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Comparison study of the volatile profiles and microbial communities of Wuyi Qu and Gutian Qu, two major types of traditional fermentation starters of Hong Qu glutinous rice wine

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ABSTRACT

Hong Qu, which mainly contains *Monascus* sp. and other microorganisms, as well as numerous microbial metabolites, is used as the fermentation starter of Hong Qu glutinous rice wine, a traditional alcoholic beverage. Two widely-used types of Hong Qu, namely Wuyi Qu (WYQ) and Gutian Qu (GTQ), were thoroughly compared for their fermentation properties, volatile profiles, and microbiota structures in this study. Significantly higher color value, glucoamylase and α-amylase activities were discovered in WYQ. And substantial variation in volatile components and microbial communities were also observed between them. It was identified that bacterial genus *Burkholderia* dominated GTQ (71.62%) and *Bacillus* dominated WYQ (44.73%), while *Monascus purpureus* was the most abundant fungal species in both types of starters (76.99%). In addition, 213 bacterial genera and 150 fungal species with low-abundance were also detected. Since the Linear Discriminant Analysis Effect Size algorithm, 14 genus-level bacterial taxa and 10 species-level fungal taxa could be utilized to distinguish these two types of starters. Moreover, the potential correlation of the volatile components and microbiota within WYQ and GTQ were further analyzed, by utilizing Partial Least Squares Discriminant Analysis. Ultimately, this study provides detailed insight into the volatile profiles and microbial communities presented in Hong Qu.

1. Introduction

Chinese yellow rice wine, which may date back as early as the seventh millennium before Christ (B.C.), is a traditional fermented alcoholic beverage that is brewed from glutinous rice or wheat (McGovern et al., 2004). Hong Qu glutinous rice wine, primarily produced in Fujian province, China, is one of the most ancient in this particular category. The distinct characteristics of Hong Qu glutinous rice wine is the utilization of Hong Qu as the fermentation starter, which produces a brilliant bright-red color, fine sweet flavor, and also offers a healthcare functionality to the wine (Park et al., 2016). Through an empirical solid fermentation process, Hong Qu is inoculated with diverse bacteria and fungi, along with numerous pigments, enzymes and other metabolites, facilitating the formation of alcohol and its unique flavor. Similar to other traditional fermented foods, Hong Qu is generally prepared under non-sterile fermentation conditions. Thus, a wide variety of microbes are presented in Hong Qu, and the quality of starters will tend to vary from region to region, resulting in the uncontrollability of the fermentation process of Hong Qu glutinous rice wine. Thus, to fully understand microbiota structures, as well as the fermentation properties and volatile profiles, and identify the key microorganism responsible for the aromatic forming or other fermentation activities, will help to establish next generation starters, which have a limited number of pure strains, and so as to improve the controllability.

Despite the variation of microbes within Hong Qu, two distinguishing types of Hong Qu can still be recognized, which are Wuyi Qu (WYQ) and Gutian Qu (GTQ). In general, WYQ presents a somewhat darker color and has a higher saccharifying and liquefying power than that of GTQ (based on experience). Moreover, a dramatic difference in flavor can easily be identified between these two types of Hong Qu, as well as the rice wines that are fermented from them. Diverse microbiota within the starters may be the primary factor or cause relating to these differences.

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Bacteria and fungi, particularly filamentous fungi and yeasts, play an essential role in the quality of Chinese rice wine fermentation starters, as their involvement in the saccharification, liquification, alcoholic fermentation and flavor formation during the wine brewing process. Therefore, numerous studies have been conducted to adequately characterize the bacterial and fungal diversity of Chinese rice wine fermentation starters (Guan et al., 2012; Lu et al., 2008). Due to the different processing methods, Hong Qu have distinct aromatic and microbial profiles with other rice wine starters. Previously our group has investigated the bacterial and fungal community structures in WYQ. Briefly, based on culture-dependent or PCR-RFLP and PCR-DGGE or MALDI-TOF mass spectrometry fingerprinting methods, 16 filamentous fungi species, including *Monascus purpureus*, *Aspergillus flavus*, *Aspergillus niger, Rhizopus oryzae* and et al. (Lv et al., 2012a), and 2 yeast species, including *Saccharomycopsis fibuligera* and *Saccharomyces cerevisiae* (Lv et al., 2013b), and 16 bacterial species belonging to 6 bacterial genera (*Bacillus, Staphylococcus, Leuconostoc, Pediococcus, Lactobacillus* and *Lactococcus*)(Lv et al., 2016) were identified from WYQ. Nevertheless, in terms of GTQ, the research is limited, although this variety is a more extensively used one in folk. The recent expansion of the next-generation sequencing technology and data mining strategies could provide a more clarifying and precise picture of the microbial community of fermentation starters and, thus, enable researchers to obtain a more global insight into the functions and interactions of various microorganisms.

In the present study, through solid phase microextraction (SPME)-GC/MS analysis and high throughput sequencing of bacterial 16S rRNA genes and fungal ITS rRNA genes upon Illumina HiSeq platform, the volatile profiles and microbial community structures of WYQ and GTQ were investigated and compared. In addition, the association between volatile components and microorganisms within these two types of starters were also calculated through Partial Least Squares Discriminant Analysis (PLS-DA) modelling, thus to preliminarily explore the roles of the microbes as they relate to the aroma formation of Hong Qu. This study may provide a better understanding of the microbial community in Hong Qu and their contribution to the fermentation process, which may be helpful for the development of new starters with pure strains and the improvement of the controllability of Hong Qu glutinous rice wine brewing.

2. Materials and methods

2.1. Sample collection

Five Wuyi Hong Qu (WYQ) were collected from local markets of Jian'ou, Fuzhou, Fuqing, Gutian, and Yongchun cities of Fujian Province, China; moreover, five Gutian Hong Qu (GTQ) were also collected from local markets of Gutian city of Fujian Province, China. All the collected samples are exhibited in Fig. 1. After collection, the samples were then ground into fine powder and appropriately stored at −20 °C until analysis.

2.2. Color value analysis

In general, three colors (red, orange and yellow), including both water-soluble and alcohol-soluble ones, are produced by *Monascus* stains, which with a maximum absorption at 510 nm, 465 nm, and 410 nm, respectively. Normally, A_{410}, A_{465}, and A_{510} are used to estimate the content of this three pigments (Yang et al., 2005; Zhang et al., 2017). In this study, distilled water or 70% (v/v) ethanol were used for pigment extraction: 0.2 g ground Hong Qu powder was suspended in 10 mL distilled water or 70% (v/v) ethanol at 60 °C for 60 min; then the extracted solution was centrifuged at 8000 rpm for 15min. Subsequently, appropriate dilution of supernate was measured for absorbance at 410 nm, 465 nm and 510 nm with a spectrophotometer (Hitachi U-1900, Tokyo, Japan). The color value was defined as the summation of absorbance units at 410 nm, 465 nm and 510 nm × dilution factor per gram of dry samples (U/g).

2.3. Fermentative power analysis

Moreover the fermentative power, including α-amylase, glucoamylase and protease, of the ten Hong Qu samples were also determined as previously described (Lv et al., 2012a). Briefly, the iodine colorimetry method was used to determine the α-amylase activities of the samples (De Moraes et al., 1995). One unit (U) of α-amylase activity was defined as the quantity of enzymes required to hydrolyze 1 mg of starch in 10 min at 40 °C. The dinitrosalicylic (DNS) acid method was then utilized to determine the glucoamylase activities of the samples (Bernfeld, 1955). One unit (U) of glucoamylase activity was defined as the amount of enzymes releasing 1 mg of glucose from the substrate in 60 min under strict assaying conditions. Next, the protease activity was determined, according to the method described by Farley and Ikasari (1992). One unit (U) of protease activity was defined as the amount of enzyme that liberated 1 μg tyrosine per min under assaying conditions. All the experiments were conducted in triplicate.

2.4. Volatile profiles analyses

Volatile profiles of the ten starters were analyzed by utilizing SPME−GC/MS methods (Luo et al., 2008). Briefly, 1.00 g of each ground Hong Qu powder, 2.00 g of NaCl and 5 mL distilled water were transferred to a 30 mL vial. This vial was then tightly capped with a silicon septum and a 50/30 μm divinylbenzene/carboxen/poly (dimethylsiloxane) (DVB/CAR/PDMS) coated fibre (Supelco, Inc., Bellefonte, PA, USA) was inserted into the headspace of the vial for the volatile compound extraction at 50 °C water bath for 40 min.

GC/MS analyses were performed on an Agilent 7890B gas chromatograph (Agilent, Palo Alto, CA, USA) coupled with an Agilent 5973C mass spectrometer (Agilent, Palo Alto, CA, USA). Separation of compounds was performed on an HP-5MS column (30.0 m × 0.25 mm × 0.25 μm, Agilent, USA). Helium was used as a carrier gas at a constant flow rate of 1 mL/min. Oven temperature was maintained at 40 °C for 5 min, programmed at 5 °C/min to 250 °C and held for 5 min. The interface temperature was set at 280 °C. The mass spectrometer was operated in electron impact mode with the electron energy set at 70 eV and a scan range of 30−550 m/z. The temperature of MS source and quadrupole was set at 230 and 150 °C, respectively. Each compound was identified using the National Institute of Standards and Technology (NIST) library (11 L). The relative percentages of the detected peaks were obtained by peak-area normalization. Afterward, based on the volatile profile data set, Principal Component Analysis (PCA) was used to visualize the differences among the starter samples, by using R software (Version 3.2.5) with the vegan, ade4 and ggplot2 packages.

2.5. Bacterial and fungal DNA extraction and illumina sequencing

Bacterial and fungal DNA was extracted from 0.2 g of each ground Hong Qu powder samples by utilizing a rapid DNA extraction kit (BioTeke Corporation, Beijing, China), following the instruction of the manufacturer. The extracted bacterial and fungal DNA were then checked by agarose gel electrophoresis.

Bacterial primers 341-F (5′-CCT AYG GGR BGC ASC AG-3′) and 806-R (5′- GGA CTA CNN GGG TAT CTA AT-3′) with specific barcode