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ARTICLE

Characterization of Chinese Liquor Starter, "*Daqu*", by Flavor Type with ^{1}H NMR-Based Nontargeted Analysis

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"*Daqu*" is a fermentation starter and substrate complex that is used to initiate fermentations for the production of Chinese liquor (alcoholic spirit). Several different types of *Daqu* are customary used, having different flavours, i.e. light, strong, or sauce flavor. With the aim to develop objective methods to characterize and distinguish such different types of *Daqu*, nontargeted analyses of extracts from three typical flavor types of *Daqu* were carried out using ^{1}H nuclear magnetic resonance (NMR) spectroscopy. A significant separation of spectra of *Daqu* of light-flavor, strong-flavor and sauce-flavor types was achieved using principal components analysis. The separation could be attributed to higher levels of glycerol, malate, acetate and N-acetylglutamine in light-flavor *Daqu*; higher levels of mannitol, betaine, trimethylamine and pyroglutamate in strong-flavor *Daqu*; and higher levels of lactate, isoleucine, leucine, isovalerate and valine in sauce-flavor *Daqu*. These metabolites were regarded as the representative metabolites or biomarkers characteristic for each type of *Daqu* and could be associated with some of the microorganisms that have been reported in *Daqu*. This study highlights the application of nontargeted analysis techniques based on NMR in process research and quality control in *Daqu* production and liquor fermentation.

KEYWORDS: *Daqu*; nontargeted analysis; ^{1}H nuclear magnetic resonance spectroscopy; flavor; Chinese liquor; fermentation starter

INTRODUCTION

Chinese liquor (or alcoholic spirit) has a long history of thousands of years, and the invention and development of its manufacturing technique is considered as one of progress in the technological history of ancient China (*1*). Nowadays, its annual production has been estimated to exceed 5 million metric tons in China. It is typically obtained from cereals such as sorghum and wheat by complex fermentation processes using natural mixed culture starters (i.e., "*Daqu*") followed by distillation. *Daqu* is a saccharifying and fermenting agent for the production of Chinese liquor and has significant impact on the flavor of the product. *Daqu* contains cereals, peas, a mixed microflora of fungi and bacteria, microbial enzymes and metabolites, and an important flavor note that will contribute to the aroma of the final distillate (*2*−*4*). According to the flavor characteristics of the liquor obtained, several types of *Daqu* can be distinguished, such as light-flavor, strong-flavor, and sauce-flavor *Daqu*. The basic reason for these differences is the different formulation of ingredients, but particularly the incubation conditions during their processing, leading to different microflora compositions and formations of metabolites and reaction products (*5*−*9*).

Nontargeted analysis refers to a qualitative and quantitative profile or fingerprint of all the organic compounds with small molecular weight (< 1 kDa) that can be measured in whole samples. Currently, nontargeted analyses are increasingly applied in differentiation, quality control, or compositional comparison of fermented foods (*10*−*14*). We hypothesize that many metabolites in *Daqu* serve as flavor compounds or flavor precursors of Chinese liquor, and the metabolites of a *Daqu* type can reflect the specific fermentation events and biochemical reactions associated with the microbial succession and metabolism taking place during the production of *Daqu*.

Nuclear magnetic resonance (NMR) is one of the important nontargeted techniques, and it can produce rapid, reproducible, stable, and unbiased metabolite profiles of a sample. It has the advantage that all kinds of small metabolite molecules can be measured simultaneously (*10*−*14*).

In this study, we aimed at differentiating three major types of *Daqu*, identifying their major flavor components, and indicating potential biomarkers that could be used to characterize the authenticity of *Daqu* types.

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MATERIALS AND METHODS

Sampling. Because of the complex process, *Daqu* is not regularly made at home but at a larger scale in factories. We selected typical *Daqu* representing the well-known flavor types to carry out our differentiation tests. Brick-shaped *Daqu* was sampled from five commercial distilleries located in northern and southwestern China and stored at −18 °C until use. An overview of the origins and types of *Daqu* is presented in **Table 1**. The outer surface layer of the *Daqu* bricks was removed, and the resulting center part was ground in a sample grinding mill.

Extraction of Polar Compounds from *Daqu*. One hundred milligrams of ground *Daqu* was transferred into a centrifuge tube. Then 1.5 mL of cold Milli-Q water (about 0 °C, ice bath) was added in the tube and vortexed at 2500 oscillations/min for 60 s using a homogenizer (Mini-Beadbeater-8, Biospec, Bartlesville, USA) without beads added. The tube was then kept in an ice bath for 10 min statically and then centrifuged for 10 min at 16060*g* at 4 °C. Finally, 1 mL of supernatant was transferred to a new tube and stored at −80 °C until analysis.

NMR Measurement. For the NMR measurements, each extract was mixed with an equal volume of cold loading buffer [0.1 M sodium phosphate, pH 7.0, containing 10% D_2O (v/v) and 1 mM TSP] and centrifuged at 16060*g* at 4 °C. Next, 200 μL of the mixture was transferred to a 3 mm NMR tube. All ^{1}H NMR spectra were measured at 300 K using an Avance III 600 NMR spectrometer (proton frequency = 600.45 MHz, 14.1 T; Bruker, Rheinstetten, Germany) with a cryogenic NMR probe. All experiments were under full automation (transfer of samples to the probe, temperature control, tuning and matching, 90 degree pulse determination, and data processing). One-dimensional ^{1}H NMR experiments were performed under the following conditions: NOESYGPPR1D pulse sequence; 90° ^{1}H NMR pulse, around 9 μs; relaxation delay, 4 s; acquisition time, 1.8 s; number of dummy scans, 4; number of transients, 256; spectral width, 18000 Hz.

Data Analysis. The spectral region δ = 0.70−9.20 of each NMR spectrum was segmented into buckets of 0.02 ppm width with AMIX software (version 3.7.10; Bruker BioSpin, Rheinstetten, Germany). The water region (δ = 4.60−5.05) was excluded from the analysis. All bucket data were scaled to total intensity of the corresponding spectrum and then analyzed by principal component analysis (PCA) with Pareto scaling. Compounds were identified and quantified with Chenomx software (version 5.0; Chenomx, Edmonton, Canada) with the reference of internal standard TSP.

Table 1. *Daqu* Samples Investigated

flavor type	sample code	no. of samples (bricks)[a]	factory location	max temps reached during incubation (°C)
light	*FHX*	1	northern China	40−50
	FQC	1		
	FHH	1		
	NQ	1		
strong	*LN*	3	southwestern China	50−60
	WN	2		
sauce	*UJ*	1	southwestern China	60−70
	LJ	2		

[a] For each flavor type of *Daqu* at least three independent samples from different batches have been used.

RESULTS

Visual Inspection of ^{1}H NMR Spectra and Assignment of Compounds. Representative ^{1}H NMR spectra of aqueous extracts of *Daqu*, representing three flavor types from five factories, are shown in **Figure 1**. The metabolite profiles of the three types of *Daqu* varied in patterns and peak intensities (spectra of outer part were not shown). About 70 metabolites were presumptively identified by Chenomx software as shown in **Table 2**.

Table 2 shows that light-flavor *Daqu* mainly contained glucose, glycerol, mannitol, acetate, *N*-acetylglutamate, glutamate, and malate; the strong-flavor *Daqu* contained mainly glucose, glycerol, mannitol, acetate, pyroglutamate, glutamate, serine, alanine, lactate, fructose, and trimethylamine *N*-oxide/betaine; and the sauce-flavor *Daqu* contained mainly glucose, glycerol, mannitol,

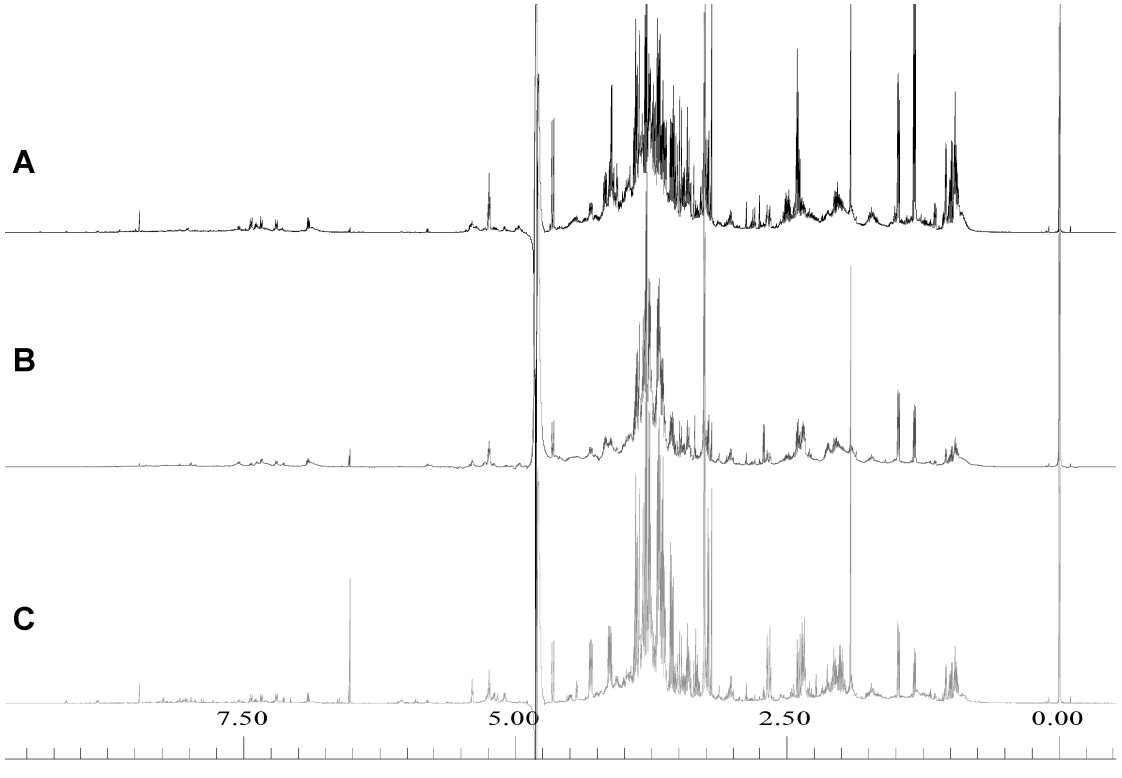

Figure 1. Representative ^{1}H NMR spectra of aqueous extracts of typical *Daqu*: (**A**) sauce-flavor type; (**B**) strong-flavor type; (**C**) light-flavor type.

Table 2. Presumptive Metabolites and Their Contents in Three Types of *Daqu* (Micromoles per Gram of *Daqu*) by Means of Chenomx

metabolite	light-flavor	strong-flavor	sauce-flavor
acetaldehyde	nd[a]	nd	0.14
acetate	8.12	7.19	11.41
N-acetylglutamate	9.56	nd	nd
adenine	nd	nd	0.41
adipate	0.99	nd	nd
alanine	3.34	12.08	10.91
allantoin	nd	nd	2.03
4-aminobutyrate	nd	4.35	2.23
3-aminoisobutyrate	nd	nd	0.94
asparagine	1.69	nd	nd
aspartate	2.26	nd	4.54
benzoate	nd	nd	0.59
betaine	3.97	7.54	10.54
caprylate	nd	nd	0.88
carnitine	1.19	1.34	3.27
choline	1.29	nd	3.18
cinnamate	0.12	nd	nd
cytosine	0.35	nd	0.27
2-deoxyadenosine	0.26	nd	nd
dimethylamine	0.21	1.32	0.80
ethanol	1.09	nd	nd
formate	1.54	1.49	9.57
fructose	nd	10.59	nd
fumarate	4.00	nd	0.11
2-furoate	nd	nd	0.56
glucose	29.39	22.99	21.58
glutamate	6.60	22.20	nd
glutamine	3.37	nd	nd
sn-glycero-3-phosphocholine	2.38	nd	1.69
glycerol	32.95	8.09	9.75
histamine	1.18	nd	nd
histidine	1.08	nd	nd
homocystine	1.28	nd	nd
2-hydroxyisocaproate	nd	0.58	nd
isobutyrate	nd	1.45	nd
isoleucine	1.94	2.20	3.11
isopropanol	nd	0.46	0.56
isovalerate	0.92	1.72	nd
lactate	nd	7.01	75.89
leucine	2.82	3.55	7.10
malate	15.47	nd	nd
nicotinate	0.36	nd	0.49
maltose	nd	nd	9.10
mannitol	4.88	36.14	32.17
methionine	1.34	nd	nd
methylhistidine	1.00	nd	nd
methylmalonate	nd	0.59	nd
oxypurinol	nd	nd	0.45
phenylalanine	1.90	2.34	3.03
O-phosphocholine	1.08	nd	nd
propionate	1.01	2.35	1.47
propylene glycol	nd	nd	1.85
4-pyridoxate	0.23	nd	nd
pyroglutamate	nd	22.90	18.57
pyruvate	nd	3.18	nd
quinolinate	nd	nd	0.96
serine	nd	12.80	nd
succinate	0.86	nd	2.27
theophylline	0.54	nd	nd
threonine	2.47	nd	nd
trigonelline	2.44	nd	nd
trimethylamine	nd	nd	0.47
trimethylamine *N*-oxide	1.22	6.66	3.70
tryptophan	0.50	nd	nd
tyrosine	0.97	nd	1.63
uracil	0.35	nd	1.15
urea	1.85	nd	nd
uridine	0.32	nd	nd
urocanate	nd	nd	0.25
valerate	1.24	nd	nd
valine	2.69	3.26	4.99

[a] nd, not detected.

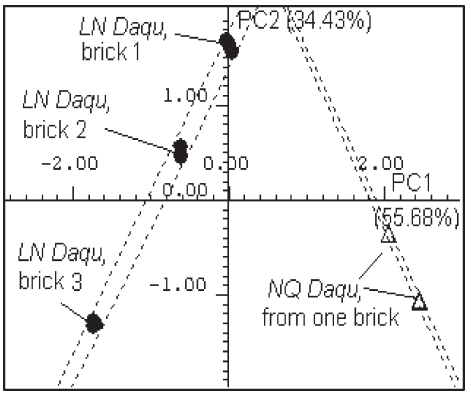

Figure 2. PCA score plot of triplicate samplings of three bricks of strong-flavor (*LN*) *Daqu* and one brick of light-flavor (*NQ*) *Daqu*.

acetate, lactate, formate, maltose, pyroglutamate, alanine, betaine, leucine, and valine.

Reliability and Accuracy of the Nontargeted Analysis. To validate the extraction method, the following comparison experiments were done. The possible effect of disrupting microbial cells on spectral patterns was investigated by vortexing the samples with or without glass beads in the Beadbeater. No differences in spectra were observed (figure not shown), and we standardized the method without glass beads accordingly.

The possible effect of enzymic modifications during extraction was investigated by extending the incubation in the ice bath from 10 min to 2 h. This did not result in detectable changes (figure not shown), so a limited delay from the standard 10 min did not lead to undesirable changes.

We also tested the need for ultrafiltration (cutoff 3 kDa) of the extract prior to NMR measurement and observed no obvious differences between spectral patterns of filtered and nonfiltered extracts. This implied that influences of possible macromolecules, for example, starch and protein, could be ignored.

Finally, we tested reproducibility by extracting three *Daqu* bricks from the same batch and carried out PCA to verify the reliability of the extraction method established. **Figure 2** shows that the three replicates of one sample group were very close to each other, but far from other triplicate samples of a different *Daqu* type. The PCA result verified that the extraction method we used in this study was reproducible and reliable.

Characteristics of Three Types of *Daqu*. Figure 3 shows the pairwise PCA comparisons of three types of *Daqu*. All score plots displayed a significant separation between the two types of *Daqu*. The loading plots indicate the metabolites that are responsible for the separations of clusters.

Figure 3A shows that strong-flavor *Daqu* contains relatively higher levels of mannitol, trimethylamine *N*-oxide/betaine, and glucose, whereas sauce-flavor *Daqu* contains high levels of lactate, leucine, isoleucine, and choline. **Figure 3B** shows that strong-flavor *Daqu* contains relatively higher levels of mannitol, trimethylamine *N*-oxide/betaine, and pyroglutamate, whereas light-flavor *Daqu* contains higher levels of lactate and propylene glycerol/isopropanol.

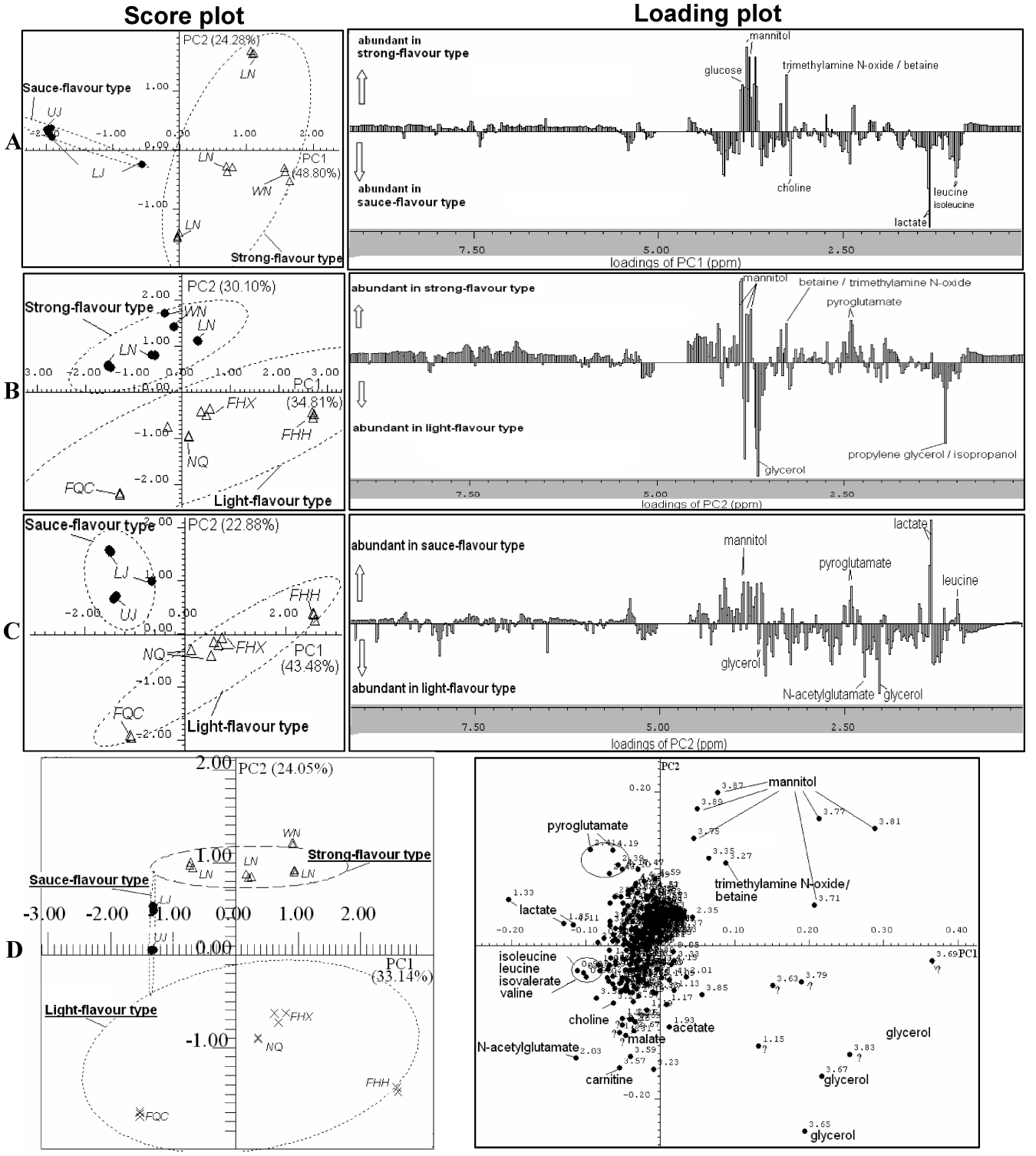

Figure 3. PCA of *Daqu* extracts: (**A**) strong-flavor type versus sauce-flavor type; (**B**) strong-flavor type versus light-flavor type; (**C**) light-flavor type versus sauce-flavor type; (**D**) PCA of three flavor types of *Daqu*.

Figure 3C shows that sauce-flavor *Daqu* contains relatively higher levels of mannitol, lactate, leucine, and pyroglutamate, whereas light-flavor *Daqu* contains higher levels of *N*-acetylglutamate and glycerol.

Figure 3D shows clearly that light-flavor, strong-flavor, and sauce-flavor types of *Daqu* are discriminated well by PC1 and PC2. The loading plot indicates the metabolites that contributed to this discrimination. The biomarkers that most significantly characterized different flavor types by their increased metabolite resonances were in A (light-flavor type *Daqu*) glycerol, acetate, malate, carnitine, and *N*-acetylglutamine; in B (strong-flavor *Daqu*) mannitol, betaine, trimethylamine *N*-oxide/betaine, and pyroglutamate; and in C (sauce-flavor *Daqu*) lactate, isoleucine, leucine, isovalerate, and valine. Those metabolites were regarded as the representative metabolites or biomarkers, respectively, for each type of *Daqu*.

Verification of Biomarkers by Direct Comparison of Their Peak Intensities. The biomarkers identified by PCA can be confirmed directly by pairwise comparison of peak intensities among the three flavor types of *Daqu*. The peaks with intensities of ≥1.5-fold higher than their counterpart were listed in descending order as below.

Strong-flavor *Daqu* contains higher intensities of peaks of pyroglutamate, betaine/trimethylamine *N*-oxide, mannitol, and dimethylamine than light-flavor *Daqu* and more betaine/trimethylamine *N*-oxide, glucose, dimethylamine, and mannitol than sauce-flavor *Daqu*.

Sauce-flavor *Daqu* contains more lactate, pyroglutamate, leucine, valine, mannitol, alanine, choline/*O*-phosphocholine, and isoleucine than light-flavor *Daqu* and more lactate, leucine, choline/*O*-phosphocholine, isoleucine, valine, propylene glycerol/isopropanol, and pyroglutamate than strong-flavor *Daqu*.

Light-flavor *Daqu* contains more propylene glycerol/isopropanol, glycerol, and *N*-acetylglutamate than strong-flavor *Daqu* and more glucose, propylene glycerol/isopropanol, glycerol, and *N*-acetylglutamate than sauce-flavor *Daqu*.

These results confirm our PCA results to a large extent.

DISCUSSION

Daqu is not only a source of inoculum but also plays an important role during the fermentation as a substrate (*4*, *6*, *15*−*17*). It could be expected that several of the biomarkers found result from microbial metabolism. Other biomarkers could be related to specific process conditions, such as the higher incubation temperatures that are used in the production of strong- and sauce-flavor *Daqu*. Such heat conditions could lead to, for example, Maillard type and other reactions. At present, knowledge of the microbiota of *Daqu* is still far from complete and is the subject of current research. A few reports (in Chinese) based on classical microbiological research methods are available (*15*−*19*).

Glucose, glycerol, mannitol, and acetate were abundant in all tested types of *Daqu*, and this reflects an active metabolism of carbohydrates in *Daqu*. Glucose is formed by the direct degradation of cereal starch by amyloglucosidase, which is produced by fungi such as *Amylomyces* and *Rhizopus* spp. (*4*, *9*, *18*, *20*−*22*), and glycerol, mannitol, and acetate are products of microbial metabolism. Some sugars and polyols such as glycerol and mannitol are also compatible solutes accumulated by a range of fungi and bacteria. Mannitol is a sugar alcohol, derived by reduction from mannose, and can be formed through the reduction of fructose by mannitol dehydrogenase by many fungi (*23*). Mannitol is known as a microbial osmoprotectant, and it could have fulfilled a protective function during the storage of *Daqu*. It also functions as a carbohydrate storage compound or as a scavenger of reactive oxygen species (*24*).

By PCA, acetate was found to be one of the biomarkers of light-flavor *Daqu*. This corresponds well with the fact that the representative aroma compounds in light-flavor liquor are mainly ethyl acetate, in balance with considerable levels of ethyl lactate (*9*). This suggests that microorganisms producing acetate play an important role in this type of *Daqu*. However, lactate was not detected in light-flavor *Daqu*, so it could be expected that this is produced during the alcoholic fermentation stage, later in the process of liquor making.

In both sauce-flavor and strong-flavor *Daqu*, acetic acid bacteria and *Bacillus* spp. were found to be the predominant microbial community (*18*). *Bacillus* spp. are well-known producers of proteases and amylases. This may explain why most biomarkers of sauce-flavor *Daqu* are amino acids, for example, isoleucine, leucine, isovalerate, and valine. However, our finding that lactate was another biomarker of sauce-flavor *Daqu* might imply that lactic acid bacteria also play an important role, rather than acetic acid bacteria as reported previously (*18*). In *LN-Daqu* (strong-flavor *Daqu*), it was found that *Bacillus* spp. were the dominant bacteria, *Rhizopus* spp. and *Mucor* spp. were the dominant molds, and *Candida* spp. was the dominant yeast genus (*16*). This microbiota composition could help to explain the biomarkers of strong-flavor *Daqu*, such as mannitol, betaine, trimethylamine, and pyroglutamate.

To this day, the manufacturing process of *Daqu* still relies on workers' experience. The *Daqu* quality cannot be kept stable even for the same batch of products. *Daqu* making involves several complex fermentation stages. Up till now, no research has been undertaken to simulate the process and make a homemade sample for experiments. We believe that the microbiota would change according to the factory locations and therefore influence the metabolite profile. However, we tried to extract the common information for each flavor type of *Daqu* in this paper. Further study of microbial ecology during the production of *Daqu* and the ensuing alcoholic fermentation will be necessary. The nontargeted analysis of metabolites based on NMR could be useful in the control of flavor development during *Daqu* production. Nontargeted and multivariate analysis can also be useful in explaining the functionality of the microbiota that develops during the process stages of *Daqu* manufacture and its use as a starter ingredient in the fermentation of sorghum for the production of liquor.

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LITERATURE CITED

(1) Hesseltine, C. W.; Rogers, R.; Winarno, F. G. Microbiological studies on amylolytic oriental fermentation starters. *Mycopathologia* **1988**, *101*, 141–155.

(2) Fu, J.-Q. Chinese ancient liquor-making site and unearthed ancient wine culture. *Liquor-making Sci. Technol.* **2004**, *6*, 90−92 (in Chinese).

(3) Kang, M.-G. *Handbook of Liquor Industry*; China Light Industry Press: China, 2007 (in Chinese).

(4) Wang, C.-L.; Shi, D.-J.; Gong, G.-L. Microorganisms in *Daqu*: a starter culture of Chinese Maotai-flavour liquor. *World J. Microbiol. Biotechnol.* **2008**, *24*, 2183–2190.

(5) Bao, Q.-A. Recognition of Qu-nie. *Liquor-making Sci. Technol.* **2003**, *6*, 28−32 (in Chinese).

(6) Zhang, W.-X.; Qiao, Z.-W.; Tang, Y.-Q.; Hu, C.; Sun, Q.; Morimura, S.; Kida, K. Analysis of the fungal community in *Zaopei* during the production of Chinese Luzhou-flavour liquor. *J. Inst. Brew.* **2007**, *113*, 21–27.

(7) Xiong, Z.-S. Research on three flavour type liquors in China (1). Introduction to Luzhou-flavour liquor. *Liquor-making Sci. Technol.* **2005**, *2*, 22−25 (in Chinese).

(8) Xiong, Z.-S. Research on three flavour type liquors in China (2). Introduction to Maotai-flavour liquor. *Liquor-making Sci. Technol.* **2005**, *4*, 25−30 (in Chinese).

(9) Xiong, Z.-S. Research on three flavour type liquors in China (3) Fen flavour liquor - Xinghuacun. *Liquor-making Sci. Technol.* **2005**, *7*, 17−21 (in Chinese).

(10) Choi, H.-K.; Yoon, J.-Y.; Kim, Y.-S.; Kwon, D.-Y. Metabolomic profiling of Cheonggukjang during fermentation by ^{1}H NMR spectrometry and principal components analysis. *Process Biochem.* **2007**, *42*, 263–266.

(11) Yang, S. O.; Kim, M. S.; Liu, K. H.; Auh, J. H.; Kim, Y. S.; Kwon, D. Y.; Choi, H. K. Classification of fermented soybean paste during fermentation by ^{1}H nuclear magnetic resonance spectroscopy and principal component analysis. *Biosci., Biotechnol., Biochem.* **2009**, *73*, 502–507.

(12) Son, H.-S.; Kim, K.-M.; van Den Berg, F.; Hwang, G.-S.; Park, W.-M.; Lee, C.-H.; Hong, Y.-S. ^{1}H nuclear magnetic resonance-based metabolomic characterization of wines by grape varieties and production areas. *J. Agric. Food Chem.* **2008**, *56*, 8007–8016.

(13) Lachenmeier, D. W.; Frank, W.; Humpfer, E.; Schäfer, H.; Keller, S.; Mörtter, M.; Spraul, M. Quality control of beer using high-resolution nuclear magnetic resonance spectroscopy and multivariate analysis. *Eur. Food Res. Technol.* **2005**, *220* (2), 215–221.

(14) Lachenmeier, D. W.; Humpfer, E.; Fang, F.; Schütz, B.; Dvortsak, P.; Sproll, C.; Spraul, M. NMR-spectroscopy for nontargeted screening and simultaneous quantification of health-relevant compounds in foods: the example of melamine. *J. Agric. Food Chem.* **2009**, *57*, 7194–7199.
(15) Wu, X.-M. Separation, purification and identification of mold in *Xiaoqu*. *Liquor-making Sci. Technol.* **2004**, *6*, 33−34 (in Chinese).
(16) Yao, W.-C.; Tang, Y.-M.; Ren, D.-Q.; Liao, J.-M.; Shen, C.-H.; Xu, D.-F.; Ying, H.; Fan, L. Study on the differences of microbes in the different layers of Guojiao *Daqu*. *Liquor Making* **2005**, *32*, 35−37 (in Chinese).
(17) Zhou, H.-L.; Yu, B. Isolation of the microorganisms from Hengyang wine drug. *Liquor-making Sci. Technol.* **2007**, *2*, 46−47 (in Chinese).
(18) Shi, A.-H.; Guan, J.-K.; Zhang, W.-P.; Xu, E.-R.; Xu, C.-X. Analysis of microbial species in Xufang *Daqu* and identification of the dominant microbes. *Liquor-making Sci. Technol.* **2001**, *6*, 26−28 (in Chinese).
(19) Xu, C.-Y.; Liu, Q.-Y.; Guo, B.; Zhou, L.; Luo, G.-W.; Jin, P.-Z.; Shen, Y.-F.; Zhuge, J. Isolation of microbes from Xiaoqu for Chi-flavour type of Chinese spirits. *Food Fermentation Ind.* **2002**, *28*, 10−12 (in Chinese).
(20) Guo, H.-Z. Isolation and identification of *Monascus* in super-mature vinegar *Daqu*. *China Food Addit.* **2006**, *3*, 99−103 (in Chinese).
(21) Zhang, L. The analysis of microbes biology in middle temperature. *J. Sichuan Food Fermentation* **1999**, *4*, 21−23 (in Chinese).
(22) Yang, D.-Y.; Fan, G.-X.; Wang, D.-Q.; Lu, Y.-H. Microbes in high temperature starter. *Liquor-making Sci. Technol.* **2007**, *5*, 37−38 (in Chinese).
(23) Van Eck, J. H.; Prior, B. A.; Brandt, E. V. The water relations of growth and polyhydroxy alcohol production by ascomycetous yeasts. *J. Gen. Microbiol.* **1993**, *139*, 1047–1054.
(24) Voegele, R. T.; Hahn, M.; Lohaus, G.; Link, T.; Heiser, I.; Mendgen, K. Possible roles for mannitol and mannitol dehydrogenase in the biotrophic plant pathogen *Uromyces fabae*. *Plant Physiol.* **2005**, *137*, 190–198.

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