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Comparison of volatile components in fermented soybean pastes using simultaneous distillation and extraction (SDE) with sensory characterisation

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ABSTRACT

In this study, the volatile compounds in nine fermented soybean pastes were extracted and analysed by simultaneous steam distillation and extraction (SDE) and gas chromatography–mass spectrometry (GC–MS), respectively. A total of 91 volatile components were identified. The differences in volatiles were observed by applying principal component analyses (PCA) to GC–MS data sets. Most of the samples did not show apparent groupings; however, a three sample clustering (CJW, SIN and HAE) was observed for pastes made by *Aspergillus oryzae* inoculation. From the PCA of the sensory data, samples are primarily separated along the first PC (explained 68% of the total variance), between samples like SUJA, CHJA and SOHI with high intensities of 'briny', 'soy sauce', 'musty' and 'astringent' and the samples (CJW and SIN) with intense levels in 'sweet-grain', 'sweet' and 'MSG' attributes. The individual concentrations of volatile compounds such as isoamyl acetate, furfuryl alcohol, maltol, pyrazines, 1-octen-3-ol and 2-methoxy-4-vinyl phenol corresponded well to the intensities of related sensory attributes by the correlation analysis.

1. Introduction

Soybean-based foods, including soy milk, tofu and fermented products, are widely consumed in Eastern countries and are expanding in consumption around the world. More specifically, varieties of fermented soybean foods such as soy sauce, tempeh, Japanese miso and natto, Thai thua nao, Chinese sufu and Korean doenjang are included (Leejeerajumnean, Duckham, Owens, & Ames, 2001; Steinkraus, 1991). Although the fermented soybean products of different countries have distinctive qualities, these products share several common characteristics such as base ingredients, fermentation and processing methods (Chung, 1999). Traditional Korean soybean paste (doenjang) is primarily made with meju, which typically uses natural flora and soybeans as the basic ingredient; whereas Japanese miso is made with koji, which utilises *Aspergillus oryzae* and soybean and grain ingredients (Park, Gil, & Park, 2003). However, due to the unequal fermentation progression that occurs with natural micro-flora, the traditional method of making Korean soybean paste (doenjang) has been adapted for mass production. Thus, large manufacturing companies are producing commercial fermented soybean pastes using wheat koji inoculated with *A. oryzae*. However, there is increasing consumer interest for traditionally made soybean pastes that possess significant health effects (Shin et al., 2001) as well as full, complex aroma characteristics.

The volatile compounds in various fermented soybean products, such as Japanese miso and natto, Chinese sufu and Thai thua nao, have been studied extensively (Chung, 1999; Chung, Fung, & Kim, 2005; Ku, Chen, & Chiou, 2000; Leejeerajumnean et al., 2001; Mori, Kiuchi, & Tabei, 1983; Sugawara, 1991), and nearly 100 different volatile compounds representing a variety of chemical classes were identified. Whilst there is wide variation in the volatile components of fermented soybean products, studies have shown that the most frequently present compounds include esters (ethyl 2-methyl butyrate, ethyl hexanoate), acids (acetic acid, 2/3-methyl butanoic acid), pyrazines and phenolic compounds. The volatile component profiles of products vary with the micro-flora involved, as well as by the processing conditions (e.g. fermentation, drying, brining, or ageing) (Chung, 1999; Leejeerajumnean et al., 2001; Sugawara, 1991). Some studies have examined the volatiles in Korean fermented soybean pastes prepared using different types of strains (Park, Lee, Kim, & Lee, 1994; Seo et al., 1996), as well as different extraction (Park et al., 2003; Shin & Joo, 1999) and ageing and mixing methods (Choi, Sohn, & Jeon, 1997). However, volatile components in commercially and traditionally manufactured fermented soybean pastes have not been quantitatively compared.

The objectives of the present study were to identify and quantify the volatile components in various traditional and mass-produced fermented soybean pastes, and to visually compare their volatile compositions by applying principal component analysis (PCA), which is a multivariate statistical tool specifically, designed to analyse and visualise complex data sets such as GC–MS data. The sensory characteristics of various fermented soybean pastes

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were also determined to investigate relationships with the volatile composition.

2. Materials and methods

2.1. Materials

Nine fermented soybean pastes (doenjang) were purchased from different manufacturing companies (Table 1). Six of the samples (ANJA, SOHI, CHJE, CHJA, MON and SUJA) were collected from local companies producing Korean soybean pastes by the traditional method, using natural micro-flora. The other three samples (SIN, CJW, HAE) were acquired from large manufacturing companies producing soybean pastes using wheat koji inoculated with *A. oryzae*. In addition, traditionally fermented meju was added to the CJW and HAE samples during production to give a traditional doenjang flavour.

Dichloromethane ($\geqslant$99.9% pure), *n*-alkane standards (C6–C24), sodium sulphate, an internal standard compound (2-methyl-1-pentanol), methanol and authentic standards were purchased from Sigma–Aldrich (St. Louis, MO). The authentic chemical standards were obtained from suppliers as follows: compound codes et1–et19, et21, al1–al9, al11–al14, ad1–ad3, ad6–ad7, ac1–ac10, ke1–ke7, py1–py3, py6–py8, phe1–phe2, phe4–phe7, ms1–ms5, ms9–ms10 and ms14 from Sigma–Aldrich Chemical Co., (St. Louis, MO), phe3 and ms5 from Paltz & Bauer, Inc. (Waterbury, CT).

2.2. Simultaneous steam distillation and extraction (SDE)

A Likens–Nickerson type (Likens & Nickerson, 1964) SDE apparatus (model 523010-000, Kontes, NJ) was used to extract the volatile compounds. For the analysis, sample (500 g each) was mixed with 1 l of distilled water and the aliquot was loaded in a 2 l round-bottom sample flask. Five millilitres of internal standard (IS) [2-methyl-1-pentanol (10 μg/ml in methanol)] was added to the sample before extraction. Each sample was extracted with 150 ml of redistilled dichloromethane. Each extraction was carried out for 2 h after the distilled water in the sample flask started to boil. The extract was dried over Na_2SO_4 overnight and concentrated to ~2 ml using a Rota Vapor (Büchi, Switzerland) equipped with a Büchi 461 water bath; the water bath temperature was 35–36 °C and the reducing pressure 100–150 mm Hg. The solvent was further removed under a purified nitrogen stream to a final volume of 1 ml. The concentrated extract was stored at −20 °C until further analysis was performed.

2.3. Gas chromatography–mass spectrometry (GC–MS)

One microlitre of each concentrated extract was analysed in duplicate on a Hewlett–Packard (HP) gas chromatograph model 6890 equipped with a split/splitless injector and a DB-WAX bonded fused capillary column (30 m × 0.32 mm i.d., film thickness = 0.25 μm, J&W Scientific Inc., Folsom, CA). The detector was a mass spectrometer (HP 5973 Mass Selective Detector, Hewlett–Packard, Palo Alto, CA). The analyses were performed under the following conditions: an inlet temperature of 220 °C; splitless time of 1 min; a purge flow rate to the split vent at 50 ml/min for 1 min; column head pressure at 14.14 psi; a helium carrier gas flow rate of 1.3 ml/min, and an average helium gas velocity of 30 cm/s; the oven temperature was held at 40 °C for 4 min and programed at 5 °C/min until 185 °C, and then held for 20 min isothermally. The mass spectra were generated in the electron impact mode (MS-EI) at 70 eV and an ion source temperature of 230 °C. The spectra were taken over the *m*/*z* range of 40–350. The total ion chromatograms (TIC) acquired via GC–MS was used for peak area integration. HP MSD Chemstation software G1701BA ver.D.02.00 was used for data acquisition.

Table 1
Materials and their ingredients.

Code	Manufacturing method[a]	Ingredients
ANJA	Traditional	Soybean 85%, salt 15%
SOHI	Traditional	Soybean 90%, salt 10%
CHJE	Traditional	Meju (soybean) 99%, salt 1%
CHJA	Traditional	Soybean 95%, salt 5%
MON	Traditional	Soybean 88%, salt 12%
SUJA	Traditional	Soybean 97%, salt 3%
SIN	Modern	Soybean 28.35%, flour, salt, wheat (koji), ethanol
CJW	Modern	Soybean 53.4%, meju 16%, salt, ethanol, koji
HAE	Modern	Meju 58%, soybean, flour, salt, wheat (koji)

[a] Traditional method using fermentation by natural micro-flora, whilst the modern method used wheat koji inoculated with *A. oryzae*.

2.4. Compound identification and quantification

The volatile compounds were positively identified by comparing Kovats retention indices (KI) (Kovats, 1965) and the MS fragmentation patterns with those of reference compounds, or with mass spectra in the Wiley 275 mass spectral database (Hewlett–Packard, Palo Alto, CA) and previously reported Kovats retention indices. The Kovats retention indices (KI) of unknown compounds were determined via sample injection with a homologous series of alkanes (C6–C24). The GC–MS conditions were the same as described above. To quantify the volatiles, the samples were run in duplicate, and the integrated areas based on the total ion chromatograms were normalised to the areas of the internal standard and averaged. The relative volatile concentrations in the nine samples were determined by comparison with the concentration of the internal standard (2-methyl-1-pentanol), assuming a response factor of 1. To determine the reproducibility of the duplicate injections and which peaks varied across samples, two-way analyses of variance (sample, injection) were performed for each volatile peak. All peaks varied significantly across samples, with only four varying significantly across replications.

2.5. Descriptive analysis (DA)

The sensory evaluation of nine soybean pastes was conducted with nine judges (6 female, 3 male) drawn from the Korea Food Research Institute, Seongnam-si, Korea. Six 1-hr training sessions were held for descriptor development, definitions and panel training. A total of 19 attributes were generated to characterise the sensory properties of soybean paste samples (Table 2). Standards used to define these aroma and taste descriptors were present during training and formal sessions. Samples were evaluated in triplicate, three samples per session; with a total of nine sessions being required. The presentation order of soybean pastes was randomized for each session. The stimuli were presented in 10 g aliquots in plastic cups marked with three-digit numbers which were covered with lids. The judges scored each attribute on a scale of 0–9, in which 9 was the highest intensity and 0 was none. Water was provided for panelists to rinse the palate between samples. All evaluations were made in sensory booths at room temperature.

2.6. Statistical analysis

All statistical analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC, USA) or XLSTAT ver.2007.1 (Addinsoft, New York, NY, USA). To evaluate significant differences in the volatile components amongst the fermented soybean pastes, analysis