | 1.86±0.11b              |
|                 | 48              | 47.01±0.69b       | 80.75±0.59b               | 4.20±0.05a | 2.82±0.14c              |
|                 | 72              | 47.92±1.27b       | 81.71±0.97b               | 3.86±0.01a | 3.33±0.10c              |

Data are expressed as mean±standard error values (n=3). Mean with different letters in each column are significantly different (p<0.05) by Duncan’s multiple range test.
The browning of dried jujube to black jujube is generated by the amino-carbonyl reaction between sugars and amino acids present in the jujube fruit. Therefore, to turn dried jujube into black jujube occurs by maturation process rather than fermentation because the microorganisms will not be able to survive (27). The color of black jujube becomes dark brown or black as the aging period becomes longer (Fig. 1). Table 2 shows the changes in colors of dried jujube and aged jujube. The brightness of dried jujube ($L$) was 19.68 before aging and then became less and less as the maturation progressed, leading to 14.23 after aging for 72 hr. The red chromaticity ($a$) and the yellow chromaticity ($b$) were recorded as 2.45 and 3.87, respectively, at the initial stage and were also significantly reduced to 1.18 and 0.77, respectively, after aging for 24 hr while still decreasing afterwards.

**Free sugar and nonvolatile organic acid contents of black jujube**

Table 3 shows the free sugar contents of dried and black jujube according to the aging period. The sugars, including fructose, glucose and sucrose, were extracted from...
dried jujube, but sucrose was disappeared after maturation for 24 hr; however, fructose and glucose gradually increased. The higher fructose and glucose contents of black jujube were thought to be caused by the decomposition of sucrose during the aging at a high temperature. According to a previous report, acid hydrolysis of sucrose occurred in continuous thermal processing at pasteurization temperature (28). Therefore, the sucrose in jujube could have converted into monosaccharides during aging at a high temperature. In conclusion, the fructose and glucose determined the sweetness of black jujube. The total sugar content of dried jujube was 370.10 mg/g, but this increased to 439.20 mg/g after aging for 72 hr. Table 4 shows the changes in nonvolatile organic acid contents according to the aging of dried jujube. Six different types of organic acids were extracted from dried jujube. The oxalic acid content was the highest with 436.08 mg%, followed by citric acid (198.92 mg%), and lactic acid (40.27 mg%). These organic acid contents, except lactic acid, appeared to increase as the aging time became longer. Although the organic acid contents were similar to those in a study by Lee (29), the citric acid content (330 mg%) was reportedly the highest in that study. Conclusively, the overall taste of aged jujube could be dependent upon the acid content which increased during the aging process. Thus, further experiments are needed to determine the optimum aging period for producing wholesome aged jujube.

5'-Hydroxymethylfurfural (5’-HMF) is a common Maillard reaction product generated during heat-processing. HMF occurs as a product of decomposing sugars, including glucose and sucrose, in the presence of acid. Although 5’-HMF has been proposed to have harmful effects, its beneficial effects include antioxidant, cytoprotective and antitumor effects and have become increasingly apparent. A recent study found that the extract of aged black garlic shows anti-inflammatory properties when administered to human umbilical vein endothelial cells (30). The 5’-HMF contents of black jujube extract significantly increased with longer aging times, and contained even higher contents in the ethanol extract than the water extract (Fig. 2). The 5’-HMF content of ethanol extract showed the highest value with 338.89 mg% after aging for 3 days. Compared to the 5’-HMF content (1.34 mg%) in non-aging jujube, that in aged jujube increased by approximately 250 times. Interestingly, Park et al. (11) reported that after 50 days of aging, HMF content of jujube extract concentrate was 38.84 mg/g. The dehydrated fruit such as prune contains about 220 mg% of HMF. The important factors in the development of HMF in the Maillard reaction are temperature and time so that the formation of browning products and HMF increase with extended heat exposure and aging time.

**Table 4.** Change in nonvolatile organic acid contents of black jujube extract according to aging times

| Organic acid contents (mg/100 g) | 0          | 24         | 48         | 72         |
|---------------------------------|------------|------------|------------|------------|
| Oxalic acid                     | 436.08±10.50<sup>a</sup> | 697.97±8.01<sup>b</sup> | 720.49±5.58<sup>b</sup> | 755.67±12.93<sup>c</sup> |
| Pyruvic acid                    | 22.03±3.36<sup>a</sup> | 44.60±2.90<sup>b</sup>   | 82.34±3.80<sup>b</sup>   | 45.44±1.58<sup>c</sup>   |
| Lactic acid                     | 40.27±2.91<sup>a</sup> | 45.56±5.83<sup>b</sup>   | 41.92±10.40<sup>a</sup>  | 42.89±3.92<sup>b</sup>   |
| Acetic acid                     | 5.24±1.95<sup>a</sup>  | 8.79±1.56<sup>b</sup>    | 8.55±0.97<sup>ab</sup>   | 12.96±2.39<sup>b</sup>   |
| Malic acid                      | 1.25±0.78<sup>a</sup>  | 3.86±0.91<sup>b</sup>    | 5.47±0.83<sup>b</sup>    | 5.86±1.05<sup>b</sup>    |
| Citric acid                     | 198.92±13.11<sup>a</sup> | 384.52±8.29<sup>b</sup> | 426.23±13.63<sup>b</sup> | 498.54±17.13<sup>c</sup> |

Data are expressed as mean±standard error values (n=3). Mean with different letters in each row are significantly different (p<0.05) by Duncan’s multiple range test.

**Fig. 2.** 5’-HMF contents of black jujube extract according to aging time. Data are expressed as mean±standard error values (n=3). Mean with different letters are significantly different (p<0.05) by Duncan’s multiple range test.
48 hr, indicating the highest levels. The reasons for the increased polyphenol contents by aging dried jujube at a higher temperature was thought to be caused by the transformation of some ingredients into soluble polyphenols or the efficient extraction of polyphenol compounds due to the softening of fruit pulp during the humid heat processing. Jummongpon et al. (31) reported that the Maillard reaction of proteins does not only result in the generation of flavors and colors, but also changes the texture of foods. Shim (32) reported that the polyphenol contents of black jujube aged for a week at a certain humidity was 2.61%, which was higher than that of dried jujube. Kim et al. (33) reported that total phenol and flavonoid contents increased when black garlic was produced by a heat treatment at high pressure.

### DPPH radical scavenging effects of BJE

DPPH (1,1-diphenyl-2-picryl-hydrazyl) can be deoxidized by ascorbic acid, tocopherol, polyhydroxy aromatic compounds and aromatic amines, decolorizing its violet color which is used to measure electron releasing levels of antioxidants (34). Electron releasing antioxidants are characterized by the ring structure containing more than one hydroxyl group and a non-polar group, such as methyl, or non-polar hydrocarbon chain. The polyphenol contents in foods are the representative means for measuring antioxidant effects.

Table 5 shows the results of anti-oxidative effects of dried jujube and black jujube according to the aging periods. The results resembled the results of DPPH radical scavenging activity of black jujube matured for 20 days were 70.90% at the concentration of 50 mg/mL and 89.42% at the concentration of 100 mg/mL, supporting the claim that the activity level was increased as the maturation period became longer (25). Generally, the reason for the higher level of DPPH radical scavenging activity of black jujube than dried jujube is thought to be stemmed from the increased polyphenol and flavonoid contents and the melanoidin generated during the aging process. As previously reported and according to our results, the Maillard reaction products provided antioxidant properties (36).

### ABTS radical scavenging effects of BJE

ABTS radical cation is eradicated by the anti-oxidative reagent resulting in the decolorization of the unique green-blue color of radicals, when ABTS and potassium persulfate are kept in the dark to generate ABTS radical cations, and can be expressed in optical density to measure the ABTS radical scavenging activity (37). Table 7 shows the results of ABTS radical scavenging activity of dried jujube and black jujube according to the aging periods. The results resembled the results of DPPH radical scavenging activity in that the activity was increased as the maturation period became longer. The IC50 value of water extract of black jujube matured for 20 days was 0.66 mg/mL after aging for 72 hr. In a previous report, the ABTS radical scavenging activity increased as dried jujube with red skin was becoming black jujube with black skin (38), which was measured in trolox equivalent antioxidant capacity after aging for 5 days with up to 3.186 mmol/L and little difference after-

#### Table 5. The total polyphenol contents of black jujube extract according to aging times

| Sample       | Aging time (hr) | Total polyphenol contents (mg/g) |
|--------------|-----------------|---------------------------------|
| Ethanol extract | 0               | 7.74±0.42^a                      |
|              | 24              | 12.44±0.71^b                     |
|              | 48              | 13.39±0.55^c                     |
|              | 72              | 13.79±0.57^d                     |
| Water extract | 0               | 8.12±0.60^a                      |
|              | 24              | 13.11±0.52^a                     |
|              | 48              | 16.82±0.58^b                     |
|              | 72              | 15.17±0.64^c                     |

Data are expressed as mean±standard error values (n=3). Mean with different letters in each column are significantly different (p<0.05) by Duncan’s multiple range test.

#### Table 6. DPPH radical scavenging effects of black jujube extract according to aging times

| Sample       | Aging time (hr) | IC50^1 (mg/mL) |
|--------------|-----------------|----------------|
| Ethanol extract | 0               | 1.08±0.10^d     |
|              | 24              | 0.68±0.03^a     |
|              | 48              | 0.56±0.03^a     |
|              | 72              | 0.54±0.02^a     |
| Water extract | 0               | 0.94±0.02^c     |
|              | 24              | 0.69±0.06^b     |
|              | 48              | 0.60±0.01^a     |
|              | 72              | 0.56±0.06^a     |
| BHT^2         |                 | 2.62±0.92 μg/mL |
| BHA^3         |                 | 9.63±0.31 μg/mL |
| Vitamin C     |                 | 6.38±0.50 μg/mL |

^1Concentration required for 50% reduction of DPPH radical at 30 min after starting the reaction.
^2Data are expressed as mean±standard error values (n=3). Mean with different letters in each column are significantly different (p<0.05) by Duncan’s multiple range test.
^3Butylated hydroxy toluene.
^4Butylated hydroxy anisole.
wards. Byun et al. (39) concluded that the anti-oxidative effects of the ethanol extract of garlic reduced to about 50% with treatment at 100°C compared to 120°C, implying that the anti-oxidative effects are related to the intermediated products of the Maillard reaction generated by the higher heat treatment. The Maillard reaction for browning can occur in most foods and this non-enzymatic browning takes place during thermal food or storage processes. Melanoidin, the brown colored polymer, is generated by the reaction between amino acids and sugars during the maturation of traditional Korean condiments and has a strong anti-oxidative effect known to prevent cancer (40). Thus, the Maillard reaction product in black jujube is a beneficial ingredient providing coloring and anti-oxidative effects.

### α-Glucosidase inhibitory activity of BJE

α-Glucosidase is the digestive enzyme in the brush-border membrane of small bowel which hydrolyzes disaccharides and polysaccharides into monosaccharides which is the absorbable form of carbohydrates (41). α-Glucosidase inhibitory substances may prevent the increase of blood glucose level after a carbohydrate intake diet. Fig. 3 shows the results of α-glucosidase inhibitory activity of black jujube at the concentration of 3.33 mg/mL according to the aging periods. Overall, the levels of α-glucosidase inhibitory activity were superior in the 70% ethanol extract compared to the water extract. Dried jujube in the water extract and 70% ethanol extract showed the inhibitory activity of 17.04% and 65.7%, respectively. As the aging progressed, the inhibitory activity level of 70% EtOH extract increased to 80.69% and after 72 hr. Hwang et al. (42) reported that the fraction isolated from the fructose-tyrosine Maillard reaction products showed strong α-glucosidase inhibitory activity. Thus, higher α-glucosidase inhibitory activity of black jujube may be related to the HMF compound which is highly extracted with 70% ethanol. Furthermore, tannins, condensed tannins and related polyphenols showed the inhibitory effects of enzymes such as xanthine oxidase. The inhibitory activity of several oligomeric hydrolyzable tanins seemed particularly low, and the degree of polymerization in proanthocyanidins was also shown to remarkably affect the strength of the inhibition (43). In addition, water soluble Maillard polymers were prepared from HMF, glucose and amino acids (44). Thus, polyphenols in jujube seem to be polymerized during the Maillard reaction and their compounds may affect enzyme activity.

Shin et al. (45) reported that α-glucosidase inhibitory activity levels of the water extract of browned garlic made by hot-air drying for 30 hr at 80°C were not much different at the concentration level less than 2.5 mg/mL; but, the inhibitory activity levels increased up to 22.22% depending on the concentration level above 2.5 mg/mL. In addition, they also reported that α-glucosidase inhibitory activities of fresh garlic, red garlic and black garlic extracts were measured at 21.23%, 21.54% and 37.84%, respectively, at the concentration of 2 mg/mL, indicating the inhibitory effect due to the increase of browning substances (46).

In diabetes, free radicals can be generated by auto-oxidation of glucose and various oxidative stresses and can result in tissue damage (47). Recently, an increasing effort exists to identify α-glucosidase inhibitors from natural materials (41). Therefore, managing diabetes milletus may be more effective when both α-glucosidase inhibitory activity and anti-oxidative activity levels in black jujube are increased. Conclusively, aged black jujube could be a valuable ingredient to help alleviate diabetes milletus indirectly. Furthermore, black jujube produced by aging for 2 days could be a natural food ingredient substituting for caramel pigment as well as providing

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**Table 7. ABTS radical scavenging effects of black jujube extract according to aging times**

| Sample         | Aging time (hr) | IC50 (mg/mL) |
|----------------|-----------------|--------------|
| Ethanol extract| 0               | 2.09±0.042   |
|                | 24              | 0.73±0.02    |
|                | 48              | 0.60±0.011   |
|                | 72              | 0.59±0.01    |
| Water extract  | 0               | 1.99±0.02    |
|                | 24              | 0.62±0.02    |
|                | 48              | 0.70±0.02    |
|                | 72              | 0.66±0.011   |
| Vitamin C      |                 | 67.29±5.94 μg/mL |
| Trolox         |                 | 57.55±1.28 μg/mL |

1) Concentration required for 50% reduction of ABTS radical.
2) Data are expressed as mean±standard error values (n=3). Mean with different letters in each column are significantly different (p<0.05) by Duncan’s multiple range test.

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**Fig. 3.** α-Glucosidase inhibitory activity of black jujube extract according to aging time. All sample concentration was 3.33 mg/mL. Data are expressed as mean±standard error values (n=3). Mean with different letters are significantly different (p<0.05) by Duncan’s multiple range test.
palatable taste.

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