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Search for potential molecular indices for the fermentation progress of soy sauce through dynamic changes of volatile compounds

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ABSTRACT

To date, the fermentation progress and maturity of soy sauce are mainly judged by olfactory and individual experiences, which are subjective and lack quality control. In this study, dynamic changes of volatile compounds during soy sauce fermentation were profiled to search for molecular indices of fermentation progression. In total, 119 volatile compounds were identified and quantified by headspace solid-phase microextraction gas chromatography–mass spectrometry. Principal component analysis (PCA) revealed the compounds that contributed most to the distinction of various stages of fermentation progression; the list included 2-methoxy-4-vinylphenol, phenylethyl alcohol, phenylacetaldehyde, and benzaldehyde. The degradation of 2-methoxy-4-vinylphenol and the generation of phenylethyl alcohol could remarkably discriminate between the discrete stages of soy sauce fermentation. It therefore suggests that their group was a refined and potential index to indicate the progression of the soy sauce fermentation. This work furthers our understanding of the molecular changes occurring during soy sauce fermentation and improves its quality control and process management.

1. Introduction

Soy sauce, a traditional fermented condiment, has been widely consumed in East Asia for centuries. Its unique flavor has created a global demand that currently requires a yield of over 10,000,000 tonnes each year (Yan, Zhang, Tao, Wang, & Wu, 2008). Soy sauce is made from soy bean and wheat flour. Its fermentation comprises two stages: koji fermentation and mash fermentation. In the koji fermentation stage, a koji mold such as *Aspergillus oryzae* is grown on the raw material, which contains a mixture of steam-cooked defatted soybean and roasted wheat flour. In the stage that produces mash, the koji is mixed with brine solution, and then subjected to long periods of aging (Chou & Ling, 1998). Although mash fermentation is a very long process (taking nearly half a year, (Yongmei et al., 2009)) it is necessary for the formation of abundant flavor compounds (Benjamin, Storkson, Nagahara, & Pariza, 1991; Kobayashi, 2005).

Due to the long-term soy sauce fermentation process, monitoring of its progression is pivotal for optimal quality control; however, objective indices and quantitative scales are still lacking. Currently, even in relatively modern factories, the progression of soy sauce fermentation is judged mainly by subjective experience, which confounds quality control and therefore compromises the consistency of final product quality. Flavor-containing compounds produced during fermentation can be considered indicators to judge fermentation progress. Although previous studies have identified several dominant flavor compounds in soy sauce (Gao et al., 2010; Lee, Seo, & Kim, 2006; Yan et al., 2008), there is still a scarcity of detailed and complete investigations into the dynamic changes of volatile compounds across the whole fermentation process. Furthermore, the field is lacking objective indices that can be used to measure the progression of fermentation. Thus, there is an urgent need for objective and quantitative indices that quantify the soy sauce fermentation process.

The aim of this study was to find molecular indices that discriminate the various stages of soy sauce fermentation. A method of headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) was used to profile the dynamic changes of volatile compounds as the fermentation progressed. Three different kinds of SPME fibers were combined to identify more compounds. Moreover, the positive identifications were confirmed using the RI (retention index) method, and partly by comparison of mass spectra with those of standards. Moreover, using principal component analysis (PCA), compounds with significant changes during fermentation were revealed, some of which can be selected as indices for the progress of soy sauce fermentation for the given experimental setup. Our work would help improve quality control, enhance product stability and safety, and provide valuable information for the optimization of production technology.

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2. Materials and methods

2.1. Materials

Three different types of SPME fibers (85-μm polyacrylate (PA), 75-μm carboxen/polydimethylsiloxane (CAR/PDMS) and 65-μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) coating fibers) were from Supelco, USA. The standards 1,2-dimethoxy-benzene, 2-methoxyphenol, benzoic acid ethyl ester, 4-ethyl-phenol, benzothiazole, 2-methoxy-4-vinylphenol, tetradecane, butylated hydroxytoluene, 4-hydroxy-3-methoxy-benzoic acid ethyl ester, hexadecane, hexadecanoic acid ethyl ester, and octadecanoic acid ethyl ester were purchased from Sigma-Aldrich, USA. Ethanol (analytical grade) was purchased from Bei Chen Chemical Reagent Factory, China. Multi-State Hydrocarbon Window Defining Standard (C8–C40, 500 μg/mL) was purchased from AccuStandard, Inc., USA.

2.2. Preparation of soy sauce

In this study, soy sauce mash samples were prepared in a factory according to a method modified from that of Röling (Röling, Apriyantono, & Van Verseveld, 1996). Briefly, the defatted yellow soybean was boiled, and then cooled to room temperature (RT, approximately 30 °C). Wheat was roasted and cooled to RT before being ground. *A. oryzae* was grown in a mixture of steamed defatted yellow soybean and ground wheat (V/V, 5.5:4.5) at 35 °C for 42 h, resulting in the formation of soy sauce koji. The soy sauce was then ripened by mixing soy sauce koji with 2.5 times the volume of salted water (18–20 °Be′) in a fermentation tank, which was held at RT for 6 months. This general practice is employed in most factories before soy sauce products are distributed for sale.

2.3. Sample collection

The soy sauce mash samples were collected at different time points during fermentation (8, 31, 56, 90, 118, and 152 days), and stored at −70 °C prior to analysis (Kang et al., 2011).

2.4. Headspace solid-phase microextraction

Soy sauce mash samples (5 g) were placed in 20-mL glass vials (Agilent, USA). Three different kinds of SPME fibers were used to extract volatile compounds. The samples pressurized in vials were pre-equilibrated for 30 min at 60 °C in a thermostatic water bath. Subsequently, SPME fibers were inserted into the headspace above the soy sauce mash sample in the vials for adsorption of the headspace volatiles for 30 min (Dajanta, Apichartsrangkoon, & Chukeatirote, 2011). After extraction, the loaded SPME fibers were immediately removed from the sample vials and inserted into the injection port of GC for further GC–MS analysis. Triplicate repeated extractions from each SPME fiber were performed for each sample.

2.5. GC–MS analysis

The GC–MS system comprised an Agilent 7890A gas chromatograph and Agilent 5975C series mass spectrometer. Helium gas (1 mL/min) was passed through the Agilent HP-5 capillary column (30 m × 250 μm × 0.25 μm). The volatiles were first desorbed from the SPME fiber by heating at 250 °C for 4 min. Separation was achieved with a temperature program of 50 °C for 5 min, then heated to 125 °C at 3 °C/min and held for 5 min, and heated to 180 °C at 2 °C/min and held for 2 min, and heated to 250 °C at 5 °C/min. The MS was operated in the electron impact mode with an ion source temperature of 230 °C, by using an ionization voltage of 70 eV, an ionization current of 100 μA, and an accelerating voltage of 2 kV. Mass scan range was 30–500 amu with a scanning rate of 1 scan/s.

2.6. Reproducibility and linearity of the HS-SPME GC–MS method

The reproducibility and linearity of the quantification method were assessed with the standard 1,2-dimethoxy-benzene, which was added in the 8-day fermented soy sauce mash at different concentrations. The standard was diluted to 1.0 mg/mL by using ethanol as the solvent. Standard solutions (1, 2, 3, 4, and 5 μL, respectively) were added into 5 equivalent 5-g samples of 8-day fermented soy sauce mash, and then analyzed by HS-SPME GC–MS with a PDMS/DVB fiber.

2.7. Identification and confirmation

The method described by Moy (Moy, Lu, & Chou, 2011) was followed for the identification of volatile compounds using MS data. Volatile metabolites were identified with Agilent's ChemStation software, based on matching the resulting mass spectra to the NIST MS 08 Library. The compounds identified by MS were further confirmed by the retention index (RI) method (Wanakhachornkrai, 2003) and partly by the comparison of mass spectra with those of their standards.

2.8. Statistical analysis

Abundances of volatile compounds were subjected to PCA. All statistical analyses were performed using SIMCA-P Software version 11.5 (Umetrics, Umea, Sweden). Hotelling's T2 test was used to determine statistically significant differences among groups; outlying samples of the ellipse region that defined the 95% confidence interval of the modeled variation were excluded from further analysis.

3. Results and discussion

3.1. Reproducibility and linearity of the HS-SPME GC–MS method

Reproducibility and linearity are the bases of accurate and precise quantitative analysis. To insure reliability, the performance of method we employed was assessed by the analysis of 5 samples containing an equal amount of 8-day fermented soy sauce mash and different concentrations of 1,2-dimethoxy-benzene. The reproducibility of retention time and peak area was assessed using the relative standard deviation (RSD). All the RSD values of retention time were distributed <0.05%, indicating the method was extremely reproducible with respect to retention time. Furthermore, the distribution of all the RSD values of peak area (except that of 1,2-dimethoxy-benzene) mainly ranged between 4 and 6%, which also indicated excellent reproducibility. Fig. 1 shows the overlay of the extracted ion chromatograms (EIC) of 1,2-dimethoxy-benzene in 5 runs at different concentrations. The retention time of 1,2-dimethoxy-benzene reached 19.113 ± 0.036 across 5 runs, reflecting high reproducibility with respect to retention time. Moreover, Fig. 1 reveals good linearity, with the R^2 value reaching 0.995. Together, these results confirm that the reproducibility and linearity of our method satisfy the requirements of this study (Zhang, Yang, & Pawliszyn, 1994).

3.2. Identification and confirmation of volatile compounds

In total, 119 volatile compounds were identified and quantified by HS-SPME GC–MS, including 17 alcohols, 9 aldehydes, 36 esters, 5 ketones, 22 miscellaneous compounds, 15 alkanes, 1 acid, 2 pyran(one)s, 2 furan(one)s, 3 sulfur-containing compounds, and 7 nitrogen-containing compounds (Supplementary table). Supporting these findings, 113 of the compounds were also confirmed by the RI method, and 11 of them were further identified by the comparison of mass spectra with those of their standards. To the best of our