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Volatile organic compounds profile during milk fermentation by *Lactobacillus pentosus* and correlations between volatiles flavor and carbohydrate metabolism

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ABSTRACT

Flavor, as one of the most important properties determining the acceptability and preference of fermented milks, is influenced by compositional and processing factors. In this study, we focused on the volatile organic compounds related to flavor during milk fermentation by *Lactobacillus pentosus* according to electronic nose analysis. Xylose (1% addition) metabolized by *Lb. pentosus* strongly affects the flavor of yogurt, with the potent volatile organic compounds of ethanol (3.08%), 2,3-butanedione (7.77%), and acetic acid (22.70%) detected using solid-phase microextraction coupled with gas chromatography-mass spectrometry analysis. Sensory analysis also showed skimmed yogurt fermented by *Lb. pentosus* with 1% xylose had the unique scores of sourness (acetic acid) and butter flavor (2,3-butanedione). Furthermore, α-acetolactate synthase and α-acetolactate decarboxylase in carbohydrate metabolism play important roles in milk fermentation. Under preferable conditions (pH 5.5, 42°C) for α-acetolactate synthase and α-acetolactate decarboxylase, the relative content of potent flavor compound 2,3-butanedione was 10.13%, which was 2.55% higher than common culture condition (pH 4.5, 37°C), revealing that xylose metabolized by *Lb. pentosus* has potential values for the milk product industry, such as the acceptability and preference of fermented milk product.

Key words: *Lactobacillus pentosus*, volatile organic component, electronic nose, solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC-MS)

INTRODUCTION

Cultured dairy products are widely consumed due to health and nutrition claims and also for their sensory properties. Naturalness and agreeable taste make yogurt an attractive food for consumption. The primary sensory attributes of yogurt include texture, taste, aroma, and flavor (Salvador and Fiszman, 2004; Sodini et al., 2006). Flavor, as one of the most important properties determining the acceptability and preference of fermented milks, is influenced by compositional and processing factors (Soukoulis et al., 2007). Milk fermentation is a complex process for the transformation of milk into yogurt. Compositional factors, namely nonvolatile acids (lactic and pyruvic), volatile acids (butyric and acetic), carbonyl compounds (acetaldehyde and diacetyl), and miscellaneous compounds, are always influenced by pasteurization, fermentation, and storage (Hugenholtz et al., 2000). Considering that the native volatile constituents in milk may change during the yogurt fermentation period, the chemical ingredients of milk base, type of milk, processing methods, and types of starter culture need to be carefully investigated for high-quality yogurt production (Kühn et al., 2006). Meanwhile, with the increasing demand for low-fat food products, more attention should be paid to yogurt with improved compositional and nutritional properties (Abete et al., 2011).

Currently, starters containing mainly *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* are used widely in the industrial production of yogurt fermentation and quality improvement (Herve-Jimenez et al., 2009). The difference in aroma has been attributed to the presence of different bacteria. The basic volatile organic compounds (**VOC**) participating in the formation of the flavor of typical Bulgarian yogurt are carbonyl compounds, such as acetaldehyde, acetone, 2-butanone, diacetyl, ethyl acetate, and ethanol (Cheng, 2010). Low-molecular VOC in milk supplemented with the strain *Bifidobacterium animalis* ssp. *lactis* Bb-12 with or without fermentation were also reported by Zareba et al. (2012). Yogurt flavor is the critical factor in both product evaluation and consumer acceptability. The correlations between flavor properties and VOC provide the basis for making qualified decisions in producing high-quality yogurt. The evolution of VOC during fermentation can provide useful

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information about the quality of the final product and the possible inefficiencies in the process of incubation (Soukoulis et al., 2010).

Milk is an extremely complicated entity comprising lipids, proteins, carbohydrates, and minerals. Over 400 volatile compounds have been identified in lactic acid bacteria (**LAB**) fermented milk products. Lactic acid bacteria have the capacity of adopting microbial, enzymatic, or chemical transformation to degrade lactose, lipids, citric acid, and proteins/amino acids in milk (Law and Haandrikman, 1997). During fermentation, the differences between the VOC of yogurt and milk are most likely generated by the metabolism of LAB. The carbon-hydrogen transport systems in LAB are specific for carbohydrate transportation and are adenosine triphosphate dependent and they are able to activate complex enzymatic systems in the milk fermentation process (Rabot et al., 2010).

When measuring the relative concentrations of a large number of metabolic enzymes in the processing of fermented milk, it is essential to come to a comprehensive understanding of evolution of VOC during fermentation. α-Acetolactate synthase (**ALS**) and α-acetolactate decarboxylase (**ALDB**) are 2 essential enzymes when pyruvate is channeled to acetoin or diacetyl (Lee et al., 2013). The progress in identifying the key flavor-related compounds and their origins will enable manufacturers to produce more uniform dairy products.

Lactose, a β-1,4-linked disaccharide of β-D-galactose and α/β-D-glucose, is a common constitution in dairy product. Xylose is also abundant in nature and supposedly has several health-promoting effects, such as being low caloric, low glycemic, low insulinemic, antimicrobial, and prebiotic. These effects could occur in milk fermentation by *Lactobacillus pentosus* (Chaillou et al., 1999). However, little attention has been paid to the VOC changes during milk fermented by *Lb. pentosus* and related metabolic pathways. The use of traditional starters limits the organoleptic variation of the end products (Ao et al., 2012). Recently, we characterized a strain of *Lb. pentosus*, which could produce specific flavor in fermented milk. For the acceptability and preference of fermented milks, we investigated VOC differences among various kinds of milk products fermented by *Lb. pentosus* using principal components analysis (**PCA**) according to an electronic nose (**E-nose**) analysis and determination of VOC by solid-phase microextraction coupled with GC-MS (**SPME-GC-MS**). Furthermore, the fermentation conditions that affect the activity of ALS and ALDB were also explored. This research will contribute to the selection and application of this new starter in the traditional milk fermentation process.

MATERIALS AND METHODS

Strain and Medium

The strain was isolated from traditional fermented milk products in Xinjiang Province (China) and classified as *Lb. pentosus* (ATCC8041) by 16S rRNA gene sequence analysis (Peng et al., 2011). *Lactobacillus pentosus* was cultured at 37°C in de Man, Rogosa, and Sharpe (MRS) medium and stored at −80°C in culture plus glycerol (20%, vol/vol) for further use.

Reagents

Milk powder and yogurt (fermented with *Lactobacillus bulgaricus* and *Strep. thermophilus*) were purchased from Bright Dairy Co. (Shanghai, China). Thiamine pyrophosphate, flavin adenine dinucleotide, oxaloacetate, and Coomassie brilliant blue G250 were purchased from Sigma-Aldrich Co. (Shanghai, China); BSA was purchased from Sangon Biotech (Shanghai, China). Other reagents were all analytical grade.

Yogurt Manufacture

Bovine milk, including cream milk (**CM**) and skim milk (**SM**; 1.5% milk fat), was heated for 30 min using batch pasteurization (75°C) and cooled at 37°C in a water bath. Each milk sample was divided into 3 groups (150 mL per flask), and inoculated with *Lb. pentosus* (2%, vol/vol) at 37°C for 12 h; when the pH reached 4.5, the cured-milk samples were stored at 4°C for further analysis. For different samples, 1% lactose or 1% xylose was added to the milk samples during milk fermentation.

E-Nose Analysis of Milk Samples

Samples were collected from CM, SM, cream yogurt (**CY**), skimmed yogurt (**SY**), skimmed yogurt fermented by *Lb. pentosus* with 1% xylose (**SYX**), and plain yogurt from the market fermented by *Lb. bulgaricus* and *Strep. thermophilus* (**PY**). Linear discriminant analysis (**LDA**) and PCA of main VOC from various fermented milk products were performed with an Airsense PEN 3 E-nose (Airsense Analytics GmbH, Schwerin, Germany). Each sample (50 mL) was placed in 125-mL Pyrex 4980 flasks with silicone caps and then introduced to the sampling apparatus of the E-nose (Yu and Wang, 2007; Mamat et al., 2011). The E-nose response to milk samples at different temperatures, including 20, 40, 60, and 80°C, were analyzed according to the LDA.

SPME-GC-MS Analysis of VOC

Volatile organic compound identification was performed by SPME-GC-MS. Carboxen/polydimethylsi-

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