Food Research International 89 (2016) 724–732

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Microbial communities related to sensory attributes in Korean fermented soy bean paste (*doenjang*)

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ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form 20 September 2016
Accepted 27 September 2016
Available online 30 September 2016

Keywords:
Soy bean paste
Doenjang
Microbial community
Next generation sequencing
Descriptive analysis
Partial least square regression

ABSTRACT

Various microbial communities play a role in generating the distinctive sensory characteristics of the traditional Korean soybean paste, *doenjang*. The objective of this study was to investigate the relationship between sensory characteristics and microbial communities in traditional *doenjang*. The results of the descriptive analysis revealed that some traditional *doenjang* (T1–T11) exhibited distinctive characteristics, such as fish sauce flavor, *meju*, bitterness, sourness, and saltiness, whereas the modified samples (M1–M3) demonstrated sweetness, *umami*, and an alcohol odor. According to the metagenomic analysis based on 16S rRNA gene sequencing, the phylum *Firmicutes* was the dominant bacterium in most *doenjang*. At the genus level, lactic acid bacteria (LAB) were frequently found in most *doenjang*. Among these LAB, the major genera of bacteria were *Tetragenococcus* in M2 (60.30%), T3 (91.20%), T8 (48.60%), and T9 (60.90%); *Enterococcus* in T1 (29.40%), T4 (34.10%), and T10 (50.50%); *Leuconostoc* in T7 (89.10%); and *Lactobacillus* in T9 (38.3%). The most frequently occurring non-LAB was *Bacillus* in M3 (50.10%), T5 (46.50%), and T6 (20.50%), and M1 and T2 contained *Staphylococcus* and *Ochrobactrum* as their major non-LAB, respectively. The results of a correlation analysis between the sensory attributes from the descriptive analysis and the microbial communities from 454 pyrosequencing provided an overview for the relationship between sensory characteristics and microbial communities. *Ochrobactrum*, *Stenotrophomonas*, *Rhodobacteraceae*, *Proteus*, and *Luteimonas* were found in samples that had a strong fish sauce characteristic. The presence of LAB—*Tetragenococcus*, *Enterococcus*, *Pediococcus*, *Carnobacterium*, *and Weissella*—was related to sourness. *Enterococcus* and *Enterobacter* were found in samples with a matured flavor and a soft mouthfeel, respectively. The overall results of the study demonstrate that microbial communities found in *doenjang* were closely related to distinct sensory attributes.

1. Introduction

Fermented soybean products are popular traditional foods in Asian countries. Common examples are soy sauce, Chinese *sufu*, Indonesian *tempeh*, Japanese *miso* and *natto*, Thai *thua nao*, and Korean *doenjang* and *chunggukjang*. Although the characteristics of fermented soybean products differ depending on their country of origin, these products are similar in terms of their ingredients and processing methods (Chung, 1999). *Doenjang*, a fermented soybean paste, is a traditional fermented soybean food that has been consumed for centuries in Korea, much like *miso* in Japan and *tempeh* in Indonesia (Golbitz, 1995; Lee, 2001). Diverse microorganisms participate in *doenjang* fermentation and produce its unique flavors by decomposing soybean proteins during fermentation. Traditional *doenjang* is primarily made with *meju*, which is typically prepared with natural flora and soybeans as its basic ingredients (Chung & Chung, 2007). However, the traditional method of making *doenjang* has not been adapted for mass production due to the difficulty of controlling fermentation with natural microflora. Instead, mass-produced fermented soybean pastes are made using wheat *koji* that has been inoculated with *Aspergillus oryzae* (Lee & Ahn, 2008).

Extensive research on *doenjang* has been previously reported; many health benefits of *doenjang*—including anti-oxidant properties (Chung & Chung, 2007), anti-mutagenicity (Hwang, Jung, Song, & Park, 2008), angiotensin converting enzyme (ACE) inhibiting characteristics (Byun, Son, Yook, Jo, & Kim, 2002), and thrombolysis (Seo & Lee, 2004)—have been reported. Generally, the flavors and textures of *doenjang* are affected by microbial activity during fermentation. To isolate the flavor and sensory characteristics of *doenjang*, Jo, Cho, Song, Shin, and Kim (2011) determined the volatile compound profiling of commercially available *doenjang* products, which were manufactured using either traditional and/or modified methods, and identified the key volatile compounds by classifying the flavors of commercial and traditional *doenjang*. Kim, Hong, Song, Shin, and Kim (2010) determined the sensory characteristics of commercially available *doenjang* products manufactured by either traditional and/or modified methods and identified the attributes that drivers of liking of commercial *doenjang* products in Korea.

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http://dx.doi.org/10.1016/j.foodres.2016.09.032

Recently, the identification of the microbial composition of fermented foods (including *doenjang*) has accelerated the development of rapid and accurate analytical techniques. The bacterial distribution and diversity of six *doenjang* samples using 16S rRNA gene amplification and sequencing revealed that *Staphylococcus equorum* was the dominant bacterium, followed by *Leuconostoc mesenteroides* and *Lactobacillus sakei* (Cho & Seo, 2007). Bacteria and fungi present in commercial and homemade *doenjang* were identified using nested polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE); however, the prevalence of each bacterium and fungus was not quantified. Next-generation sequencing (NGS) has also been used to identify the microbial communities present in fermented foods (Nam, Lee, & Lim, 2012a; Nam, Park, & Lim, 2012c; Nam, Yi, & Lim, 2012b). The 454 pyrosequencing procedure enables the identification of microbial communities, as well as the rapid quantification of the abundance of microorganisms (Cardenas & Tiedje, 2008). Nam et al. (2012b) compared microbial communities and presented a comprehensive analysis of microbial diversity in the Korean fermented soy product, *cheonggukjang*. For *doenjang* products, 454 pyrosequencing with sample-specific barcoded primers that target the V1/V2 hyper-variable regions of the 16S rRNA gene was applied to *doenjang* products and used to identify the microbial communities in commercial and traditional *doenjang* samples (Nam et al., 2012a). Various microorganisms are involved in the fermentation of *doenjang* and influence the profiling of both volatile and non-volatile compounds (Jo et al., 2011; Shukla et al., 2010). However, no information is available on the relationship between the microbial communities that are present and the sensory attributes of *doenjang*. Information regarding how microbial communities affect the sensory quality of *doenjang* can be valuable to the production of high-quality *doenjang*. Therefore, the objective of this study was to investigate the relationship between sensory characteristics and microbial communities in *doenjang*.

2. Materials and methods

2.1. Materials

Fourteen fermented soybean pastes (*doenjang*) were used in this study (Table 1). Three of the samples (M1–M3) were acquired from large manufacturing companies that produce *doenjang* from *koji* that has been inoculated with *Aspergillus* sp. or *Bacillus* sp. Eleven samples (T1–T11) were obtained from local companies that produce Korean soybean pastes by traditional methods. These samples were certified as “Traditional Korean Food” by the Ministry of Agricultural, Food, and Rural Affairs in the Republic of Korea.

2.2. Descriptive analysis

Twenty-five participants who were interested in the descriptive analysis of *doenjang* were recruited by the Korea Food Research Institute (Seongnam-si, Korea). They were screened by their ability to discriminate between five basic tastes (sweet, salty, sour, bitter, and umami) using 10 sets of a triangle test (Meilgaard, Civille, & Carr, 1999). Twelve participants who answered >60% correctly were selected as the panelists for the descriptive analysis. The training sessions were held eight times a week for two weeks. Each training session lasted for approximately 30 min. In all, 31 attributes, including appearance, odor, taste/flavor, mouthfeel, and aftertaste were developed and defined according to a consensus among panelists during the training sessions. The definitions and references for the 31 descriptive attributes of *doenjang* are presented in Table 2. The *doenjang* samples were kept at 4 ± 1 °C in a refrigerator until they were tested. One hour before the experiment, 5 g of each sample were transferred to an opaque plastic container (diameter: 3.5 cm, height: 2.5 cm) and covered with a lid. Each sample was coded with a three-digit number that had been randomly generated by a sensory data collection program (custom-made program, Korea Food Research Institute, Seongnam-si, Korea). The samples were served monadically to the panelists in individual sensory evaluation booths. During each session, only four or five sample evaluations were presented to minimize the incidence of sensory fatigue. The sample evaluation procedure followed the same protocol established during the training periods.

To evaluate the odor of the *doenjang* samples, the panelists opened the lid of the container halfway, sniffed three times, and closed the lid immediately. The panelists then opened the lid of the sample container to evaluate the appearance attributes of the *doenjang* samples. The taste/flavor, mouthfeel, and aftertaste attributes of the *doenjang* samples were assessed by tasting the sample using small spoons (with a maximum volume of 0.4 mL). The panelists evaluated the intensities of the 31 descriptive attributes using the evaluation procedure, which had been developed during the training periods. The intensities of each attribute were rated on a 15-cm line scale labeled with “weak” on the left side and “strong” on the right side. The panelists were not permitted to eat or drink anything other than water for at least 1 h prior to the descriptive analysis sessions. The panelists cleansed their palates with a piece of white bread (2 × 2 × 1.5 cm, width × length × height) and purified tap water (20 ± 2 °C) between samples. Each session took approximately 30 min, and the evaluations were performed in triplicate.

Table 1
Information on the 14 *doenjang* samples used in this study.

Sample	Region	Salinity (%)[1]	Major Ingredients[2]
M1	Commercial	10.8^{h} [3]	*Meju*, Soybean, wheat flour, refined salt, ethanol, starter, purified water
M2	Commercial	15.8^{a}	*Meju*, Soybean, refined salt, ethanol, starter
M3	Commercial	11.4^{gh}	Soybean, sun-dried salt, ethanol, starter
T1[4]	Andong, Southeast of Korea	13.3^{cde}	Soybean, sun-dried salt
T2	Cheongsong, Southeast of Korea	13.1^{de}	*Meju*(black bean), sun-dried salt
T3	Gyeongsan, Southeast of Korea	12.7^{ef}	*Meju*, refined salt, purified water
T4	Yangpyeong, Capital area	14.0^{b}	Soybean, salt, purified water
T5	Anseong, Capital area	14.1^{b}	*Meju*, saline solution
T6	Yangyang, Northeast of Korea	8.6^{i}	*Meju*, barley, malt, salt
T7	Sunchang, Southwest of Korea	14.0^{bc}	Soybean, sun-dried salt, purified water
T8	Haenam, Southwest of Korea	14.2^{b}	Soybean, sun-dried salt, soybean sauce, purified water
T9	Jeju Island, Far south of Korea	13.7^{bcd}	Soybean, sun-dried salt
T10	Sejong, Central of Korea	12.4^{f}	*Meju*, sun-dried salt
T11	Andong, Southeast of Korea	11.8^{g}	*Meju*, sun-dried salt

[1] Salinity was measured by Mohr method.
[2] Major ingredients listed on product labels.
[3] Different superscripts within a column meant significantly different at $P < 0.05$.
[4] T1–T11 were certified as a traditional food and had Traditional Food Quality Certification by the Ministry of Agricultural, Food and Rural Affairs, Republic of Korea.