Effect of Drying Conditions on Nutritional Quality and In Vitro Antioxidant Activity of Traditional Doenjang

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ABSTRACT: Doenjang, a major traditional Korean condiment, is often dried to reduce volume and thereby shipping cost while increasing shelf life. However, changes of nutritional and sensory properties of doenjang during processing have not been well understood. Therefore, this study aimed to evaluate how drying processes influence the nutritional and chemical properties of doenjang. When two drying methods, hot air drying and freeze drying were compared from the nutritional point of view, air-dried doenjang at 60°C or lower showed similar quality parameters including sensory scores, proximate composition, antioxidant capacity, amino acid composition, amino nitrogen, and acid value to freeze-dried doenjang. In contrast, the sample dried at 80°C and 100°C showed lower quality parameters than the freeze-dried one. Ferric reducing antioxidant potential (FRAP), total phenolics content, amino acid composition, and acid value were shown to reflect the sensory and physical properties of dried doenjang. In particular, the FRAP value of dried doenjang was sensitively responsive to drying temperatures and may be utilized as an early biomarker for quality deterioration of dried doenjang.

Keywords: traditional doenjang, drying, quality, antioxidant, nutrients

INTRODUCTION

Traditionally, fermented soybean products including doenjang (fermented soybean paste) and ganjang (soy sauce) are the base for various kinds of soups, sauces for Korean style salad, and a major seasoning in Korean dishes. Doenjang has traditionally been manufactured using fermented meju pre-soaked in brine (20% salt solution) for 30 days or longer. Meju, in turn, is manufactured by soaking soybeans in clean natural water, steaming, crushing, and subsequent molding into rectangular blocks which are being exposed to air so as to collect airborne microorganisms (mainly Aspergillus and Bacillus species) from the natural environment to ferment it. While commercial doenjang (equivalent to Japanese miso) manufactured by the fermentation of cooked soybeans with koji (or Aspergillus oryzae) is predominant in the market, traditional doenjang increasingly attracts consumers due to its unique flavor and taste (1,2).

Recently, Korean traditional doenjang received much attention due to its health-promoting benefits such as its antioxidant activity, anti-cancer effects, and associated anti-mutagenicity (3-9). While soybeans contains a variety of bioactive components, fungal fermentation could enhance the biological activities of bioactive compounds through enzymatic bioconversion. For instance, isoflavones present mostly in glycoside forms in raw soybeans could be converted, by fungal enzymes during fermentation, into aglycones, which are more bioavailable forms (10,11).

Doenjang contains approximately 60% of water and therefore has a relatively short shelf life. To improve its storage stability, it needs to be dried or processed to lower its water activity. However, drying at elevated temperatures could cause quality deterioration through chemical reactions. In particular, Maillard reactions may lead to darkening, increased acidity, generation of unique flavor and taste, while increasing the antioxidant potential of doenjang (12).

Thus, this study aims to investigate how drying conditions affect the nutritional properties and antioxidant potential of traditionally prepared doenjang.

MATERIALS AND METHODS

Materials
All chemicals and reagents used were of American Chem-
ical Society grade. Gallic acid, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridylstilbazone (TPTZ) solution were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and isoflavones (genistein, daidzein, and glycitein) were obtained from Chengdu Biopurify Phytochemicals (Chengdu, China).

Drying of doenjang

Traditional doenjang aged for more than one year was obtained from Andong Jebiwon Agricultural Corp. (Andong, Gyeongbuk, Korea), and 100 g of the sampledried under different conditions including conventional hot-air drying at 40, 60, 80, and 100°C (HB-502S, Hanbaek Scientific Co., Ltd., Bucheon, Gyeonggi, Korea), and freeze-drying (FD 8512; Ilshin BioBase Co., Ltd., Yangju, Gyeonggi, Korea).

Preparation of doenjang extract for antioxidant activity assays

Dried doenjang samples were extracted with 10 volumes of 80% (v/v) ethanol, filtered, concentrated to a final concentration of 10 mg/mL, and filtered through a 0.2 μm sterile syringe filter (Sartorius, Göttingen, Germany) before assays.

FRAP assay

The FRAP of doenjang extract was determined as described previously (13). Briefly, 30 μL of H₂O and 30 μL of ferrous sulfate as standard, or samples were incubated at room temperature with 1 mL of FRAP reagent, containing 300 mmol/L acetate buffer (pH 6.3), 10 mmol/L TPTZ solution, 20 mmol/L FeCl₃ solution, and H₂O. The absorbance at 593 nm was recorded after 4 min. FRAP values of unknowns were calculated by extrapolation of standard curves.

DPPH radical scavenging assay

The DPPH radical scavenging activity of doenjang extract was evaluated as previously described (14). Briefly, 50 μL of sample solution or dimethyl sulfoxide was added to 200 μL of 200 μM DPPH radical solution, which was freshly made. After 30 min of incubation at room temperature, the absorbance at 515 nm was measured. Synthetic antioxidant reagent, L-ascorbic acid, was used as a positive control, and all tests were conducted in triplicates.

ABTS radical cation decolorization assay

The ABTS solution (a mixture of 5 mL of 7 mM ABTS and 80 μL of 2.45 mM potassium persulfate) was allowed to react in the dark at room temperature for 12 h before use (15). The solution was diluted with ethanol so that its absorbance was adjusted to 0.7±0.02 at 734 nm. ABTS (1 mL) was mixed with a sample (50 μL) in a glass test tube by vortexing for 30 s. Absorbance was measured at 734 nm after 5 min. The percentage of radical scavenging activity was calculated by comparing the absorbance values of the control without samples. All determinations were triplicated (16).

Analyses of nutritional composition and chemical components

Proximate composition, pH, titratable acidity, acid value, salinity, amino nitrogen, total free amino acids, and biogenic amines of doenjang dried under different conditions were assayed according to AOAC methods (17). For the pH measurement of the samples, 25 mL of deionized distilled water was added to 5 g of doenjang samples and then homogenized and filtered with Whatman paper (No. 2, Advantec Toyo Kaisha Ltd., Tokyo, Japan). The pH of the sample was measured using a pH meter (MP220, Mettler-Toledo, Greifensee, Switzerland).

The total free amino acid composition was analyzed by an amino acid analyzer (Hitachi L-8900, Hitachi, Tokyo, Japan) after extracting amino acids from doenjang samples as previously described (16).

Determination of total phenolic, flavonoid, and total isoflavone contents

Total phenolics were determined using the Folin-Ciocalteu reagent (18). Briefly, 100 μL of extract was mixed with 50 μL of sodium bicarbonate solution [10% (w/v)], followed by the addition of 15 μL of Folin-Ciocalteu reagent (previously diluted 5-fold with distilled water). After 5 min at room temperature, the sample mixture was transferred to a 96-well microplate, and the absorbance at 655 nm was measured using a microplate reader (Sunrise™, Tecan Group Ltd., Männedorf, Switzerland). Results are expressed as gallic acid equivalents.

Total flavonoid content was determined by aluminum chloride using a colorimetric method previously described with slight modifications (19). Briefly, 25 μL of the sample was mixed with 75 μL of 95% methanol in a 96-well microplate. Then, 5 μL of 10% AlCl₃·6H₂O, 1 M potassium acetate, and 14 μL of distilled water were added, and the mixture was incubated for 40 min at room temperature. Absorbance readings were obtained at 415 nm with a microplate reader. The total flavonoid content of the samples was extrapolated from standard curves plotted with naringin at 0~50 μg/mL.

High-performance liquid chromatography (HPLC) analyses for total isoflavones including genistein, daidzein, and glycine, were performed by the procedure described elsewhere (20). Briefly, 1 g of dried doenjang sample was placed in a glass test tube containing 10 mL of acetonitrile and 1 mL 0.1 N HCl. After shaking the test tube for 90 min at room temperature, the supernatant was filtered.
Table 1. Operating conditions for HPLC analysis of isoflavones

| Description                      | Condition                  |
|----------------------------------|----------------------------|
| Column                           | Gemini C<sub>18</sub>, 5 μm, 2.0×150 mm |
| Column oven temp.                | 25°C                       |
| Detector                         | Diode array detector, 254 nm |
| Mobile phase                     | A: 0.1 % acetic acid in water |
|                                  | B: 0.1 % acetic acid in acetonitrile |
| Flow rate                        | 0.8 mL/min                 |
| Injection volume                 | 10 μL                      |
| Composition of mobile phase      | A:B=85:15 (0 min)→60:40 (30 min)→85:15 (40 min) |

Fig. 1. Drying curve of traditional doenjang. Fifty gram of traditional doenjang obtained from Andong Jебiwon Agricultural Corp. was dried in convection oven at 45, 60, 80, and 100°C and weighed every 30 min until reached constant weight.

RESULTS

Drying curve for traditional doenjang at different drying temperatures

Korean traditional doenjang prepared at Andong Jебiwon Agricultural Corp. was subjected to conventional air drying using a convection oven at various temperatures. As shown in Fig. 1, doenjang with 60% moisture content showed typical drying curves. Total drying times at 45, 60, 80, and 100°C were approximately 36, 15, 9, and 6 h, respectively.

Antioxidant activity of doenjang dried under different conditions

The antioxidant capacity of doenjang was compared among the drying conditions, freeze drying and hot air drying at 45, 60, 80, and 100°C (Fig. 2). While doenjang dried under different conditions was not significantly different in DPPH radical scavenging activity (Fig. 2A), doenjang dried at relatively high temperatures showed a stronger antioxidant activity than the other drying conditions (P<0.05) as assayed by ABTS<sup>+</sup> radical scavenging activity and FRAP (Fig. 2B and 2C). In particular, the extract prepared from doenjang dried in the convection oven at 100°C showed significantly higher antioxidant activity (P<0.05) than the samples dried at lower temperatures and a dose-dependent FRAP activity (Fig. 2C).

The content of total phenolics was significantly higher in doenjang dried at 100°C while the isoflavone content was higher in doenjang dried at 80 and 100°C than the samples dried at 45, 60°C, or freeze dried (Table 2).

Proximate composition and quality parameters of doenjang dried under different conditions

The general composition of traditional doenjang dried at different temperatures or by freeze drying was not significantly different from each other. Doenjang dried in
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Fig. 2. Antioxidant activity of doenjang dried under different conditions. Doenjang dried under different conditions was extracted with 80% ethanol and then subjected to antioxidant activity assays such as DPPH (A) and ABTS\(^+\) (B) radical scavenging assays, and FRAP assay (C). Data are mean±SD (n=3). Bars not sharing a common letter (a-d) in same concentration group represent statistically significantly different values from each other (\(P<0.05\)). NS, not significant.

the convection oven at 100\(^\circ\)C had significantly lower pH, high acidity, and acid value (\(P<0.05\)) (Table 3). However, the concentrations of biogenic amines and amino nitrogen were not significantly affected by drying temperature while those were slightly higher in freeze-dried doenjang.

Free amino acid composition of traditional doenjang was compared according to the drying conditions (Table 4). The concentrations of most free amino acid including threonine, histidine, proline, and glutamic acid were increased in doenjang dried at 100\(^\circ\)C, compared to the samples dried at lower temperatures or freeze-dried. A few amino acids such as aspartic acid, cystine, and arginine were reduced in doenjang dried at 100\(^\circ\)C.

Meanwhile, the air-dried sample at 100\(^\circ\)C had a significantly lower L value and higher a value than the other samples, indicating darker color than the samples including freeze-dried one (Table 5).

Sensory evaluation by 10 trained panelists on doenjang dried at different drying temperatures showed that doenjang dried at 60\(^\circ\)C had the highest overall acceptability among the sample groups (Table 6).
DISCUSSION

Doenjang, one of the most consumed Korean traditional seasonings and condiments, is traditionally manufactured by soaking meju in a salt solution for 2~3 months (9). Meju, a fermented rectangular block of crushed cooked soybeans, is made by allowing airborne microorganisms to grow on the surface of the cooked soybean block. The major microorganisms involved in meju fermentation are reported to be Bacillus subtilis and molds such as Rhizopus, Mucor, and Aspergillus species (1,9). Despite its high salt concentration, doenjang containing approximately 60% moisture is liable to microbial spoilage, so it is usually recommended to be stored at refrigerated temperature.

Removal of free water from doenjang by the drying process is one of the best ways to extend its shelf life and make a long distance shipping possible with the minimum quality change. However, the quality of doenjang is significantly affected by drying conditions including drying temperatures. Although the proximate composition of doenjang was little changed at drying temperatures lower than 100°C, some minor components such as free amino acids, amino nitrogen, and total phenolics showed significant change at drying temperatures as high as 100°C (Table 2 and 3). For instance, the total phenolic content significantly increased in doenjang dried at 100°C (Table 2), probably due to increased chemical reactions at high temperatures. In addition, the acid value, which is a biomarker of lipid oxidation, was increased at drying temperatures of 100°C due to increased chemical reactions at high temperatures. The parameters other than proximate composition and acid value were values from a single measurement.

| Total phenolics (µg/g) | Total flavonoids (µg/g) | Isoflavones (µg/g) |
|------------------------|------------------------|-------------------|
| Freeze dried | 499.7±10.9 | 1,493.2±8.4 | 766.9±6.3 |
| Hot air drying | 45°C | 391.7±9.1 | 1,457.2±70.6 | 744.1±11.4 |
| 60°C | 428.3±13.8 | 1,700.0±46.3 | 704.9±19.5 |
| 80°C | 355.1±18.2 | 1,529.7±33.5 | 825.7±108.0 |
| 100°C | 579.9±15.7 | 1,634.5±39.8 | 808.6±15.7 |

Total phenolic, total flavonoid, and isoflavone contents of doenjang dried under various conditions were assayed according to the protocols described in ‘MATERIALS AND METHODS’. Data are mean±SD (n=3).

Table 3. Proximate composition and quality parameters of doenjang dried under different conditions

| Ash (%) | 12.9±0.0 | 12.9±0.0 | 12.9±0.0 | 12.9±0.0 |
| Crude protein (%) | 21.5±0.0 | 19.1±0.0 | 17.6±0.0 | 25.9±0.0 |
| Crude fat (%) | 22.6±0.0 | 17.4±0.0 | 17.3±0.0 | 16.4±0.0 |
| Salt concentration (%) | 60 | 60 | 56 | 60 |
| pH | 5.9 | 5.9 | 5.7 | 5.3 |
| Total acids (%) | 60 | 60 | 3.2 | 3.6 |
| Acid value (mg KOH/g) | 29.6±2.0 | 31.6±1.7 | 30.6±1.2 | 27.4±0.0 |
| Amino N (mg %) | 865.8 | 799.4 | 772.7 | 798.5 |
| Total free amino acids (mg/g) | 83.4 | 83.8 | 84.2 | 88.6 |
| 2-Phenylethylamine (mg/g) | 2.35 | 3.02 | 2.66 | 2.23 |
| Histamine (mg/g) | 0.06 | 0.05 | 0.02 | 0.05 |

Data are mean±SD (n=3). Values not sharing a common letter (a-c) are statistically significantly different from each other (P<0.05).
Table 4. Free amino acid composition of *doenjang* dried under different conditions (unit: μg/g)

| Free amino acids | Freeze dried | 45°C | 60°C | 80°C | 100°C |
|------------------|--------------|------|------|------|-------|
| Phosphoserine    | ND           | ND   | ND   | ND   | ND    |
| Taurine          | ND           | ND   | ND   | ND   | ND    |
| Phosphoethanol amine | ND       | ND   | ND   | ND   | ND    |
| Urea             | ND           | ND   | ND   | ND   | ND    |
| Aspartic acid    | 2,104.63     | 2,128.95 | 2,058.92 | 2,077.93 | 2,042.94 |
| Threonine        | 3,312.99     | 3,349.12 | 3,551.97 | 4,407.07 | 4,420.17 |
| Serine           | 1,321.68     | 1,318.81 | 1,482.59 | 1,921.58 | 1,895.72 |
| Glutamic acid    | 3,789.57     | 3,715.36 | 3,719.62 | 4,219.95 | 4,055.26 |
| Sarcosine        | 15.04        | 17.22  | 16.27 | 12.83 | 29.04 |
| α-Amino adipic acid | 773.04    | 762.69 | 766.19 | 825.79 | 813.16 |
| Glycine          | 4,398.86     | 4,390.67 | 4,228.85 | 4,273.12 | 4,236.02 |
| Alanine          | 9,787.03     | 9,781.97 | 9,608.61 | 9,746.25 | 9,701.89 |
| Citrulline       | ND           | ND   | ND   | ND   | ND    |
| α-Amino-n-butyric acid | 1,989.76 | 2,119.67 | 1,385.04 | 972.48 | 1,019.12 |
| Valine           | 5,993.62     | 6,125.71 | 6,137.41 | 6,360.48 | 6,096.60 |
| Cystine          | 597.80       | 651.26 | 641.16 | 545.16 | 436.60 |
| Methionine       | 2,232.14     | 2,190.96 | 2,141.32 | 2,168.67 | 2,106.26 |
| Cystathionine    | ND           | ND   | ND   | ND   | ND    |
| Isoleucine       | 5,899.42     | 5,968.42 | 5,953.27 | 6,132.27 | 6,222.91 |
| Leucine          | 11,232.13    | 11,331.96 | 11,224.81 | 11,299.74 | 11,391.40 |
| Tyrosine         | 1,728.97     | 1,719.12 | 2,344.58 | 3,715.82 | 2,992.21 |
| Phenyllalanine   | 6,614.60     | 6,657.76 | 6,981.22 | 7,372.41 | 7,202.92 |
| β-Alanine        | 183.94       | 283.71 | 338.16 | 321.29 | 214.88 |
| β-Amino isobutyric acid | 126.85 | 128.41 | 622.78 | 704.24 | 572.89 |
| γ-Amino-n-butyric acid | 12,777.12 | 12,926.22 | 12,609.79 | 13,031.51 | 12,720.77 |
| Ethanolamine     | 132.62       | 133.42 | 126.18 | 129.82 | 126.75 |
| Hydroxylysine    | ND           | ND   | ND   | ND   | ND    |
| Ornithine        | 2,183.28     | 2,156.84 | 2,075.23 | 2,204.57 | 2,164.97 |
| Lysine           | 9,160.52     | 9,085.56 | 8,737.37 | 9,146.46 | 9,047.05 |
| 1-Methylhistidine | ND         | ND   | ND   | ND   | ND    |
| Histidine        | 623.42       | 601.86 | 735.15 | 1,066.10 | 1,013.18 |
| 3-Methylhistidine | ND         | ND   | ND   | ND   | ND    |
| Anserine         | 216.64       | 299.41 | 338.42 | 389.99 | 377.84 |
| Carnosine        | 11.50        | 10.76  | 11.79 | 8.91  | 11.12 |
| Arginine         | 204.87       | 205.73 | 205.71 | 186.41 | 187.80 |
| Hydroxyl proline | ND           | ND   | ND   | ND   | 1.48  |
| Proline          | 5,313.56     | 5,389.03 | 5,512.10 | 5,230.70 | 6,301.28 |
| Total free amino acid | 92,726.61 | 93,180.60 | 93,554.52 | 98,471.56 | 97,402.23 |

ND, not detected.

Table 5. Color of *doenjang* powder dried under different conditions

| Color values | Freeze dried | 45°C | 60°C | 80°C | 100°C |
|--------------|--------------|------|------|------|-------|
| L            | 71.33±0.24   | 53.71±0.16 | 52.74±0.03 | 54.42±0.36 | 49.29±0.12 |
| a            | 4.50±0.03    | 7.39±0.03 | 8.38±0.10 | 8.66±0.06 | 9.05±0.01   |
| b            | 21.36±0.14   | 21.06±0.07 | 19.09±0.07 | 21.67±0.23 | 18.28±0.08   |
| ∆E*<sub>ab</sub> | 30.98±0.26   | 43.85±0.17 | 43.74±0.03 | 44.13±0.38 | 46.39±0.14   |

∆E*<sub>ab</sub>=√((ΔL)<sup>2</sup>+(Δa)<sup>2</sup>+(Δb)<sup>2</sup>)

Data are mean±SD (n=3). Values not sharing a common alphabetical letter are statistically significantly different from each other (P<0.05).
to the DPPH and ABTS\(^+\) assays, FRAP measures the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions of known concentrations. Ferric to ferrous ion reduction at low pH causes a colored ferrous-triprydyltriazine complex to form (23). The results from this study imply that FRAP is a relatively sensitive biomarker for the quality change of doenjang by hot air drying.

Meanwhile, sensory evaluation by 10 trained panelists on doenjang dried under different conditions showed that doenjang powder prepared by drying at 60\(^\circ\)C had the highest sensory score among all hot air drying temperatures used in the study, with similar overall sensory score for the freeze-dried samples (Table 6). Doenjang dried at 80 and 100\(^\circ\)C was shown to have a burnt aroma, with concurrent changes in FRAP value, total free amino acid content, and acid value. In addition, the air-dried sample at 100\(^\circ\)C had a darker color than the samples dried at lower temperatures of less than 80\(^\circ\)C, while freeze-dried doenjang showed the brightest color (Table 5). The Maillard reaction is a mixture of chemical reactions between reducing sugars and amino groups of protein, peptides, or amino acids. The reaction rate is mainly affected by initial pH, temperature, time, and water activity (24,25). Among these factors, temperature is one of the most important parameters that affects the reaction rates and aroma characteristics of foods (26,27). Our study also demonstrated that drying at temperatures as high as 80\(^\circ\)C and 100\(^\circ\)C caused a significant decline in sensory score and changes in some components including total phenolics and free amino acids probably due to the Maillard reaction. In particular, doenjang powder prepared by drying at a high temperature of 100\(^\circ\)C showed a significantly strong antioxidant activity and high FRAP values which can probably be mediated by Maillard reaction products.

In conclusion, hot air drying at 60\(^\circ\)C or lower resulted in doenjang powder with similar sensory and nutritional qualities to that of freeze-drying. Also, the quality change of dried doenjang can be monitored most sensitively by the FRAP assay as well as sensory evaluation.

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### AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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