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Effects of salinity on bacterial communities, Maillard reactions, isoflavone composition, antioxidation and antiproliferation in Korean fermented soybean paste (*doenjang*)

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ABSTRACT

The purpose of this study was to investigate bacterial communities and health-benefit-related parameters in *doenjang* prepared with various brine concentrations (8, 12, 16, and 20%). Phenolic, flavonoid, melanoidin and isoflavone contents, antioxidation, and antiproliferation of *doenjang* (AD8, AD12, AD16, and AD20) aged for 3 months were compared with those of initial *doenjang* (ID8, ID12, ID16, and ID20). The ID8 and AD8 *doenjang*, made with 8% brine, contained higher phenolics, melanoidins, and isoflavones than those with high salinity. As results of bacterial communities, *Enterococcus* was a dominant bacterium in most *doenjang*, while *Lactobacillus* was predominant in AD8. Trolox equivalent antioxidant capacity (32.5 μmol TE/g dwb) and DPPH radical-scavenging capacity (57 μmol TE/g dwb) values of AD8 were the highest among samples. The EC_{50} of HT-29 cell proliferation treated with AD8 was 0.47 mg/mL, while EC_{50} of AD20 was 1.07 mg/mL, indicating stronger antiproliferative activity in low-salinity *doenjang*.

1. Introduction

Soybean (*Glycine* max) is an important plant protein source consumed worldwide as fermented and nonfermented foods (Omoni & Aluko, 2005). Fermented soybean products are widely consumed in China, Korea, Japan, and other countries (Kuligowski, Pawlowska, Jasinska-Kuligowska, & Nowak, 2017; Lee, Lai, & Wu, 2015; Wang et al., 2008). *Doenjang* and *cheonggukjang* from Korea, *miso* and *natto* from Japan, *sufu* from China, *thua nao* from Thailand, and *tempeh* from Indonesia are common examples of fermented soybean products (Kim, Kwak, Jung, & Kim, 2016). Although the fermented soybean foods from each country have distinctive flavor, texture and taste, the products have several common properties, such as the basic ingredients, fermentation process and nutritional value (Chung & Chung, 2007). *Doenjang* is the major traditional fermented soybean product in Korea. The main ingredient of *doenjang* is *meju*, which normally is made by fermenting cooked soybean with airborne fungi or through artificial inoculation with *Aspergillus oryzae* or *Aspergillus sojae* (Kang et al., 2016). *Doenjang* prepared by the traditional method is naturally fermented under high salt conditions without inoculation of a specific microorganism (Jeon et al., 2016).

Previously, antioxidant (Shukla), anti-inflammatory (Kim et al., 2014), anti-obesity (Cha et al., 2014), and anticarcinogenic activities (Jung, Park, & Park, 2006; Seol, Youn, Koo, Kim, & Choi, 2016) of *doenjang* have been reported. The main preventive action related with these diseases is scavenging or neutralizing free radicals by donating electrons or hydroxyl atoms. Antioxidant activity of fermented soybean foods such as *doenjang* was significantly correlated with isoflavone contents, Maillard reaction products, and total phenolic content (Kim & Kim, 2014; Shukla et al., 2016; Wittanalai, Deming, & Rakariyatham, 2012). Additionally, bacterial communities might be important indices to understand the functionalities of *doenjang*. Recently, several studies have reported that various microbial communities play an important role in *doenjang*'s sensory characteristics and metabolite changes (Jung, Jung, Lee, & Jeon, 2016; Kim et al., 2016). Especially, *Lactobacillus* among dominant bacterium was associated with the increase of metabolites such as γ-aminobutyric acid (GABA) (Jung et al., 2016). Even though numerous scientific reports have demonstrated the beneficial health effects of *doenjang*, such as antioxidant, anti-inflammatory, anti-obesity and anticarcinogenic (Cha et al., 2014; Kim & Kim, 2014; Kim et al., 2014; Seol et al., 2016; Shukla et al., 2016), there might be some controversy, due to the detrimental effect of the high salt concentration in *doenjang* (Hwang, Kim, Moon, Yang, & Kim, 2017). Diets with high salt are not beneficial to human

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health because they can cause diseases such as hypertension (He & MacGregor, 2009). Recently, consumers and researchers have begun to pay attention to the nutritional value of a salt-reduced diet. As part of efforts to produce low-salinity *doenjang*, Jeon et al. (2016) reported screening and characterization of potential starter cultures for effective fermentation under low salt conditions (approximately 6.5–7.5%). However, no information is available on beneficial health effects and bacterial communities of salt-reduced fermented soybean products. For utilization of salt-reduced fermented soybean products, information about their health-related functional properties and bacterial communities is important.

Therefore, the purpose of this study was to investigate the effect of salinity on bacterial communities and health-benefit-related parameters, such as browning reactions, antioxidants, and antiproliferation of cancer cells, using *doenjang* prepared with various brine concentrations (8, 12, 16, and 20%) initially and after early stages of fermentation (about 3 months). Additionally, bioactive components of *doenjang* samples, phenolic, flavonoid, and isoflavone contents were measured.

2. Materials and methods

2.1. Materials

2.1.1. Doenjang preparation and storage

The *doenjang* samples used in this study were made by the traditional method. In brief, soybean was soaked in water overnight and then steamed for 90 min at 120 °C. The steamed soybean was crushed roughly, and shaped into brick-sized blocks. Shaped blocks were fermented for 2 weeks at 28–30 °C. Then, the *meju* blocks were soaked in various salt solutions (8, 12, 16, and 20%) and fermented for 40 days. After 40 days of fermentation, the mixtures of *meju* and salt solutions were separated into solid and liquid parts. The solid part is called *doenjang* (fermented soybean paste), and the liquid part is called *ganjang* (soy sauce). After separation, *doenjang* samples prepared with various brine concentrations were fermented in a traditional way by storing in porcelain pots located at the Korea Food Research Institute (Gyeonggi-do, Korea). The tops of the *doenjang* samples in the porcelain pots were covered with polyethylene vinyl film, and coarse sea salt (with 1-cm thickness) was placed on the vinyl film to prevent exposure to air. In order to consider the sensory characteristics of low salinity *doenjang*, samples used in this study were aged for 3 months according to the study of Byun, Nam, and Lee (2015). During 3-month fermentation, temperature and humidity in the porcelain pots were recorded using a thermo-recorder (TR-72wf; T & D Corporation, Nagano, Japan).

Identification of samples is described in Table 1. The *doenjang* samples aged for 3 months (AD8, AD12, AD16, and AD20) and initial *doenjang* (ID8, ID12, ID16, and ID20) were used to analyze salinity, pH, bacterial communities, isoflavonoid, total phenolic content (TPC) and total flavonoid content (TFC), antioxidant capacities, and antiproliferative activity.

2.1.2. Chemicals

Folin-Ciocalteu, Trolox, quercetin, gallic acid, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, ethanol, acetic acid, water, aluminum chloride, sodium carbonate, sodium nitrite, and potassium persulfate were obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan). Daidzein, glycitein, genistein, daidzin, glycitin and genistin were purchased from Wako Chemical (Richmond, VA). HT-29 cells were purchased from the American Type Culture Collection (ATCC) (Manassas, VA). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FSB) were purchased from HyClone Laboratories Inc., (South Logan, UT).

2.2. Methods

2.2.1. Salinity and pH values of doenjang

The salinity (NaCl concentration) of the *doenjang* samples was measured using the Mohr method (AOAC., 2000). For pH measurements, a mixture of distilled water (45 mL) and the *doenjang* samples (5 g) was vortexed and then measured with a pH meter (720 A; Orion Research Inc., Boston, MA).

2.2.2. Bacterial communities of doenjang

The *doenjang* samples were extracted using a stool Power Water® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) and quantified using the PicoGreen assay (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. DNA extracts from the *doenjang* were amplified, sequenced and analyzed as described by Kim et al. (2016).

For barcoded pyrosequencing, the 16S rRNA gene was amplified with a primer set 27F (5′GAGTTTGATCMTGGCTCAG N3′) and 518R (5′WTTACCGCGGCTGCTGG3′) using a PCR System (Roche Life Science, Indianapolis, IN). The PCR was conducted as follows: initial denaturation step for 3 min at 94 °C, followed by 35 cycles of denaturation for 15 s at 94 °C, annealing for 45 s at 55 °C, extension for 30 s at 72 °C, and a final extension step for 8 min at 72 °C. After amplification, purification was carried out using AMPure beads (Beckman Coulter, Brea, CA), and then next-generation sequencing (NGS) was performed with a Roche-454 GS-FLX+ (454 Life Sciences) by a DNA sequencing and metagenome analysis company (Macrogen, Seoul, Korea).

The sequences generated from pyrosequencing were analyzed with the CD-HIT-OUT and QIIME programs for pre-processing (quality-adjustment, barcode split), identification of operational taxonomic units (OTUs), taxonomic assignment and community comparison (Nam, Lee, & Lim, 2012).

2.2.3. Color characteristics of doenjang

The color values of the *doenjang* samples were measured using a colorimeter (CR-300; Minolta, Tokyo, Japan) and color parameters were calculated according to Palou, López-Malo, Barbosa-Cánovas,

Table 1
The salinity and pH values of initial and aged *doenjang* prepared with various brine concentrations (8, 12, 16, and 20%).

samples	Abbreviation	Salinity (%)***	pH***
initial *doenjang* prepared with 8% brine	ID8	7.68 ± 0.18^{g}	5.93 ± 0.03^{a}
initial *doenjang* prepared with 12% brine	ID12	10.23 ± 0.18^{e}	5.86 ± 0.03^{b}
initial *doenjang* prepared with 16% brine	ID16	12.08 ± 0.18^{c}	5.84 ± 0.03^{b}
initial *doenjang* prepared with 20% brine	ID20	14.23 ± 0.47^{b}	5.90 ± 0.01^{b}
aged *doenjang* prepared with 8% brine	AD8	8.44 ± 0.02^{f}	5.57 ± 0.03^{d}
aged *doenjang* prepared with 12% brine	AD12	11.38 ± 0.06^{d}	5.74 ± 0.02^{c}
aged *doenjang* prepared with 16% brine	AD16	12.14 ± 0.01^{c}	5.69 ± 0.02^{c}
aged *doenjang* prepared with 20% brine	AD20	14.92 ± 0.32^{a}	5.68 ± 0.04^{c}

All values are means of three replications ± standard deviation. Values with same letter within a column are not significantly different.
*** Significantly different at $p < .001$.