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

## Seung Min Park, Jisun Oh, Jung Eun Kim, and Jong-Sang Kim

School of Food Science and Biotechnology (BK21 plus program) and Institute of Agricultural Science and Technology, Kyungpook National University, Daegu 41566, Korea

**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-

Received 21 February 2018; Accepted 10 May 2018; Published online 30 June 2018

Correspondence to Jong-Sang Kim, Tel: +82-53-950-5752, E-mail: vision@knu.ac.kr

Copyright © 2018 by The Korean Society of Food Science and Nutrition. All rights Reserved.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

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-tripyridylstriazine (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  $\mu$ m sterile syringe filter (Sartorius, Göttingen, Germany) before assays.

### **FRAP** assay

The FRAP of *doenjang* extract was determined as described previously (13). Briefly, 30  $\mu$ L of H<sub>2</sub>O and 30  $\mu$ L 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 FeCl<sub>3</sub> solution, and H<sub>2</sub>O. 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  $\mu$ L of sample solution or dimethyl sulfoxide was added to 200  $\mu$ L of 200  $\mu$ M 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  $\mu$ L 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  $\mu$ L) 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  $\mu$ L of extract was mixed with 50  $\mu$ L of sodium bicarbonate solution [10% (w/v)], followed by the addition of 15  $\mu$ L 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<sup>TM</sup>, 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  $\mu$ L of the sample was mixed with 75  $\mu$ L of 95% methanol in a 96-well microplate. Then, 5  $\mu$ L of 10% AlCl<sub>3</sub>·6H<sub>2</sub>O, 1 M potassium acetate, and 14  $\mu$ L 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  $\mu$ g/mL.

High-performance liquid chromatography (HPLC) analyses for total isoflavones including genistein, daidzein, and glycitein, 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

Description Condition Column Gemini C<sub>18</sub>, 5 µm, 2.0×150 mm Column oven temp. 25°C Diode array detector, 254 nm Detector 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 A:B=85:15 (0 min)→60:40 (30 min)→ mobile phase 85:15 (40 min)

Table 1. Operating conditions for HPLC analysis of isoflavones

through a syringe filter (polyvinylidene difluoride membrane, 0.45  $\mu$ m pore size, 13 mm diameter; Whatman<sup>TM</sup>, Maidstone, UK). The filtrate (10  $\mu$ L) was injected into a Jasco HPLC system equipped with a pump (PU-2089 Plus Quaternary Gradient Pump, Jasco International Co., Ltd., Tokyo, Japan) and a photodiode array detector (MD-2010 Plus Multi-wavelength Detector, Jasco International Co., Ltd.). The operating conditions of HPLC are specified in Table 1.

#### Surface color measurement

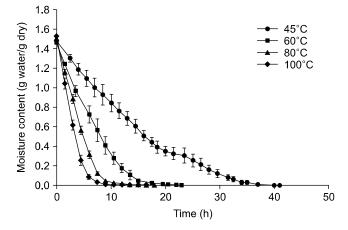
The color of dried powder of *doenjang* was measured using a chromatometer (CR 400, Minolta, Osaka, Japan), which provided CIE L\*, a\*, and b\* values. Negative a\* values indicate green and higher positive a\* values represent red color. Higher positive b\* values indicate a more yellow color (21).

#### Sensory analysis

The samples were organoleptically assessed by 10 panelists, using a sensory rating scale of  $1 \sim 5$  for color, odor, smooth taste, umami taste, bitterness, saltiness, and overall acceptability, as described previously (22). The organoleptic properties evaluated included: six attributes for color (5, bright; dark, 1), odor (5, like; 1, dislike), umami taste (5, strong; 1, weak), bitterness (5, strong; 1, weak), salty taste (5, strong; 1, weak), and overall acceptability (5, like; 1, dislike). The panel of assessors was an external panel of non-smokers who were very familiar with fermented soy products and were checked on the basis of sensory acuity and consistency. The protocol for sensory evaluation was approved by the Institutional Review Board (IRB) of Kyungpook National University (IRB #KNU-2018-0075).

#### Statistical analysis

Statistical significance of data was tested by analysis of variance, followed by Duncan's multiple range test, using SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA). Data were presented as mean $\pm$ standard deviation (SD). Mean values not sharing a common letter indicate statistically significant differences (*P*<0.05).



**Fig. 1.** Drying curve of traditional *doenjang*. Fifty gram of traditional *doenjang* obtained from Andong Jebiwon 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 Jebiwon 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.

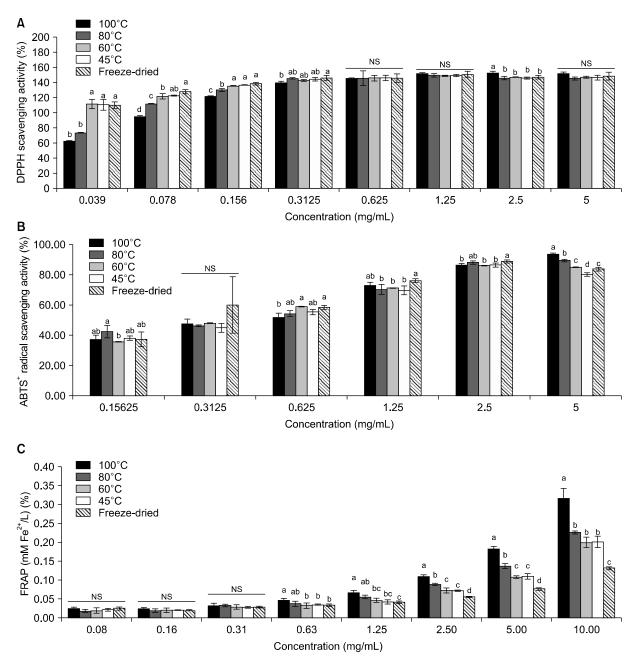
## Antioxidant activity of *doenjang* dried under different conditions

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 *doen-jang* 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



**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 $\pm$ 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°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°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°C.

Meanwhile, the air-dried sample at 100°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°C had the highest overall acceptability among the sample groups (Table 6).

	Total phenolics <sup>1)</sup>	Total	Total Isoflavones			
		flavonoids <sup>2)</sup>	Genistein	Daidzein Glycite		
Freeze dried Hot air drying	499.7±10.9 <sup>b</sup>	1,493.2±8.4 <sup>b</sup>	766.9±6.3 <sup>ab</sup>	559.8±5.9 <sup>ab</sup>	283.0±4.9 <sup>b</sup>	
45°C	391.7±9.1 <sup>d</sup>	1,457.2±70.6 <sup>b</sup>	744.1±11.4 <sup>ab</sup>	533.5±10.0 <sup>b</sup>	297.6±29.5 <sup>ab</sup>	
60°C	428.3±13.8 <sup>c</sup>	1,700.0±46.3 <sup>a</sup>	704.9±19.5 <sup>b</sup>	511.2±6.2 <sup>b</sup>	274.2±25.3 <sup>b</sup>	
80°C	355.1±18.2 <sup>e</sup>	1,529.7±33.5 <sup>b</sup>	825.7±108.0 <sup>a</sup>	602.9±71.8 <sup>ª</sup>	332.7±33.6ª	
100°C	579.9±15.7ª	1,634.5±39.8 <sup>a</sup>	808.6±15.7ª	621.7±15.5 <sup>a</sup>	329.6±15.4ª	

Table 2. Total phenolics and flavonoids contents and isoflavone composition of dried doenjang(unit: µg/g db)

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).

Values not sharing a common letter (a-e) are statistically significantly different from each other (P<0.05).

<sup>1)</sup>Gallic acid equivalent.

<sup>2)</sup>Naringin equivalent.

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

		Hot air drying				
	Freeze dried -	45°C	60°C	80°C	100°C	
Ash (%)	12.9±0.0	12.9±0.0	12.9±0.0	12.9±0.0	12.9±0.0	
Crude protein (%)	21.5±0.0 <sup>b</sup>	25.5±0.0 <sup>ª</sup>	19.1±0.0 <sup>c</sup>	17.6±0.0 <sup>d</sup>	25.9±0.0 <sup>ª</sup>	
Crude fat (%)	22.6±0.0 <sup>a</sup>	17.4±0.0 <sup>b</sup>	17.3±0.0 <sup>b</sup>	16.4±0.1 <sup>c</sup>	16.7±0.1 <sup>c</sup>	
Salt concentration (%)	60	62	60	56	60	
рН	5.9	5.9	5.7	5.3	4.9	
Total acids (%)	2.6	3.3	3.0	3.2	3.6	
Acid value (mg KOH/g)	29.6±2.0 <sup>bc</sup>	31.6±1.7 <sup>ab</sup>	30.6±1.2 <sup>b</sup>	27.4±0.0 <sup>c</sup>	33.5±1.4ª	
Amino N (mg %)	865.8	799.4	772.7	798.5	739.4	
Total free amino acids (mg/g)	83.4	83.8	84.2	88.6	87.6	
2-Phenylethylamine (mg/g)	2.35	3.02	2.66	2.23	2.13	
Histamine (mg/g)	0.06	0.05	0.02	0.05	0.03	

Data are mean $\pm$ SD (n=3).

Values not sharing a common letter (a-c) are statistically significantly different from each other (P < 0.05).

The parameters other than proximate composition and acid value were values from a single measurement.

## DISCUSSION

Doenjang, one of the most consumed Korean traditional seasonings and condiments, is traditionally manufactured by soaking *meju* in a salt solution for  $2 \sim 3$  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 labile 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 was significantly increased in *doenjang* dried at 100°C (P <0.05), and the concentrations of free isoflavones such as genistein, daidzein, and glycitein were elevated, maybe 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 (Table 3). Interestingly, the content of total free amino acids was significantly increased in *doenjang* samples dried at 80 and 100°C, probably due to thermal degradation of proteins and peptides (Table 4).

In particular, *doenjang* samples dried at temperatures higher than 80°C showed elevated FRAP values, one of antioxidant capacity marker, while all samples dried under different drying conditions had similar DPPH and ABTS<sup>+</sup> radical scavenging activities (Fig. 2). In contrast

Table 4. Free amino acid composition of *doenjang* dried under different conditions

(unit:	μg/g)
(anne	mg, g,

Free amino acids	Freeze dried	Hot air dried				
Free amino acius	Freeze uneu -	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	3,313.99	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	
$\alpha$ -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	
$\alpha$ -Amino- <i>n</i> -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	
Phenylalanine	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	
$\beta$ -Amino isobutyric acid	126.85	128.41	622.78	704.24	572.89	
$\gamma$ -Amino- <i>n</i> -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	<i>doenjang</i> powde	r dried under	different conditions
-------------------	-----------------------	---------------	----------------------

			Drying method				
Color values	France deird	Hot air dried					
	Freeze dried	45°C	60°C	80°C	100°C		
L	71.33±0.24 <sup>ª</sup>	53.71±0.16 <sup>c</sup>	52.74±0.03 <sup>d</sup>	54.42±0.36 <sup>b</sup>	49.29±0.12 <sup>e</sup>		
а	4.50±0.03 <sup>e</sup>	7.39±0.03 <sup>d</sup>	8.38±0.10 <sup>c</sup>	8.66±0.06 <sup>b</sup>	9.05±0.01 <sup>ª</sup>		
b	21.36±0.14 <sup>b</sup>	21.06±0.07 <sup>c</sup>	19.09±0.07 <sup>d</sup>	21.67±0.23 <sup>ª</sup>	18.28±0.08 <sup>e</sup>		
$\Delta E*_{ab}$	30.98±0.26 <sup>c</sup>	43.85±0.17 <sup>b</sup>	43.74±0.03 <sup>b</sup>	44.13±0.38 <sup>b</sup>	46.39±0.14ª		

 $\begin{array}{l} \Delta \textit{E}^{*}{}_{\textit{ab}}\text{=}\sqrt{(\bigtriangleup L)^{2}+(\bigtriangleup a)^{2}+(\bigtriangleup b)^{2}}\\ \text{Data are mean}\text{\pm}\text{SD (n=3)}. \end{array}$ 

Values not sharing a common alphabetical letter are statistically significantly different from each other (P<0.05).

	Freeze dried	Hot air dried				
		45°C	60°C	80°C	100°C	
Color	2.9±1.2 <sup>ª</sup>	3.3±1.1ª	3.3±1.3ª	2.9±1.4 <sup>ª</sup>	2.5±1.0 <sup>b</sup>	
Odor	2.7±1.3 <sup>NS</sup>	3.0±0.7	3.1±0.7	3.2±1.5	3.0±1.2	
Umami taste	3.0±0.7 <sup>a</sup>	2.9±1.0 <sup>ª</sup>	2.4±1.1 <sup>b</sup>	2.1±1.2 <sup>c</sup>	2.4±1.0 <sup>c</sup>	
Salty taste	3.0±1.0 <sup>a</sup>	2.6±0.7 <sup>ª</sup>	3.0±0.9 <sup>a</sup>	$1.5 \pm 1.1^{b}$	2.4±1.0 <sup>ª</sup>	
Bitterness	1.8±1.1ª	1.9±0.9ª	2.2±1.1ª	$2.7 \pm 1.2^{ab}$	2.9±1.0 <sup>b</sup>	
Overall acceptability	3.0±1.05 <sup>a</sup>	2.5±0.9 <sup>b</sup>	3.2±1.1ª	2.4±1.4 <sup>b</sup>	2.0±0.9 <sup>c</sup>	

Table 6. Sensory characteristics of *doenjang* dried under different conditions

Data are mean $\pm$ SD (n=10).

Values not sharing a common letter (a-c) are statistically significantly different from each other (P<0.05).

The samples were organoleptically assessed by ten panelists, using a sensory rating scale of  $1 \sim 5$  for six parameters. NS, not significant.

to the DPPH and ABTS<sup>+</sup> 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-tripyridyltriazine 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°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°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°C had a darker color than the samples dried at lower temperatures of less than 80°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°C and 100°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°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°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.

## ACKNOWLEDGEMENTS

This research was supported by the Agriculture and Fisheries R&D Promotion Grant (Grant no. 201701430100) of Gyeongsangbuk-do and a grant from INNOPOLIS, Ministry of Science, ICT and Future Planning, Republic of Korea (2017).

## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

## REFERENCES

- Lee S, Lee S, Singh D, Oh JY, Jeon EJ, Ryu HS, Lee DW, Kim BS, Lee CH. 2017. Comparative evaluation of microbial diversity and metabolite profiles in *doenjang*, a fermented soybean paste, during the two different industrial manufacturing processes. *Food Chem* 221: 1578-1586.
- Song YR, Jeong DY, Baik SH. 2015. Monitoring of yeast communities and volatile flavor changes during traditional Korean soy sauce fermentation. *J Food Sci* 80: M2005-M2014.
- 3. Kang SJ, Seo JY, Cho KM, Lee CK, Kim JH, Kim JS. 2016. Antioxidant and Neuroprotective effects of *doenjang* prepared with *Rhizopus*, *Pichia*, and *Bacillus*. *Prev Nutr Food Sci* 21: 221-226.
- Cha YS, Park Y, Lee M, Chae SW, Park K, Kim Y, Lee HS. 2014. Doenjang, a Korean fermented soy food, exerts antiobesity and antioxidative activities in overweight subjects with the PPAR-γ2 C1431T polymorphism: 12-week, double-blind randomized clinical trial. J Med Food 17: 119-127.
- 5. Hwang KM, Jung KO, Song CH, Park KY. 2008. Increased antimutagenic and anticlastogenic effects of *doenjang* (Korean fermented soybean paste) prepared with bamboo salt. *J Med Food* 11: 717-722.
- Jeong JK, Chang HK, Park KY. 2014. Doenjang prepared with mixed starter cultures attenuates azoxymethane and dextran sulfate sodium-induced colitis-associated colon carcinogenesis in mice. J Carcinog 13: 9.
- Lee DE, Lee KW, Byun S, Jung SK, Song N, Lim SH, Heo YS, Kim JE, Kang NJ, Kim BY, Bowden GT, Bode AM, Lee HJ, Dong Z. 2011. 7,3',4'-Trihydroxyisoflavone, a metabolite of the soy isoflavone daidzein, suppresses ultraviolet B-induced skin cancer by targeting Cot and MKK4. J Biol Chem 286:

14246-14256.

- 8. Nam YR, Won SB, Chung YS, Kwak CS, Kwon YH. 2015. Inhibitory effects of *Doenjang*, Korean traditional fermented soybean paste, on oxidative stress and inflammation in adipose tissue of mice fed a high-fat diet. *Nutr Res Pract* 9: 235-241.
- 9. Park KY, Jung KO, Rhee SH, Choi YH. 2003. Antimutagenic effects of *doenjang* (Korean fermented soypaste) and its active compounds. *Mutat Res* 523-524: 43-53.
- Nam DH, Kim HJ, Lim JS, Kim KH, Park CS, Kim JH, Lim J, Kwon DY, Kim IH, Kim JS. 2011. Simultaneous enhancement of free isoflavone content and antioxidant potential of soybean by fermentation with *Aspergillus oryzae*. J Food Sci 76: H194-H200.
- 11. Kim JH, Hwang CE, Lee CK, Lee JH, Kim GM, Jeong SH, Shin JH, Kim JS, Cho KM. 2014. Characteristics and antioxidant effect of garlic in the fermentation of *Cheonggukjang* by *Bacillus amyloliquefaciens* MJ1-4. *J Microbiol Biotechnol* 24: 959-968.
- Heuberger AL, Broeckling CD, Lewis MR, Salazar L, Bouckaert P, Prenni JE. 2012. Metabolomic profiling of beer reveals effect of temperature on non-volatile small molecules during short-term storage. *Food Chem* 135: 1284-1289.
- 13. Benzie IFF. 1996. An automated, specific, spectrophotometric method for measuring ascorbic acid in plasma (EFTSA). *Clin Biochem* 29: 111-116.
- Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anuurad E, Shiwaku K, Yamane Y. 2004. Screening for antioxidant activity in edible plant products: comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Folin-Ciocalteu assay. J Agric Food Chem 52: 2391-2396.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26: 1231-1237.
- 16. Zhang H, Wang ZY, Yang X, Zhao HT, Zhang YC, Dong AJ, Jing J, Wang J. 2014. Determination of free amino acids and 18 elements in freeze-dried strawberry and blueberry fruit using an amino acid analyzer and ICP-MS with micro-wave

digestion. Food Chem 147: 189-194.

- 17. Schonfeldt HC, Pretorius B. 2011. The nutrient content of five traditional South African dark green leafy vegetables—a preliminary study. *J Food Compos Anal* 24: 1141-1146.
- Kuhnen S, Moacyr JR, Mayer JK, Navarro BB, Trevisan R, Honorato LA, Maraschin M, Pinheiro Machado Filho LC. 2014. Phenolic content and ferric reducing-antioxidant power of cow's milk produced in different pasture-based production systems in southern Brazil. J Sci Food Agric 94: 3110-3117.
- Chung HS, Chang LC, Lee SK, Shamon LA, van Breemen RB, Mehta RG, Farnsworth NR, Pezzuto JM, Kinghorn AD. 1999. Flavonoid constituents of *Chorizanthe diffusa* with potential cancer chemopreventive activity. J Agric Food Chem 47: 36-41.
- 20. Wang HJ, Murphy PA. 1994. Isoflavone content in commercial soybean foods. *J Agric Food Chem* 42: 1666-1673.
- Ayala-Zavala JF, Wang SY, Wang CY, González-Aguilar GA. 2004. Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *LWT-Food Sci Technol* 37: 687-695.
- Akin MB, Akin MS, Kırmacı Z. 2007. Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic icecream. *Food Chem* 104: 93-99.
- 23. Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 239: 70-76.
- 24. Ajandouz EH, Tchiakpe LS, Dalle Ore F, Benajiba A, Puigserver A. 2001. Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Food Chem Toxicol* 66: 926-931.
- Jing H, Kitts DD. 2002. Chemical and biochemical properties of casein-sugar Maillard reaction products. *Food Chem Toxicol* 40: 1007-1015.
- 26. Baisier WM, Labuza TP. 1992. Maillard browning kinetics in a liquid model system. *J Agric Food Chem* 40: 707-713.
- 27. van Boekel MAJS. 2008. Kinetic modeling of food quality: a critical review. *Compr Rev Food Sci Food Saf* 7: 144-158.