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# GC–MS based metabolomics approach of Kimchi for the understanding of *Lactobacillus plantarum* fermentation characteristics

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ABSTRACT

GC–MS datasets, coupled with multivariate statistical methods, were used to investigate metabolic changes in Kimchi during fermentation. In order to evaluate the effect of the starter culture (*Lactobacillus plantarum*) on Kimchi fermentation, the Kimchi was sterilized in an autoclave before inoculation. Increased levels of lactic acid, glycerol, pyrotartaric acid, pentanedioic acid, 2-keto-1-gluconic acid, ribonic acid, isocitric acid, and palmitic acid were observed in Kimchi during fermentation, along with decreased levels of valine, leucine, propanoic acid, threonine, proline, glutamine, citric acid, adenine, fructose, glucose, galactose, myo-inositol, and sucrose in Kimchi were observed during fermentation. Principal component analysis (PCA) score plot also showed clear differences in metabolites among Kimchi according to the starter culture. This study highlights the applicability of GC–MS based metabolomics for monitoring Kimchi fermentation and evaluating the fermentative characteristics of starter culture.



## 1. Introduction

Kimchi is the best-known Korean traditional fermented food and is made through the fermentation of vegetables such as Chinese cabbage and radish seasoned with various spices including red pepper powder, garlic, ginger, green onion, fermented seafood and salts (Jung et al., 2012). As kimchi is usually produced without the use of starter cultures by spontaneous fermentation, the environment encourages the growth of various microorganisms. The main microbial groups in spontaneously fermented Kimchi are lactic acid bacterias (LABs), which contribute considerably to the safety and shelf-life as well as the organoleptic and nutritional properties of Kimchi (Jung et al., 2013). Many previous studies have reported that LABs, such as *Leuconostoc*, *Lactobacillus*, *Weissella*, *Lactococcus*, and *Pediococcus* are closely associated with the microbial communities in Kimchi (Cho et al., 2006; Jung et al., 2012; Kim & Chun, 2005).

Kimchi metabolites, such as free sugars (glucose and fructose), organic acids (lactic and acetic acids), and other flavoring compounds (mannitol and amino acids) are important determinants of Kimchi taste and flavor (Jeong, Lee, Jung, Choi, & Jeon, 2013). The LAB that arises from the fermentation of Kimchi contributes significantly to the metabolites of kimchi products. However, it is difficult to identify a clear relationship between the microbial community and the metabolites of Kimchi since spontaneous fermentation without sterilization of the raw materials allows for the growth of various LABs during fermentation (Jeong et al., 2013). Jung et al. reported that the microbial community formed during Kimchi fermentation was similar regardless of the starter inoculation (Jung et al., 2012).

Little is known about the dynamics of the microbial communities and metabolites present during Kimchi fermentation. The LABs that participate in the fermentation of Kimchi are dominant at different stages of fermentation (Jang, Lee, Jung, Choi, & Suh, 2014). To demonstrate the role of LABs in Kimchi fermentation, it is essential to investigate the dynamics of the whole bacterial community (Lee et al., 2005) and the relationships between the microbial populations and metabolites.

Metabolomics are applied in various technologies, including liquid chromatography–mass spectrometry (Albrecht et al., 2012; Xu, Hu, Wang, Wan, & Bao, 2015), gas chromatograph–mass spectrometry (Namgung et al., 2010; Yang et al., 2011), capillary electrophoresis-mass spectrometry (Hasunuma et al., 2011;

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Muroya, Oe, Nakajima, Ojima, & Chikuni, 2014), nuclear magnetic resonance (Lee et al., 2011; Son, Hwang, Park, Hong, & Lee, 2009), etc. One of the most widely used analytical methods in metabolomics is the gas chromatography–mass spectrometry (Gullberg, Jonsson, Nordström, Sjöström, & Moritz, 2004). GC–MS is low cost compared to other analytical methods (CE-MS, LC-MS, and NMR) and has high reproducibility, high resolution, and few matrix effects (Park et al., 2010). Many previous studies employed metabolomics techniques combined with GC–MS and multivariate statistical analysis to obtain the metabolite profiles of various foods (Namgung et al., 2010; Yang et al., 2011). Metabolomics has been applied in food science to monitor the quality, processing, safety, and microbiology of raw materials and final products (Mozzi, Ortiz, Bleckwedel, De Vuyst, & Pescuma, 2013).

There are a few existing reports regarding the fingerprinting or metabolic profiling of Kimchi using analytical methods such as NMR (Jeong et al., 2013; Jung et al., 2012) and GC–MS (Shim et al., 2012). Also, studies of microbial succession and metabolic changes were analyzed using NMR and GC (Jeong, Lee, Jung, Choi, et al., 2013). However, little is known about the relationships between the microorganisms and metabolites during the entire Kimchi fermentation process since a rational approach to the control of the microbial community is currently almost impossible (Jeong, Lee, Jung, Choi, et al., 2013). Therefore, an investigation of the changes in metabolites during fermentation with selected microorganisms is needed. In this paper, metabolite changes in Kimchi fermented using *Lactobacillus plantarum* were identified and quantified. In order to investigate the effect of the starter (*L. plantarum*) on Kimchi fermentation, the Kimchi was sterilized in an autoclave before inoculation and the metabolite profile in Kimchi during fermentation was investigated by applying a multivariate statistical method to GC–MS datasets.

## 2. Materials and methods

### 2.1. Bacterial cultures

Two *L. plantarum* strains were used to evaluate how the incorporation of different strains affects the metabolic change in Kimchi. *L. plantarum* MLK 14-2 isolated from Kimchi (Yoo, Seo, Park, & Son, 2014) and *L. plantarum* KCCM 11322 purchased from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) were used for the fermentation of Kimchi. The strains were inoculated into Man Rogasa Sharpe (MRS, Difco, Sparks, MD, USA) broth and grown at 37 °C for 48 h to obtain a final cell count of log 9–10 CFU/mL.

### 2.2. Preparation of kimchi

The Kimchi model system was prepared according to standardized methods from the Pusan National University Kimchi Research Institute (Cho, Lee, Rhee, & Park, 1998). Vegetables and seasonings were added at the following ratio: Chinese cabbage: water: radish: red pepper powder: welsh onion: garlic: ginger: salt: sugar = 100: 100: 13: 3.5: 2.0: 1.4: 0.6: 2.7: 1.0. The materials were mixed, followed by homogenization using a blender. The mixtures were distributed into 13 2-L glass bottles, producing five batches for each strain and three batches for control. Autoclaving was conducted a temperature of 121 °C for 15 min and the mixture was air-cooled to room temperature for inoculation of the starters. The control Kimchi was not sterilized for the spontaneous fermentation by various microorganisms.

### 2.3. Fermentation

The Kimchi was inoculated with 1% (v/w) of LAB starter cultures except for the control and fermentation was performed at 25 °C for 24 h, then at 4 °C until 50 days. Samples were taken on the 1st, 3rd, 5th, 10th, 20th, 30$^{th}$ and 50$^{th}$ days of fermentation for chemical and microbiological analyses.

### 2.4. Microbiological analysis

The LAB count was determined after growing LAB in MRS agar (Difco, Sparks, MD, USA) and incubating at 37 °C for 48 h. Tests for every sample were carried out in duplicate and the results were expressed as log CFU/g.

### 2.5. Physico-chemical analysis

After centrifuging for 15 min at 8000 rpm, the supernatant liquid was used for all the tests. pH was determined using a pH meter (pH-250L, ISTEK, Seoul, Korea) and the means of three measurements were recorded. Titratable acidity as lactic acid was determined by titrating to pH 8.3 with 0.1 N NaOH. Results are shown as mean values of 5 measurements from each treatment.

### 2.6. Derivatization

Samples containing 150 μL of the above supernatant and 30 μL internal standard (ribitol in water, 20 mg/mL) were dried in a vacuum centrifuge dryer. 80 μL of methoxyamine hydrochloride (MEOX) dissolved at 20 mg/mL in pyridine was added to the dried extract and agitated for 12 h at 37 °C. For derivatization, 100 μL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) containing trimethylchlorosilane (20%, v/v) was added to the samples, and the mixtures were incubated at 37 °C for 1 h. 800 μL of *n*-hexane was added for dissolving derivatized compounds. After vortexing, samples were centrifuged at 13,000 rpm for 10 min, and 600 μL of supernatant was transferred into 2 mL clear glass autosampler vials.

### 2.7. GC–MS analysis

Chromatography was performed on a 6890N gas chromatography (Agilent, Santa Clara, USA) equipped with a 5973N mass selective detector. Separation was achieved using a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Sample volumes of 1 μL were injected with a split ratio of 20:1 by the autoinjector. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injection temperature was 250 °C, and the temperature of the interface and the ion source was set to 280 °C. The GC oven temperature was heated to 80 °C for 2 min, raised to 300 °C at a rate of 10 °C/min and maintained at 300 °C for 14 min. Electron impact ionization (70 eV) at full scan mode (m/z 50–550) and a rate of 20 scans/s was used. Ribitol served as an internal standard to monitor batch reproducibility and to correct for minor variations that occurred during sample preparation and analysis. MSD ChemStation software (Agilent, USA) was used to acquire mass spectrometric data. The mass spectra of all detected compounds were compared with spectra in the NIST and Wiley library. Metabolites were initially identified by comparison of mass spectra to the spectra library using a cut-off value of 90%. All metabolite identifications were manually validated to reduce deconvolution errors during automated data-processing and to eliminate false identifications. For quantitation methods, the MS was operated in the selected ion monitoring (SIM) mode with the quantification ions presented in Table 1. The most specific fragment ion in the spectra of each identified metabolite was determined to the quantification ion, and their summed abundance was integrated; fragment ions due to trimethylsilylation (i.e. *m/z* 73.1 and 147.1)