



Characterization of volatile compounds in fermented milk using solid-phase microextraction methods coupled with gas chromatography-mass spectrometry

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ABSTRACT

Lactic acid bacteria (LAB) are industrially important bacteria that are widely used in the fermented food industry, especially in the manufacture of yogurt. Characteristic flavors are produced by LAB during fermentation and storage that affect the quality and acceptability of fermented milk products. In this study, the volatile compounds in milk fermented by *Streptococcus thermophilus* IMAU80842 alone, *Lactobacillus delbrueckii* ssp. *bulgaricus* IMAU20401 alone, or both species together were identified using solid-phase microextraction methods coupled with gas chromatography-mass spectrometry. A total of 53, 43, and 32 volatile compounds were identified in milk fermented by *S. thermophilus* alone, *L. delbrueckii* ssp. *bulgaricus* alone, or both species together, respectively. The presence of some important flavor compounds was confirmed: acetic acid, acetaldehyde, acetoin, 2,3-butanedione, ethanol, and 1-heptanol. Our results demonstrate that the composition of the volatile compounds in fermented milk depends on the species of LAB used and whether they are used alone or in combination. This is important for the selection of appropriate starter cultures for the production of different types of fermented milk product with particular flavors.

Key words: fermented milk, volatile compounds, solid-phase microextraction methods, gas chromatography/mass spectrometry

INTRODUCTION

Lactic acid bacteria (LAB) are industrially important bacteria that are widely used in the fermented food industry, especially in the manufacture of yogurt.

Yogurt is the most popular fermented milk, and usually produced using mixtures of homofermentative LAB such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* as the starter culture (Herve-Jimenez et al., 2009; Muramalla and Aryana, 2011; Kaneko et al., 2014).

During fermentation, *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* generate lactic acid and a variety of volatile organic aroma compounds. These compounds impart particular flavors to fermented milk (Beshkova et al., 1998). The overall flavor of any fermented milk product is formed by a large number of these volatile compounds. Among these compounds, carbonyl compounds and organic acids such as acetaldehyde, diacetyl, and acetic acid play a crucial role and can be used to evaluate the flavor quality of dairy products (Zha et al., 2015). Ott et al. (1997) found that acetaldehyde provided the typical flavor of dairy products such as yogurt and buttermilk. Erkus et al. (2013) also reported that diacetyl contributed the characteristic sour buttery flavor to some fermented dairy products.

Solid-phase microextraction methods (SPME) were developed in the 1990s by Arthur and Pawliszyn (1990) as a fast and useful technique for the analysis of volatile compounds. The SPME methods coupled with GC-MS can provide high sensitivity with a small sample volume; therefore, it can be used to analyze the flavor profile of a wide variety of substances. Most recently, this technique has been used to study the volatile profiles of fermented camel milk (Ning et al., 2011), grapes and wine (Panighel and Flamini, 2014), and dry fermented sausage (Corral et al., 2016).

In this study, SPME pretreatment coupled with GC-MS was used to study the volatile compounds in fermented milk compounds during fermentation and storage. The aim of this study was to investigate the composition of the volatile compounds in milk that had been fermented by either *S. thermophilus* IMAU80842 alone, *L. delbrueckii* ssp. *bulgaricus* IMAU20401 alone, or both species together.

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MATERIALS AND METHODS

Sample Preparation

Sterile milk was prepared by reconstituting 10% (wt/vol) skimmed milk powder in distilled water and autoclaving at 95°C for 5 min. It was stored at 4°C before use.

The *S. thermophilus* IMAU80842 and *L. delbrueckii* ssp. *bulgaricus* IMAU20401 isolates from the Lactic Acid Bacteria Collection Center of Inner Mongolia Agricultural University were used throughout this study. These isolates were from traditionally produced yogurt and kurut from Mongolia and from the Gansu province of China. Frozen cells of these isolates were activated by 3 subcultures using de Man, Rogosa, Sharpe broth (Becton, Dickinson and Co., Sparks, MD) and then inoculated into 100 mL of reconstituted 10% (wt/vol) milk medium and incubated for 24 h at 42°C. Inoculations were made to achieve a final concentration of approximately 5×10^6 cfu/mL. Three inoculation treatments were made: pure *S. thermophilus*, pure *L. delbrueckii* ssp. *bulgaricus*, and a 1:1 mixture of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*. After inoculation, the milk was fermented at 42°C until the pH value fell to 4.5, and was then stored at 4°C. Samples were taken from each culture after 0, 1, 3, 7, and 14 d during the storage period. The samples collected were stored at -20°C until the volatile compounds were analyzed.

Isolation of Volatile Compounds

Volatile compounds from the fermented milk were isolated using the headspace SPME technique (Ning et al., 2011). All extractions were performed using 50/30 μ m divinylbenzene/carboxen/polydimethylsiloxane fibers. The SPME fiber was purchased from Supelco Inc. (Bellefonte, PA). Five-milliliter samples of each of the fermented milk products were placed into 15-mL glass vials (Supelco Inc.) with micro-stirring bars and stirred for 60 min at 55°C to allow the samples to reach equilibrium. The fiber was inserted into the injection port of the Agilent 7890B gas chromatograph (Agilent Technologies Inc., Palo Alto, CA), held for 5 min for preconditioning, and then inserted into the vial and exposed in the headspace for 60 min under the above conditions. After absorbing the volatile compounds, the fibers were inserted into the GC-MS injector port for desorption (3 min) at 270°C to desorb volatile compounds into the gas chromatograph.

Volatile compounds from the fermented milk were identified using an 7890B gas chromatograph equipped with an 5977A mass selective detector (both from Agilent Technologies Inc.). Volatile compounds absorbed

onto the SPME fiber were passed through an HP-5MS column (30 m length, 0.25 mm inside diameter, 0.25 μ m film thickness; Agilent Technologies Inc.) with helium as the carrier gas at 1 mL/min. The gas chromatograph temperature was maintained at 35°C for 5 min, then increased to 140°C at a rate of 4°C/min for 5 min, and finally gradually increased to 250°C at a rate of 10°C/min for 5 min. The transfer line temperature was set to 250°C. The mass detector was operated at 150°C in electron impact mode at a voltage of 70 eV and an ion source temperature of 230°C. Mass spectra of different treated samples were recorded with a mass range of 40 to 400 m/z, with 5 scans and no solvent delay.

Identification of Volatile Compounds

Volatile compounds were identified by comparing their mass spectra with those from a published database (NIST version 11 mass spectral database; Agilent Technologies Inc.). To calculate the retention indices (RI) of detected compounds by the NIST 11 database in the same capillary column, a series of *n*-alkanes C3–C25 (AccuStandard Inc., New Haven, CT) were run under the same chromatographic conditions. Furthermore, acetaldehyde, hydroxyacetic acid, acetic acid, 2,3-butanedione, ethanol, propylene glycol, formic acid ethenyl ester, and acetic acid ethenyl ester were used as standards to confirm the identifications. Acetaldehyde, hydroxyacetic acid (99.5%), acetic acid (99.9%), and ethanol (99.9%) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and the other compounds were obtained from Sigma-Aldrich (Steinheim, Germany).

RESULTS AND DISCUSSION

Volatile compounds from fermented milk are very diverse and have an effect on flavor. We used SPME GC-MS techniques to compare the volatile flavor compounds of fermented milk from pure cultures and mixed cultures. Results of volatile compounds profile and their relative contents are summarized in Tables 1 to 6.

Separation of Acids

Volatile acid compounds extracted with SPME and analyzed by GC-MS were from different fermented milks produced by *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* that were cultured alone or as a mixture. Seven acid compounds were identified in the volatile fraction of *S. thermophilus*-fermented milk (Table 1). The quantity of each acid compound increased after 1 d of storage. This increase was particularly evident for acetic acid, 4-chlorobutanoic acid, 3-methylbutanoic

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