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Characteristics of traditional Chinese acidic rice soup (rice-acid) prepared with different fermentation methods

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ABSTRACT

Rice-acid, a Chinese traditional acidic rice soup (rice-acid), is widely accepted by consumers due to its unique flavor and anti-oxidation, anti-aging and immunity enhancement functions. This study confirmed that *L*-lactic acid and malic acid were the main organic acids in rice-acid. Low-temperature rice-acid samples produced by enterprises had the highest signal intensity of sour taste. The total content of free amino acids in different fermented rice-acid samples were in the range of 0.003–0.468 mg/g. 42 key volatile flavor compounds were identified in rice-acid. 8 volatile compounds with a higher contribution to the aroma of rice-acid were respectively acetic acid, 1-octen-3-ol, 2-heptanol, ethyl acetate, propyl propionate, hexanal, nonanal, and 2,3-butanedione. The interaction between lactic acid bacteria (3.00×10^3–7.02×10^6 CFU/mL) and yeasts (5.04×10^4–2.25×10^8 CFU/mL) affected the formation of taste and aroma components in rice-acid. The physicochemical characteristics including titratable acidity, pH, reducing sugars, amino acid nitrogen, gamma-aminobutyric acid showed significant differences between low-temperature fermentation samples and high-temperature fermentation samples. In addition, relationships linking all data through Pearson coefficient correlation were also reported. In summary, the study can be used to improve the quality of rice-acid products.

1. Introduction

Sour soup, as a unique traditional food of Miao and Dong nationalities, is popular in China. It can be classified into red sour soup and white sour soup according to fermentation raw materials. The latter is fermented with glutinous rice, rice or flour and also called rice-acid. Rice is one of the human's main foods and has rich nutrition components [1]. For example, rice bran polysaccharide can enhance the activity of antioxidants [2]. Moreover, fermentation can improve the functional properties and nutritional quality of rice. Rice-based fermented cereal products are widely accepted by consumers in the world, such as fermented rice vinegar, rice wine, and rice noodle [3]. Rice-acid as another rice-based fermented food has unique flavor components and various physiologically active substances. It is believed that rice-acid has the anti-fatigue, anti-aging and immunity functions and probiotic flora, so it can be used to adjust the intestinal micro-ecological balance and prevent digestive diseases [4].

In general, rice-acid is produced by spontaneous fermentation in a jar. Fig. 1 shows rice-acid production processes, including simple artisanal process and large-scale industrial process. After adding rice-acid produced by the first fermentation into rice soup, new rice-acid can be obtained after the second fermentation at 28–35 °C for 4–7 days. Rice-acid product is a milky or light yellow liquid and its bottom layer turns slightly turbid after the settlement. The main raw material of rice-acid is glutinous rice containing many polymer organic matters, minerals, dietary fibers and flavonoids. Interestingly, rice-acid is also a unique seasoning product obtained through the spontaneous fermentation process with lactic acid bacteria and

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yeasts. Yeast starters were better producers of volatile alcohols and bacterial starters of acid compounds [5]. Flavor substances produced by lactic acid bacteria mainly include organic acids, alcohols, esters, aldehydes, and ketones, such as ethanol, acetic acid, ethyl acetate, and 2,3-butanedione [6]. Cappello et al. [7] indicated that lactic acid bacteria in wine were strongly correlated with the generation of aroma components and flavors. Yeasts are the most studied and commonly used fermentation microbes in fermented foods due to their favorable contributions to the flavor. Yeast autolysis release sugars, proteins, amino acids, organic acids, enzymes, vitamins and inorganic salts, thus providing carbon and nitrogen sources for fermentation and promoting the growth of other microorganisms [8]. Yang et al. [9] screened the fermentation yeasts with the superior ethanol tolerance and fermentation activity in order to improve the flavor profiles of Chinese rice wine and assessed the F23 wine in terms of fruit aroma, alcohol aroma and taste. An important reason why people like rice-acid is due to its unique flavor, 3 types of rice-acid are available: low-temperature rice-acid produced by farmers, low-temperature rice-acid produced by enterprises and high-temperature rice-acid produced by farmers, but the flavor comparison of different types of rice-acid products has not been reported.

Rice-acid can be produced through low-temperature or high-temperature fermentation. During low-temperature fermentation, microorganisms grow at 5–15 °C. High-temperature fermentation is performed beside the stove, so the microorganism composition varies with the stove temperature from 35 °C to 80 °C. However, the previous studies on rice-acid only focused on the processing technology. Due to the differences in fermentation materials, methods and conditions, different types of rice-acid products have different tastes, aroma components and nutrients. In order to develop rice-acid products with excellent quality, it is necessary to further explore the flavor profiles and physicochemical characteristics of rice-acid produced by different fermentation methods.

Therefore, the study aims to determine the taste substances and aroma components of traditional Chinese fermented rice-acid with HPLC, electronic tongue and solid-phase micro-extraction-gas chromatography-mass spectrometry (SPME-GC-MS) assay and analyze the quality of rice-acid in different fermentation methods.

2. Materials and methods

2.1 Materials

Samples were obtained from 9 different production producers in 3 different regions and represented the main types of rice-acid produced by 3 different fermentation methods. The low-temperature and high-temperature fermentation samples were collected in February 2019 and December 2018, respectively. The preparation methods and these samples are shown in Table S1 and Fig. 1. M1, M2 and L1 were sampled from two rice-acid workshops of the most two famous rice-acid enterprises in Kaili (Guizhou Province, China). M1 and M2 were sampled from the same enterprise after different fermentation time. The low-temperature fermentation samples of H1, H2, H3 and H4 were respectively produced by 4 farmers from the same geographical region (Huangping, Guizhou Province). The high-temperature fermentation samples of D1, D2 and D3 were 3 homemade samples by farmers in the same geographical region (Congjiang, Guizhou Province). To determine the effect of the duration of the second fermentation on the characteristics (microbial community and flavor) of rice-acid, M1 and M2 were respectively sampled after 7-days and 6-days fermentation. The samples were produced locally by individuals (farmers) or generated via a standardized process (enterprises) and contained different unique flavors due to different fermentation temperatures, time and raw materials.

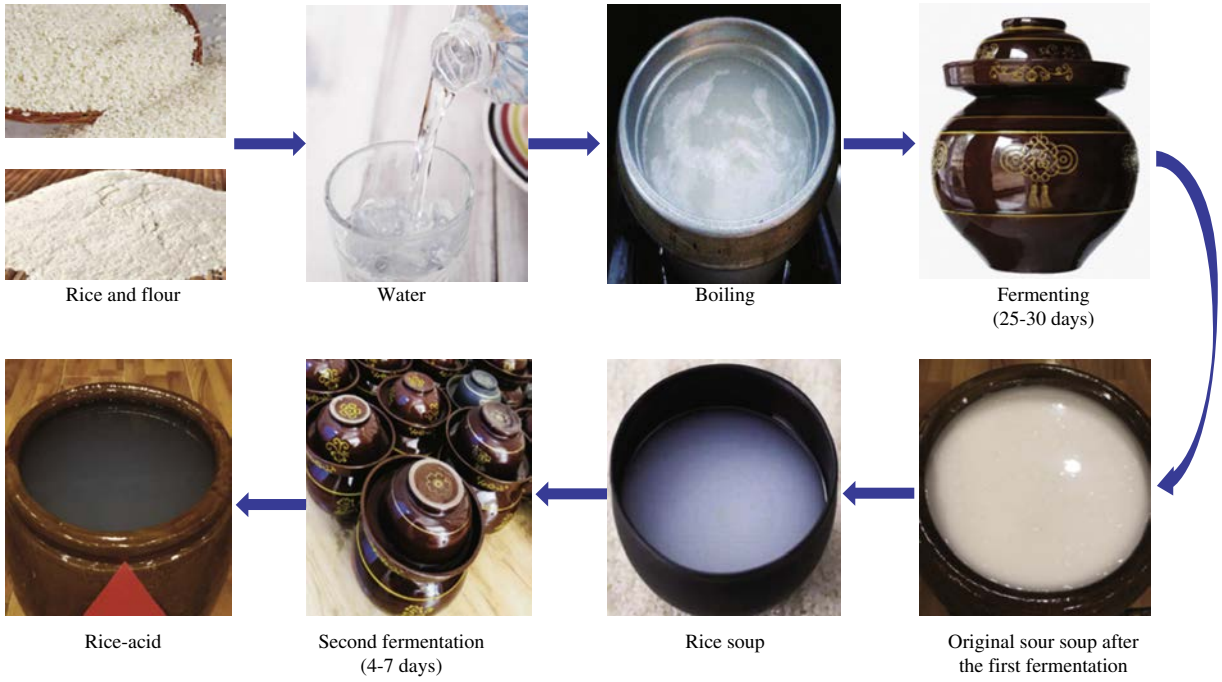

Fig. 1 Rice-acid processing process.

Lactobacilli MRS agar (MRS) and YPD agar medium (YPD) media were obtained from Bio-way Biotechnology Co., Ltd. (Shanghai, China). Other chemicals were purchased from Sigma Aldrich Co., Ltd. (USA).

2.2 Determination of organic acids

The samples of rice-acid were filtered with double-layer filter paper (Medium Speed, Taizhou Aoke Inc., China). The obtained filtrate was filtered through 0.22-μm microporous membrane (13 mm in diameter, Tianjin Linghang Co., Ltd., China) and then passed through a ZORBAX SB-AQ column (4.6 mm × 250 mm, 5 μm, American Agilent Corporation) solid phase cartridge for HPLC analysis with HPLC (Agilent 1260 Infinity, Agilent Corporation, USA) (equipped with G1329B autosampler, G1311C quaternary low pressure ladder, G1316A column oven, and G1315D diode array UV-visible light detector) under the conditions of the mobile phase of 0.02 mol/L NaH_2PO_4 (pH 2.7), the injection volume of 10 μL, flow rate of 0.9 mL/min, column temperature of 35 °C, and detector wavenumber (UV) of 210 nm. Lactic acid (*L*-lactic acid), malic acid, citric acid, acetic acid, and tartaric acid in rice-acid samples were determined. The concentration of *L*-lactic acid was measured with the Amplite™ Colorimetric *L*-Lactate Assay Kit (AAT Bioquest Inc. USA) and the purity of *L*-lactic acid was measured according to the method of Moon et al. [10]. Firstly, 0.5, 1.0, 2.0, 5.0, and 10.0 mL of organic acid standards (oxalic acid, malic acid, citric acid, acetic acid, and tartaric acid) were respectively taken, then diluted to a volume of 25 mL in a volumetric flask with ultrapure water, and then filtered through 0.22-μm aqueous phase membrane to obtain organic acids with different concentrations. With the peak areas and concentrations of acid standard solutions, a standard curve was drawn to obtain the linear range regression equation and the concentration of each organic acid in rice-acid was calculated.

2.3 Determination of free amino acids

Free amino acids were determined according to the method by Dominguez et al. [11]. The sample solution was prepared according to the following procedure. Firstly, 8 mL of rice-acid sample was transferred into a centrifuge tube and centrifuged at 3 000 r/min for 5 min. Then, 1 mL of the supernatant was transferred into another centrifuge tube. Then, 9 mL of 2% sulfosalicylic acid was added. The mixture solution was mixed well, stood for 15 min, and centrifuged at 10 000 × *g* for 10 min. Then the supernatant was acquired and filtered through 0.22-μm membrane. L-8800 automatic amino acid analyzer was used to determine the contents of 17 free amino acids in rice-acid. The determination conditions were set as follows: LCAK06/Na (4.6 mm × 150 mm) as the column, 0.12 mol/L sodium citrate solution (pH = 3.45) as mobile phase A, 0.2 mol/L sodium citrate solution (pH = 10.85) as mobile phase B, column temperature was gradient temperature control between 58 °C and 74 °C, reactor temperature was 130 °C, elution rate was 0.45 mL/min, flow rate of derivative pump was 0.25 mL/min, and detection wavelength was 570 nm (only the detection wavelength of Proline was 440 nm). Each free amino acid in the samples to be tested was determined based on the elution spectrum and the standard curve obtained with the amino acid standard solution under the same conditions.

2.4 Determination of taste characteristics

Rice-acid filtrate (8 mL) was diluted to the volume of 80 mL for the analysis with electronic tongue (Insent SA-402B, Atsugi-chi, Japan) according to the following method. The electronic sensor was firstly cleaned in the cleaning solution for 90 s. Then the first reference solution was added to clean the sensor for 120 s. Then, the second reference solution was added to clean the sensor for 120 s. After cleaning, the sensor was zeroed at the equilibrium position for 30 s and then entered the sample cup for 30-s test. After each test, the sensor was sequentially washed in two reference solutions for 3 s. The same sample was tested for 4 times. After excluding the first repeated data, the average of the remaining 3 data was used in the subsequent analysis.

2.5 Determination of volatile flavor compounds

Aroma compounds in samples were determined with SPME-GC-MS. HP6890/5975C GC-MS (Agilent, USA) and manual solid phase microextraction device (Supelco, USA) were used in the determination of volatile flavor compounds (VFCs).

SPME was performed according to the following procedure. The mixed sample (10 g) was placed in a 50-mL SPME sample bottle and a manual injector equipped with 2 cm-50/30 μm DVB/CAR/PDMS StableFlex was inserted. After the plate was heated at 60 °C for 50 min, the extraction head was removed, immediately inserted into the gas chromatograph inlet (250 °C) and thermally decomposed for 6 min.

GC-MS analysis was performed according to the following procedure. The analysis of GC was performed with an FB-5MS elastic quartz capillary column (30 m × 0.25 mm × 0.25 μm). The column temperature was firstly maintained at 40 °C for 4 min. Then the temperature was gradually raised to 163 °C at a rate of 3 °C/min and then raised to 251 °C at a rate of 8 °C/min. Running time was 56 min. The injector temperature was set at 250 °C. The carrier gas was high-purity He (99.999%). The pre-column pressure was 48.75 kPa. The flow rate of the carrier gas was 1.0 mL/min and the splitless injection mode was adopted. Solvent delay time was 1 min and the ion source was EI source at 230 °C. The quadrupole temperature was 150 °C. Electron energy was 70 eV and emission current was 34.6 μA. Multiplier voltage was 1 847 V. Interface temperature was 280 °C and the mass range was 29–500 U. The peaks in the total ion flow map were retrieved by the mass spectrometer computer data system and identified through the comparison with reference mass spectra in the Nist14 and Wiley275 databases. Finally, VFCs were determined and the relative mass fraction of each chemical component was determined with the peak area normalization method.

The relative odor activity value (ROAV) index was measured according to the method by Zhang et al. [12].

$$\mathrm{ROAV}_i = 100 \times \frac{C_i}{C_{max}} \times \frac{T_{max}}{T_i} \quad (1)$$

where C_i and T_i are respectively the percentage and threshold of a flavor component; C_{max} and T_{max} are respectively the highest percentage of the volatile component and the corresponding threshold.

2.6 Determination of physicochemical and microbiological indicators

Titratable acidity/total acid was determined with the previous method by Cao et al. [13]. The pH was determined with a pH meter (Testo 205, Titisee-Neustadt, Germany). Amino acid nitrogen was determined with Chinese National Food Safety Standard GB/T 5009.235-2016 (2016) [14]. The reducing sugar was measured with DNS method (3,5-dinitrosalicylic acid method) with some revisions based on the method of Saqib and Whitney [15]. Firstly, glucose standard solutions were prepared to draw the standard curve according to the method of Chen [16]. Then, 1 mL of sample was transferred to a 100-mL volumetric flask. Then, 1 mL of rice-acid sample, 1 mL of distilled water and 1.5 mL of the DNS reagent was added in a test tube. The test tube was heated in a boiling water bath for 5 min and immediately cooled with cold water. Then the solution was diluted with distilled water to a volume of 25 mL and shaken to determine the absorbance at 520 nm.

The microbes in samples were cultured according to the previous method by Yang et al. [17] with some modifications based our samples. Firstly, a sample (5 g) was mixed with 45 mL of 0.85% (*m*/*V*) NaCl solution and shaken at 150 r/min for 30 min at room temperature. Then, the mixed solution was serially diluted (10^3, 10^4, and 10^5 times) with 0.85% (*m*/*V*) NaCl solution and spread on MRS solid medium and YPD agar medium. Lactic acid bacteria and yeasts were cultured in constant-temperature incubators at 37 °C and 30 °C for 48 h and 72 h, respectively.

Sensory indicators were evaluated according to the following procedure. According to the method of Geng et al. [18], a panel composed of 10 members who regularly ate rice-acid participated in the sensory evaluation and these members should rinse their mouths with room-temperature purified water. The samples of rice-acid were randomly numbered for sensory evaluation in terms of flavor, color, tissue morphology, and taste (Table S2).

2.7 Determination of gamma-aminobutyric acid (GABA)

The content of GABA was determined with the method by Xu et al. [19].

2.8 Data analysis

Experimental data were processed in SPSS20.0 and Origin Pro 2018. All the analyses were carried out in triplicate and data were reported as means ± standard deviation (SD). Duncan's multiple range test and *t*-test were carried out to analyze significant differences in SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant and $P < 0.01$ was considered to be extremely significant.

3. Results and discussion

3.1 Effects of different fermentation methods on organic acids

Organic acids significantly affect the balance of flavor, chemical stability, pH and quality in fermented foods [20]. Six organic acid standards were used to determine their retention time (Fig. S1). The concentrations of 6 organic acids were the highest in the sample M1 and the lowest in the sample H3 and the concentrations of organic acids were different between enterprises' and farmers' rice-acid (Fig. 2). Lactic acid (mainly *L*-lactic acid) was the main organic acid in rice-acid (29.39%–73.33% of the total acid), followed by malic acid, acetic acid, citric acid and oxalic acid and the concentration of tartaric acid was the lowest. The organic acid composition showed no significant difference among the 3 rice-acid samples produced by enterprises (L1, M1, and M2) ($P > 0.05$). *L*-lactic acid was produced by lactic acid bacteria. However, the concentrations of lactic acid in the two samples (H3 and D2) were low probably due to the interaction between lactic acid bacteria and yeasts in their unique fermentation conditions. The number and type of microorganisms in the fermentation environment might have a significant effect on the contents of organic acids. It was consistent with the report by Vimercati et al. [21].

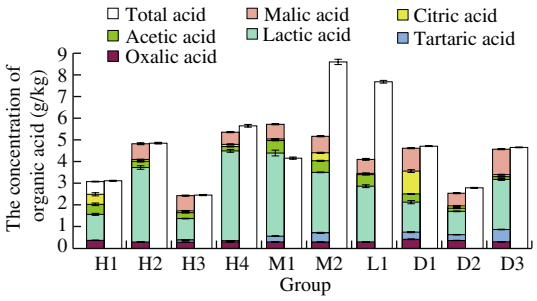

Fig. 2 The concentration of organic acid in different low-temperature rice-acid (H1, H2, H3, H4, L1, M1 and M2) and high-temperature rice-acid sample (D1, D2 and D3). Results are expressed as mean ± standard deviation.

The production of organic acids provided an acidic environment, which was not conducive to the growth of many spoilage microorganisms [22]. Malic acid has a stimulating and refreshing sour taste and its metabolism facilitates amino acid absorption and prevents fat accumulation. In addition, malic acid can be ascribed to malolactic fermentation, in which malic acid was converted to lactic acid. Acetic acid widely exists in fruits and vegetable oil, the content of acetic acid in all rice-acid samples was low since acetic acid mainly existed in the form of esters. Citric acid can produce the acid for rice-acid and prevent the oxidation caused by enzyme catalysis and metal catalysis as well as the discoloration and deterioration of potatoes [23]. Glucosidase, esterase and protease produced by lactic acid bacteria had a close relationship with citrate metabolism. Although only a low content of tartaric acid existed in rice-acid, it played the indispensable role in the flavor of rice-acid. Interestingly, the concentration of lactic acid was the highest in rice-acid, thus it might lead to the mild acidity of rice-acid. It is worth mentioning that *L*-lactic acid can form poly(*L*-lactic acid), which can be combined with functional additives to improve mechanical and biological properties for cardiovascular implant applications [24]. *L*-lactic acid-based rice-acid products will be further developed in the future.

3.2 Effects of different fermentation methods on free amino acids

Amino acids in foods can be roughly divided into two parts: non-free amino acids and free amino acids. Non-free amino acids cannot be immediately hydrolyzed and do not contribute much to the taste of foods. Therefore, it is necessary to determine the composition and content of free amino acids in the study on the contribution of amino acids to the taste of foods. Free amino acids were classified

Table 1
The average distribution (%) of free amino acids (savory sour, sweet savory, bitter sweet, bitter, salty) in different low-temperature rice-acid (H1, H2, H3, H4, L1, M1 and M2) and high-temperature rice-acid sample (D1, D2 and D3).

Taste	H1	H2	H3	H4	L1	M1	M2	D1	D2	D3
Savory, Sour	26.51	30.25	20.00	51.76	0	0	0	23.91	15.30	17.40
Sweet, Savory	16.27	36.48	80.00	30.63	33.33	100	43.75	33.70	40.30	44.03
Bitter, Sweet	43.98	13.99	0	8.10	66.7	0	31.25	43.48	52.23	2.73
Bitter	13.25	19.09	0	9.51	0	0	25	38.04	39.17	35.84
Salty	0	0.19	0	0	0	0	0	0	0	0

into 5 categories and the compositions of free amino acids of different samples were different (Table 1, Fig. S2). The total content of savory and sour amino acids in the sample H4 was the highest, accounting for 51.7%. The samples H2 and H3 had the highest contents of sweet and savory amino acids (80% and 36.48%) and the sample H1 had the strongest bitter sweet taste. The highest contents of sweet and savory amino acids were detected in low-temperature rice-acid samples produced by enterprises, whereas the highest contents of bitter and sweet amino acids were detected in high-temperature rice-acid samples produced by farmers.

The total contents of free amino acids in 10 rice-acid samples were between 0.003 mg/g and 0.529 mg/g (Table 2). The taste difference was caused by the hydrolysis of cereal proteins [25]. Sixteen amino acids were detected and their contents decreased according to the following order: glutamic acid (Glu) > alanine (Ala) > glycine (Gly) > aspartic acid (Asp) > lysine (Lys) > valine (Val) > leucine (Leu) > serine (Ser) > tyrosine (Tyr) > methionine (Met) > threonine (Thr) > histidine (His) > arginine (Arg) > proline (Pro) > isoleucine (Ile) > cysteine (Cys). These free amino acids existed in rice-acid due to the actions of microbial activity and protein hydrolysis, but glutamine (Gln) or asparagine (Asn) was not detected in any sample. Although the contents of free amino acids were low, the contents of various amino acids showed significant differences among rice-acid samples produced by different fermentation methods ($P < 0.05$). In the low-temperature samples produced by farmers (H4 and H2), the contents of Glu were the highest, accounting for 34.15% and 15.31% of total amino acids. The contents of Glu in the samples H3, L1, M1, and M2 were low, because the degree of metabolism of Glu exceeded the degree of proteolysis and glutamate might be metabolized into other acids [26]. The contents of essential amino acids (EAA) were 0.002 mg/g to 0.178 mg/g, accounting for about half of the total amino acid content. The contents of non-essential amino acids (NEAA) were 0.001 mg/g to 0.290 mg/g. Enterprises' samples (M1, M2 and L1) contained the high percentage of sweet and savory component, but the content of sweet and savory component was low. The percentage of bitter amino acids in D2 was the highest and the bitterness might be ascribed to the uncoordinated proteolysis during rice-acid fermentation. The samples D2 and D3 had the same concentration of bitter amino acids, indicating that the high temperature affected the composition of amino acids. Tyr in bitter amino acids could enhance the umami taste of foods [27]. The contents of salty amino acid in the 10 samples were the lowest, indicating that salty amino acids might slightly contribute to the difference in the flavor of rice-acid. Notably, the content of raw materials (rice and flour) in rice-acid was only 3%–8%. This may be the reason for the low amino acid content and needs to be verified in future experiments.

3.3 Effects of different fermentation methods on volatile compounds

Eight chemical classes of volatile substances were detected in 10 rice-acid samples (Fig. 3 and Fig. S3). The total number of the volatile flavor substances was 42. These compounds exhibited different characteristic aroma and worked together to form a unique aroma of rice-acid. There were 3 acids, 13 alcohols, 4 esters, 6 aldehydes, 6 ketones, 7 alkanes, 2 alkenes and 1 polyphenol. Flavor perception is related to the concentrations and thresholds of volatile compounds. Relative odor activity value (ROAV) > 1 means the higher contribution to the flavor (Table 3). Eight volatile compounds with the higher contribution to rice-acid included acetic acid, 1-octen-3-ol, 2-heptanol, ethyl acetate, propyl propionate, hexanal, nonanal, and 2,3-butanedione. Four aroma compounds (acetic acid, ethanol, 1-propanol, and ethyl acetate) existed in the 10 samples and had been previously identified in fermented condiments. Eleven common aroma compounds existed in enterprises' samples. Thirty-four common aroma compounds existed in enterprises' samples (M1 and M2) and 16 common aroma compounds existed in high-temperature rice-acid samples. Eight common aroma compounds existed in low-temperature rice-acid samples produced by farmers. Aroma compounds showed significant differences among rice-acid samples produced by different fermentation methods ($P < 0.01$). The quantity of aroma compounds in low-temperature rice-acid was more than that in high-temperature rice-acid. Alcohols, esters and ketones were the key aromatic components in rice-acid. In addition, 85 compounds could be the non-shared potentially aroma compounds (Table S3). These aroma compounds showed significant differences between rice-acid and red sour soup [28]. It could be inferred that the different aroma compounds of fermented foods could be used to distinguish different products.

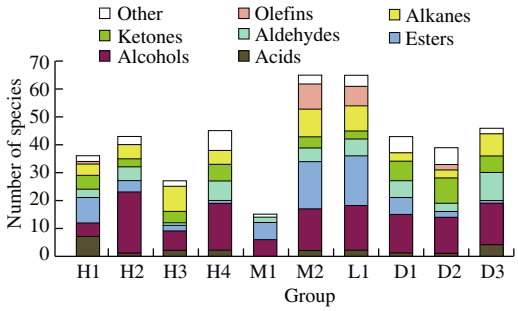

Fig. 3 Composition of volatile components: acid, alcohol, esters, aldehydes, ketones, alkanes, olefins and other in different low-temperature rice-acid (H1, H2, H3, H4, L1, M1 and M2) and high-temperature rice-acid sample (D1, D2 and D3). Results are expressed as mean.

Table 2
Analysis of free amino acid composition (mg/g) of rice-acid under different low-temperature rice-acid (H1, H2, H3, H4, L1, M1 and M2) and high-temperature rice-acid sample (D1, D2 and D3).

Taste	Free amino acid	H1	H2	H3	H4	L1	M1	M2	D1	D2	D3	Average value
Savory Sour	Glu	0.044 ± 0.002^{c}	0.081 ± 0.003^{b}	< 0.001^{f}	0.097 ± 0.002^{a}	< 0.001^{f}	< 0.001^{f}	< 0.001^{f}	0.015 ± 0.001^{d}	0.010 ± 0.001^{e}	0.041 ± 0.002^{c}	0.029 ± 0.004
	Asp	< 0.001^{f}	0.079 ± 0.005^{a}	0.002 ± 0.001^{d}	0.050 ± 0.002^{b}	< 0.001^{f}	< 0.001^{f}	< 0.001^{f}	0.007 ± 0.001^{a}	0.031 ± 0.001^{c}	0.010 ± 0.001^{e}	0.018 ± 0.003
	Gln	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND
	Asn	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND
	Total	0.044 ± 0.002^{d}	0.16 ± 0.004^{a}	0.002 ± 0.001^{e}	0.147 ± 0.006^{b}	< 0.001^{f}	< 0.001^{f}	< 0.001^{f}	0.022 ± 0.002^{e}	0.041 ± 0.002^{d,e}	0.051 ± 0.003^{c}	0.047 ± 0.006
Sweet Savory	Thr	< 0.001^{f}	0.01 ± 0.001^{e}	< 0.001^{f}	0.008 ± 0.003^{b}	< 0.001^{f}	< 0.001^{f}	< 0.001^{f}	0.006 ± 0.001^{c}	0.004 ± 0.001^{d}	0.009 ± 0.002^{a}	0.004 ± 0.005
	Ser	< 0.001^{d}	0.022 ± 0.004^{a}	< 0.001^{d}	0.018 ± 0.001^{b}	< 0.001^{d}	< 0.001^{d}	< 0.001^{d}	0.004 ± 0.001^{c}	0.005 ± 0.001^{c}	0.023 ± 0.002^{a}	0.007 ± 0.004
	Gly	0.016 ± 0.003^{d}	0.081 ± 0.006^{a}	0.001 ± 0.000^{e}	0.016 ± 0.003^{d}	< 0.001^{f}	0.002 ± 0.001^{c}	0.002 ± 0.001^{c}	0.01 ± 0.001^{e}	0.025 ± 0.007^{b,c}	0.03 ± 0.005^{b}	0.018 ± 0.002
	Ala	0.011 ± 0.006^{d}	0.08 ± 0.004^{a}	0.007 ± 0.001^{e}	0.042 ± 0.003^{c}	0.001 ± 0.000^{f,g}	0.002 ± 0.001^{f,g}	0.005 ± 0.001^{f}	0.011 ± 0.004^{e}	0.061 ± 0.003^{b}	0.057 ± 0.004^{b}	0.028 ± 0.003
	Pro	< 0.001^{c}	< 0.001^{c}	< 0.001^{c}	0.003 ± 0.001^{b}	< 0.001^{c}	< 0.001^{c}	< 0.001^{c}	< 0.001^{c}	0.013 ± 0.002^{a}	0.01 ± 0.002^{a}	0.003 ± 0.004
	Total	0.027 ± 0.002^{e}	0.193 ± 0.009^{a}	0.008 ± 0.001^{f}	0.087 ± 0.002^{c}	0.001 ± 0.001^{h}	0.004 ± 0.002^{g}	0.007 ± 0.002^{g}	0.031 ± 0.004^{d}	0.108 ± 0.008^{b,c}	0.129 ± 0.009^{b}	0.060 ± 0.004
Bitter Sweet	His	< 0.001^{f}	0.014 ± 0.002^{a}	< 0.001^{e}	0.003 ± 0.001 ^{c}	0.002 ± 0.001^{d}	< 0.001^{f}	0.002 ± 0.001^{d}	0.002 ± 0.001^{d}	0.004 ± 0.001^{c}	0.008 ± 0.002^{b}	0.004 ± 0.003
	Lys	0.058 ± 0.004^{a}	0.06 ± 0.006^{a}	< 0.001^{c}	0.02 ± 0.003^{b}	< 0.001^{c}	< 0.001^{c}	< 0.001^{c}	0.002 ± 0.001^{b}	< 0.001^{c}	< 0.001^{c}	0.014 ± 0.003
	Arg	0.015 ± 0.002^{a}	< 0.001^{d}	< 0.001^{d}	< 0.001^{d}	< 0.001^{d}	< 0.001^{d}	0.003 ± 0.001^{c}	< 0.001^{d}	0.01 ± 0.005^{b}	< 0.001^{d}	0.003 ± 0.002
	Total	0.073 ± 0.003^{a}	0.074 ± 0.0045^{a}	< 0.001^{f}	0.023 ± 0.002^{b}	0.002 ± 0.001^{e}	< 0.001^{f}	0.005 ± 0.001^{e}	0.004 ± 0.001^{e}	0.014 ± 0.004^{c}	0.008 ± 0.002^{d}	0.020 ± 0.001
Bitter	Val	0.01 ± 0.001^{c}	0.021 ± 0.002^{b}	< 0.001^{e}	0.004 ± 0.001^{d}	< 0.001^{e}	< 0.001^{e}	< 0.001^{e}	0.011 ± 0.004^{c}	0.023 ± 0.005^{b}	0.07 ± 0.002^{a}	0.014 ± 0.001
	Met	0.004 ± 0.001^{c}	0.015 ± 0.005^{a}	< 0.001^{e}	0.006 ± 0.001^{b}	< 0.001^{e}	< 0.001^{e}	0.004 ± 0.001^{c}	0.003 ± 0.001^{c}	0.004 ± 0.001^{c}	0.001 ± 0.001^{d}	0.004 ± 0.008
	Ile	< 0.001^{d}	0.007 ± 0.001^{b}	< 0.001^{d}	< 0.001^{d}	< 0.001^{d}	< 0.001^{d}	< 0.001^{d}	0.003 ± 0.001^{c}	0.010 ± 0.004^{a}	0.004 ± 0.001^{c}	0.002 ± 0.001
	Leu	0.008 ± 0.001^{c}	0.031 ± 0.003^{a}	< 0.001^{d}	0.008 ± 0.001^{c}	< 0.001^{d}	< 0.001^{d}	< 0.001^{d}	0.01 ± 0.001^{b}	0.028 ± 0.00^{a}	0.018 ± 0.003^{b}	0.010 ± 0.002
	Tyr	< 0.001^{e}	0.027 ± 0.003^{a}	< 0.001^{e}	0.009 ± 0.001^{c}	< 0.001^{e}	< 0.001^{e}	< 0.001^{e}	0.008 ± 0.001^{c}	0.004 ± 0.001^{d}	0.012 ± 0.005^{b}	0.006 ± 0.001
	Total	0.022 ± 0.002^{c}	0.101 ± 0.001^{a}	< 0.001^{e}	0.027 ± 0.002^{c}	< 0.001^{e}	< 0.001^{e}	0.004 ± 0.001^{d}	0.035 ± 0.003^{b}	0.105 ± 0.006^{a}	0.105 ± 0.007^{a}	0.274 ± 0.009
Salty	Cys	< 0.001^{b}	0.001 ± 0.001^{a}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001
	Total	< 0.001^{b}	0.001 ± 0.001^{a}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001^{b}	< 0.001
Total amino acids		0.166 ± 0.004^{b,c}	0.529 ± 0.006^{a}	0.010 ± 0.001^{e}	0.284 ± 0.004^{c}	0.003 ± 0.001^{f}	0.004 ± 0.001^{f}	0.016 ± 0.004^{e}	0.092 ± 0.004^{d}	0.268 ± 0.008b	0.293 ± 0.009^{b}	0.401 ± 0.004
EAA		0.080^{c}	0.178^{a}	< 0.001^{h}	0.055^{d}	0.002^{g}	< 0.001^{h}	0.006^{f}	0.045^{e}	0.088^{c}	0.120^{b}	0.057
NEAA		0.042^{c}	0.290^{a}	0.010^{d}	0.138^{b}	0.001^{e}	0.004^{d}	0.010^{d}	0.040^{c}	0.149^{b}	0.142^{b}	0.083

Note: ND: not detected. Results are expressed as mean ± standard deviation, three repetitions. $^{a–h}$ Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3
ROAV and the relative content (%) of aroma compounds of different fermented low-temperature rice-acid (H1, H2, H3, H4, L1, M1 and M2) and high-temperature rice-acid sample (D1, D2 and D3).

aroma compounds	Threshold	RI	H1		H2		H3		H4		L1		M1		M2		D1		D2		D3	
			%	ROAV	%	ROAV	%	ROAV	%	ROAV	%	ROAV	%	ROAV	%	ROAV	%	ROAV	%	ROAV	%	ROAV
Acids (3)																						
Acetic acid	10	610	7.28	1.18	3.45	0.02	11.14	0.20	5.45	0.02	< 0.01	< 0.01	5.93	0.58	5.48	0.44	11.06	0.40	3.46	0.01	4.77	0.05
Propanoic acid	2.19	700	19.59	14.38	–	–	15.18	1.23	–	–	< 0.01	< 0.01	4.97	2.23	6.30	2.28	–	–	–	–	–	–
Pentanoic acid	3	903	36.64	19.63	0.001	< 0.01	0.001	< 0.01	0.001	< 0.01	< 0.01	< 0.01	–	–	–	–	–	–	–	–	–	–
Alcohol (13)																						
Ethanol	3.5	427	0.02	< 0.01	9.84	0.15	3.49	0.18	3.30	0.03	23.84	0.54	1.75	0.49	0.88	0.20	2.48	0.26	0.95	0.01	3.36	0.11
1-Propanol	600	555	< 0.01	< 0.01	0.53	< 0.01	28.55	< 0.01	3.30	< 0.01	23.84	< 0.01	1.75	< 0.01	0.88	< 0.01	2.48	< 0.01	0.95	< 0.01	3.36	< 0.01
2-Butanone	N	598					12.83	N	0.16	N	16.59	N	23.19	N	< 0.01	N	32.34	N	0.25	N	0.06	N
1-Hexanol	0.820 5	868	–	–	21.68	1.43	0.79	0.17	21.53	0.90	–	–	0.24	0.29	0.14	0.14	4.42	1.97	1.76	0.09	14.36	2.01
2-Heptanol	0.002 8	901	–	–	1.17	22.56	–	–	0.24	2.93	–	–	0.15	52.55	0.09	25.52	0.48	62.57	6.67	100	2.33	95.65
1-Octen-3-ol	0.01	980	–	–	18.38	99.25	0.52	9.20	29.24	100	–	–	0.12	11.78	0.06	4.76	0.87	31.76	–	–	8.25	94.83
2-Ethylhexanol	0.3	1 030	–	–	–	–	–	–	–	–	3.20	0.84	0.71	2.32	0.75	1.99	–	–	–	–	–	–
1-Octanol	0.13	1 071	–	–	6.60	2.74	0.49	0.67	1.92	0.51	–	–	0.13	0.98	0.41	2.50	0.81	2.27	4.59	1.49	2.79	2.47
1-Nonen-4-ol	N	1 103	–	–	–	–	–	–	–	–	–	–	0.51	N	0.46	N	–	–	–	–	2.73	N
Benzeneethanol	N	1 116	–	–	4.00	N	–	–	0.19	N	–	–	–	–	–	–	1.04	N	0.24	N	2.42	N
4-Terpineol	N	1 182	–	–	1.01	N	–	–	–	–	–	–	1.04	N	1.78	N	–	–	–	–	–	–
α-Terpineol	N	1 189	–	–	–	–	–	–	–	–	–	–	1.71	N	1.72	N	0.14	N	–	–	0.24	N
2-Undecanol	0.008 6	1 308	0.17	31.78	–	–	–	–	–	–	–	–	0.18	20.53	0.10	9.23	–	–	–	–	–	–
Esters (4)																						
Ethyl acetate	0.005	612	0.17	54.67	0.37	4	0.50	17.7	0.04	0.27	6.33	100	0.51	100	0.30	47.65	1.37	100	0.87	7.31	0.49	11.26
Propyl acetate	2.7	708	1.30	0.77	–	–	–	–	–	–	7.98	0.23	14.72	5.35	13.26	3.40	9.97	1.35	–	–	–	–
Propyl propionate	0.44	807	4.67	17.06	–	–	0.31	0.12	–	–	2.58	0.46	17.82	39.73	18.98	34.25	6.03	5.00	–	–	–	–
Propyl caproate	N	1094	0.31	N	–	–	–	–	–	–	–	–	0.05	N	0.14	N	–	–	–	–	–	–
Aldehyde (6)																						
Hexanal	0.005	800	–	–	0.38	4.10	–	–	1.67	11.42	–	–	0.21	41.20	0.24	38.12	–	–	0.12	1.01	4.35	100
Heptanal	0.005	901	–	–	0.12	1.30	–	–	0.40	2.74	–	–	0.05	9.81	0.18	28.59	0.22	16.06	–	–	0.94	21.61
Benzaldehyde	1	962	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06	0.02	0.85	0.04	0.54	0.06
Octanal	0.35	1 003	1.22	5.60	–	–	–	–	2.06	0.20	1.03	0.23	0.14	0.39	2.25	5.10	1.14	1.19	–	–	3.83	9.14
Nonanal	0.045 5	1 104	2.83	100	–	–	1.42	5.52	4.47	3.36	11.16	19.38	–	–	5.73	100	6.53	52.38	–	–	8.64	21.83
Decanal	0.045 5	1 206	0.19	6.71	–	–	–	–	–	–	–	–	0.14	3.02	0.11	1.92	0.23	1.85	–	–	–	–
Ketones (6)																						
2,3-Butanedione	0.006	595	–	–	–	–	3.39	100	–	–	–	–	–	–	–	–	0.07	4.26	0.04	0.28	5.03	
2-Butanone	N	598	0.60	N	–	–	9.59	N	–	–	–	–	0.02	N	0.01	N	0.85	N	0.02	N	–	–
2-Pentanone	1.38	685	0.05	0.06	0.11	< 0.01	–	–	0.44	0.01	–	–	–	–	–	–	0.28	0.07	0.38	0.01	0.23	0.02
2-Heptanone	0.14	891	0.32	3.67	0.46	0.18	–	–	2.56	0.63	–	–	–	–	–	–	3.09	8.06	17.09	5.13	4.64	3.81
4-Methyl-2-heptanone	N	943	–	–	–	–	0.43	N	–	–	–	–	0.04	N	0.04	N	0.33	N	0.29	N	–	–
2-Octanone	0.041	990	–	–	–	–	0.64	2.76	1.59	1.33	–	–	–	–	–	–	0.52	4.63	25.46	26.08	1.56	4.37
Alkanes (7)																						
2,4-Dimethylheptane	50	821	0.38	0.01	0.05	< 0.01	1.29	< 0.01	0.24	< 0.01	–	–	0.09	< 0.01	0.18	< 0.01	–	–	0.06	< 0.01	1.08	< 0.01
4-Methyloctane	N	863	0.10	N	–	–	0.55	N	0.19	N	–	–	0.06	N	0.15	N	–	–	–	–	0.85	N
2-Methylnonane	10	964	–	–	–	–	0.21	< 0.01	–	–	–	–	0.05	< 0.01	0.06	< 0.01	–	–	–	–	–	–
Decane	10	1 000	–	–	–	–	0.43	< 0.01	–	–	–	–	0.10	0.01	0.16	0.01	–	–	–	–	–	–
Dodecane	10	1 200	–	–	0.48	< 0.01	0.29	< 0.01	0.32	< 0.01	–	–	0.10	0.01	0.07	< 0.01	0.30	0.01	–	–	0.31	< 0.01
Tetradecane	0.06	1 400	0.09	2.41	0.53	0.48	0.62	1.83	0.34	0.20	–	–	0.08	1.31	0.07	0.93	0.22	1.34	0.05	0.04	0.15	0.29
Pentadecane	3.6	1 500	0.13	0.06	0.14	< 0.01	–	–	–	–	–	–	0.03	0.01	0.05	0.01	–	–	0.05	< 0.01	0.40	0.01
Alkenes (2)																						
1,3-Di-tert-butylbenzene	7	1 247	1.41	0.32	1.44	0.01	3.47	0.09	2.18	0.01	–	–	1.49	0.21	1.44	0.16	1.67	0.09	0.56	< 0.01	3.61	
α-Cedrene	N	1 411	0.14	N	–	–	–	–	–	–	–	–	0.04	N	0.04	N	–	–	0.03	N	–	–
Polyphenols (1)																						
2,4-Di-tert-butylphenol	0.7	1 519	0.03	0.07	0.55	0.04	–	–	0.30	0.01	0.51	0.06	0.06	0.08	0.04	0.05	0.29	0.15	0.10	0.01	–	–

Note: –: not detected, N (Threshold): not found. Results are expressed as mean.

3.3.1 Acids

Acetic acid had a certain effect on the flavor of rice-acid because its ROAV value was larger than that of other acids. The content of aroma acids in the sample L1 was significantly lower than that in other 9 samples ($P < 0.05$) since less acid-producing microorganisms in L1 affected the acid production ability and significantly reduced the contents of volatile acids, such as acetic acid, propanoic acid and pentanoic acid. Interestingly, due to the different fermentation method, butyric acid, 2-methylbutyric acid, 3-methyl butanoic acid and hexanoic acid were also detected in the sample H1 with the unique fermentation taste. According to our previous study and other studies, the difference in acid compounds were probably due to different microbial compositions in the starter cultures [29].

3.3.2 Alcohols

Alcohols were the most abundant type of volatile substances in rice-acid. Alcohols mainly originated from the alcohol fermentation stage and provided the precursors for the synthesis of organic acids. Some substances in the sample L1 were similar to those in the two samples (M1 and M2), including ethanol, 1-propanol, 2-butanol, and 2-ethylhexanol. Ethanol has a special scent and is slightly irritating and accompanied by a pungent spicy taste. Ethanol was reported as the largest group of alcohol compounds in many fermented foods. 1-Propanol had a smell similar to a mixture of ethanol and acetone whereas 2-butanol had alcoholic aroma [30]. 2-Ethylhexanol might be produced in a fermenter, but it did not affect the flavor of rice-acid.

3.3.3 Ester compounds

Ester compounds are produced in the reactions with acids in the post-fermentation stage of rice-acid. Esters are mainly formed by the enzymatic condensation of organic acids and alcohols during the fermentation process [31]. The ROAV and relative content values of ethyl acetate, propyl acetate, propyl propionate and propyl caproate were large, indicating that these 4 components had the high contribution to the flavor in rice-acid samples. Interestingly, ethyl acetate was the only common ester compound in 10 rice-acid samples and the contents of ester compounds showed no significant difference among the only 3 samples produced by enterprises and high-temperature rice-acid samples produced by farmers (L1, M1, and D1, $P > 0.05$). Ethyl acetate showed the significant difference in other 7 rice-acid samples. Esters are the most prevalent aroma category in vinegar and contribute to fruity and floral fragrances to products [30]. Ethyl acetate as the main contributer could promote the formation of the flavor of rice-acid.

3.3.4 Aldehydes and ketones

Aldehydes contribute to unique flavors of fermented products because of their low odor thresholds. The ROAV values of hexanal and heptanal were large, indicating that they had an important contribution to the formation of the flavor of rice-acid compared with other aldehydes. Ketones are mainly produced by the degradation of amino acids and the thermal degradation and oxidation of unsaturated fatty acids and have a creamy or fruity flavor. Some ketones are important intermediates for the formation of heterocyclic compounds and can enhance the formation of aroma [32]. Most aldehydes and ketones were detected in low-temperature rice-acid samples produced by enterprises and high-temperature rice-acid samples produced by farmers. Although the contents of ketones were low, ketones had an important contribution to the flavor of rice-acid, especially 2-heptanone, 2-octanone, and 2-nonanone.

3.3.5 Hydrocarbons

Hydrocarbons usually have high threshold values. Normal alkane and its methyl branched chain derivatives are mainly derived from the cleavage of fatty acid alkoxy radicals. The contents of 2,4-dimethylheptane and tetradecane were relatively high in the low-temperature samples produced by farmers. Interestingly, olefins were concentrated in the samples (M1 and M2, Table S3). Olefin compounds played a critical role in the flavor. Sung et al. [33] had studied the properties of ethylene/α-olefin copolymers with excellent processability and surface properties. *D*-limonene is a monoterpenoid with a lemon-like aroma and γ-terpinene has citrus- and lemon-like aromas. The contents of the two components were high in the low-temperature samples (M1 and M2) and the two components were beneficial to the formation of the aromatic flavor of rice-acid.

3.3.6 Polyphenols

Polyphenols are reported to be closely related to the antioxidant activity of traditional rice-acid. 2,4-Di-tert-butylphenol was a common polyphenol component in 8 rice-acid except samples H3 and D3. Alonso et al. [34] studied the correlation between the antioxidant power and the content of polyphenols in Sherry vinegar and the correlation coefficient r was 0.920 1. Polyphenols can capture free radicals with high potential energy and convert those free radicals to inactive or more stable compounds by providing hydrogen protons. In addition, polyphenols can be oxidized into stable phenolic radicals. Polyphenols can eliminate free radicals via direct electron transfer.

3.4 Effects of different fermentation methods on taste substances

The taste signal intensities of different rice-acid samples detected by an electronic tongue were different (Fig. 4A). Low-temperature rice-acid samples produced by enterprises (M1, M2 and L1) had the highest signal intensity of sour taste. The high-temperature rice-acid samples produced by farmers had the highest signal intensity of umami and bitterness, followed by astringency. Low-temperature rice-acid samples produced by farmers (H1, H2, H3, and H4) had the highest signal intensity of bitterness, followed by umami. The signal intensity of sweetness showed no significant difference among 10 rice-acid samples since the ability of lactic acid bacteria to break down starch was limited at the end of fermentation. The signal intensity of saltiness was the weakest because salts were not added into rice-acid samples in the fermentation process and the salty taste could not be generated by lactic acid bacteria and yeasts in the fermentation system. The signal intensity of the acidity also verified the highest acidity of three rice-acid samples (M1, M2 and L1). The highest acidity was related to the high content of *L*-lactic acid, the high

titratable acidity, low pH, and the low content of reducing sugars. The acidity was mainly related to the fermentation processes of rice-acid from saccharification to alcoholization and acidification under the actions of lactic acid bacteria and yeasts. The signal intensity of the tastes of rice-acid might depend on the interactions among different taste substances [35]. Umami taste of the rice-acid samples might be ascribed to rich Glu and Asp as well as the synergistic effect among a large number of umami peptides. The content of amino acid nitrogen was related to both total free amino acids and the umami taste [36]. Bitterness was mainly caused by bitter amino acids, hydrophobic bitter peptides, and calcium ions.

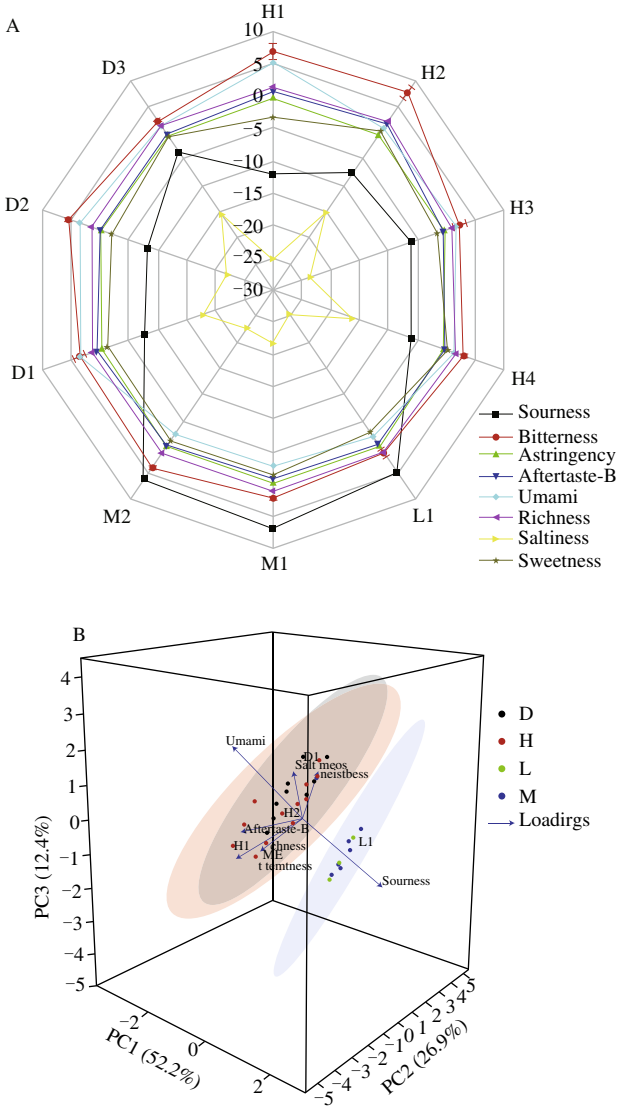

Fig. 4 Taste signal radar chart (A) and principal component analysis of 3D (B) in different low-temperature rice-acid (H1, H2, H3, H4, L1, M1 and M2) and high-temperature rice-acid sample (D1, D2 and D3). Results are expressed as mean ± standard deviation.

The results of principal component analysis showed that the contribution rates of three principal components were respectively 52.2%, 26.9% and 12.4% (Fig. 4B). Three principal components could better reflect the features of all flavor components. The flavor characteristics of ten rice-acid samples were significantly different in the first main component PC1. Ten rice-acid samples could be better classified into 3 groups: low-temperature fermentation group by enterprises (M1, M2, and L1), high-temperature fermentation group (D1, D2, and D3) and low-temperature fermentation group by farmers (H1, H2, H3, and H4). The above results indicated that the electronic tongue could distinguish the three groups of rice-acid produced by different fermentation methods.

3.5 *Effects of different fermentation methods on physicochemical, microbiological and functional characteristics*

Enriched acids in rice-acid might be interpreted as follows. Multiple microorganisms were involved into the complex interaction and alternation, which led to the degradation of oligosaccharides and polysaccharides into reducing sugar, and the growth of microorganisms also consumed partial reducing sugar [37]. The pH values of low-temperature rice-acid samples by enterprises (M1, M2 and L1) were lower than those in the samples produced by farmers (Table 4). The change trend of titratable acidity in the above samples was opposite to that of pH. The pH values in M1, M2 and L1 samples were lower because they had the higher temperature and longer fermentation time than the samples produced by farmers. The two samples (M1 and M2) had the highest titratable acidity probably due to the interaction between lactic acid bacteria and yeasts. The two samples (D2 and H3) had the lowest titratable acidity due to the difference in fermentation temperature. The sample D2 was high-temperature fermentation rice-acid, whereas the sample H3 was low-temperature fermentation rice-acid by farmers. The titratable acidity showed significant differences among the 10 samples ($P < 0.05$). Low-temperature samples produced by enterprises had more acids than low-temperature and high-temperature samples produced by farmers. The contents of reducing sugars in M1 and M2 samples showed no significant difference. The contents of reducing sugars in the two samples (H3 and D2) were respectively 8.67-fold and 7.00-fold higher than those in M1, and 4.80-fold and 3.80-fold higher than those in M2, respectively. The samples of rice-acid with the higher sugar content realized the low pH and high titratable acidity value. The result was consistent with the report by Chiş et al. [38]. Previous studies reported that nitrogen-containing compounds (proteins, peptides and amino acids) could endow fermented foods with the umami taste [39]. The low-temperature samples had the higher content of amino acid nitrogen than high-temperature samples. Therefore, it could be speculated that low-temperature samples had the better umami taste.

Low-temperature rice-acid samples (M1 and M2) had the higher sensory scores (Table 4). Sensory scores showed significant differences among the high-temperature fermentation rice-acid samples (D1, D2 and D3) ($P < 0.05$). Likewise, the numbers of lactic acid bacteria in the 10 samples showed significant differences ($P < 0.01$). There were more quantity yeasts (5.04×10^4–2.25×10^8 CFU/mL) than lactic acid bacteria (3.00×10^3–7.02×10^6 CFU/mL). Previous studies reported that the synergistic interaction among these microorganisms in fermented foods could improve the utilization of nutrients [40]. It could be inferred that lactic acid bacteria and yeasts affected the flavor (taste and aroma components) and quality of fermented rice-acid. This result was consistent with the previous report on the correlation between microbiota and flavor [41]. *Lactobacillus* and *Kluyveromyces* were the dominant genera in rice-

Table 4
pH, titratable acidity, reducing sugar, amino nitrogen, sensory score, number of lactic acid bacteria, number of yeast and GABA of rice-acid with different low-temperature rice-acid (H1, H2, H3, H4, L1, M1 and M2) and high-temperature rice-acid sample (D1, D2 and D3).

Sample	pH	Titratable acidity (g/kg)	Reducing sugar (mg/mL)	Amino nitrogen (g/100 g)	Sensory score	Number of lactic acid bacteria (CFU/mL)	Number of yeast (CFU/mL)	GABA (mg/L)
H1	3.79 ± 0.01^{ab}	5.52 ± 0.44^{f}	0.24 ± 0.01^{a}	0.02 ± 0.00^{a}	6.37 ± 0.31^{d}	$(1.80 \pm 0.06) \times 10^{4\,h}$	$(2.90 \pm 0.04) \times 10^{6\,c}$	61.76 ± 1.03^{b}
H2	3.44 ± 0.03^{c}	8.61 ± 0.24^{d}	0.11 ± 0.01^{c}	0.03 ± 0.00^{a}	8.86 ± 0.06^{b}	$(3.25 \pm 0.07) \times 10^{6\,c}$	$(1.75 \pm 0.02) \times 10^{6\,d}$	68.36 ± 2.07^{a}
H3	3.92 ± 0.01^{a}	4.32 ± 0.07^{g}	0.29 ± 0.02^{a}	$< 0.01^{c}$	7.52 ± 0.09^{c}	$(7.02 \pm 0.19) \times 10^{6\,a}$	$(7.05 \pm 0.15) \times 10^{6\,b}$	59.83 ± 0.19^{c}
H4	3.14 ± 0.03^{d}	10.05 ± 0.21^{c}	$0.21 \pm 0.01^{a,b}$	0.02 ± 0.00^{a}	8.94 ± 0.11^{b}	$(7.45 \pm 0.17) \times 10^{4\,f}$	$(1.92 \pm 0.03) \times 10^{6\,d}$	72.18 ± 3.72^{a}
L1	2.94 ± 0.02^{e}	7.38 ± 0.45^{e}	0.11 ± 0.01^{c}	$< 0.01^{c}$	$8.27 \pm 0.15^{b,c}$	$(2.50 \pm 0.07) \times 10^{5\,d}$	$(1.30 \pm 0.02) \times 10^{6\,d}$	$54.41 \pm 0.01^{d,e}$
M1	2.83 ± 0.01^{ef}	15.30 ± 0.01^{a}	0.03 ± 0.01^{de}	0.01 ± 0.00^{b}	9.65 ± 0.22^{a}	$(4.40 \pm 0.09) \times 10^{6\,b}$	$(2.25 \pm 0.03) \times 10^{8\,a}$	56.12 ± 0.86^{d}
M2	2.86 ± 0.02^{ef}	13.65 ± 0.21^{b}	0.05 ± 0.04^{de}	0.02 ± 0.00^{ab}	9.32 ± 0.14^{a}	$(3.95 \pm 0.04) \times 10^{6\,c}$	$(5.50 \pm 0.11) \times 10^{5\,e}$	55.56 ± 0.22^{d}
D1	3.53 ± 0.02^{b}	8.37 ± 0.25^{d}	0.09 ± 0.01^{d}	$< 0.01^{c}$	7.40 ± 0.31^{c}	$(4.60 \pm 0.08) \times 10^{4\,g}$	$(3.10 \pm 0.09) \times 10^{5\,f}$	63.36 ± 0.05^{b}
D2	3.85 ± 0.01^{ab}	4.95 ± 0.22^{g}	0.24 ± 0.01^{a}	$< 0.01^{c}$	6.28 ± 0.13^{d}	$(9.50 \pm 0.12) \times 10^{4\,e}$	$(1.15 \pm 0.01) \times 10^{5\,g}$	60.70 ± 0.02^{b}
D3	3.58 ± 0.01^{b}	8.25 ± 0.48^{d}	0.18 ± 0.02^{b}	$< 0.01^{c}$	7.14 ± 0.28^{cd}	$(3.00 \pm 0.03) \times 10^{3\,i}$	$(5.04 \pm 0.07) \times 10^{4\,h}$	60.03 ± 3.72^{b}

Note: Results are expressed as mean ± standard deviation, three repetitions. $^{a–h}$ Different letters in the same column indicate significant differences ($P < 0.05$).

acid in our previous study [42]. Stefanovic et al. [43] obtained diverse cheese flavors with different lactic acid bacteria strains, which were identified as the most dissimilar strains in two model systems. Another study reported that the highest content of extracellular polysaccharides produced by *Kluyveromyces* was beneficial to the formation of the flavor of whole wheat bread [44]. These results proved that rice-acid might had a potential probiotic ecosystem and contribute to the unique tastes and flavors.

GABA is a major inhibitory neurotransmitter in the mammalian central nervous system and can regulate various physiological functions such as blood pressure and heart rate. The contents of GABA in the samples obtained by two different fermentation methods (low-temperature and high-temperature fermentation) were between 54.41 mg/L and 72.18 mg/L ($P < 0.05$, Table 4). The commercially available low-temperature fermented rice-acid samples had relatively low content of GABA probably because the sterilization method before leaving the factory inhibited the growth of microorganisms. The difference in the content of GABA in high-temperature fermentation rice-acid samples (D1, D2, and D3) was not significant ($P > 0.05$). The higher content of GABA in low-temperature rice-acid samples produced by farmers might be ascribed to the accumulation of lactic acid bacteria in the cooling procedure. Carafa et al. [45] reported that fermented foods produced with different strains were different in the ability to produce GABA. Lactic acid bacteria promoted GABA production. *Lactocooccus lactis* subsp. and *Bacillus licheniformis* could produce GABA in the anaerobic environment of food fermentation [46]. Interestingly, the content of GABA in this unique low-temperature fermentation rice-acid was higher than that in high-temperature fermentation rice-acid. The cause for the difference requires to be further explored in subsequent experiments.

3.6 Correlations between taste and aroma compounds and nutritional components in rice-acid

Based on Pearson's coefficient, the correlations between the key components in rice-acid were explored. Sour taste (lactic acid) was associated with titratable acidity and sensory score and sourness had a negative correlation with pH, reducing sugar, GABA, bitterness and umami (Fig. 5). Sweetness was positively associated with GABA, amino acid (Asp, Ala and His), richness and saltiness. Bitterness (Val) was associated positively with amino nitrogen, aftertaste and umami. Umami had a positive correlation with pH, reducing sugar and bitterness, but it had a negative correlation with titratable acidity, sensory score and sourness. Saltiness had a positive correlation with amino acids (Glu, Asp, Ala, and His) and GABA. GABA, as a nutritional component, was positively correlated with savory sour amino acids (Glu and Asp). Glu showed the close relationship with amino nitrogen. Ethyl acetate showed the significant correlation with ethanol ($P < 0.01$). Interestingly, lactic acid bacteria and yeasts had a positive correlation with sensory score (lactic acid and titratable acidity) and were negatively correlated with Val. Chiş et al. [38] also reported that fermentation of gluten free rice with lactic acid bacteria and yeasts could produce flavor compounds to form specific aroma profiles and odorant compositions, which influenced the quality aroma of the final fermented goods. This result proved that the microbial community played a crucial role in the formation of flavor. The study on the relationship between the flavor and microorganisms could improve the quality of fermented foods. In addition to food-self characteristics and eating qualities, microbiota compositions were also reported to exert significant influences on the flavor profiles of rice products [47,48]. It is the first time to explore the flavor profiles and physicochemical characteristics in rice-acid and we will further investigate the correlation between the microbial community and flavor compounds in the fermentation process.

4. Conclusion

The study revealed the taste and aroma compounds and nutritional components in traditional fermented rice-acid. Rice-acid produced by different fermentation methods could be distinguished by its unique flavor profiles. Especially, taste substances could be used to distinguish rice-acid produced by different fermentation methods. Sourness (lactic acid) is a crucial evaluation index of the flavor quality of rice-acid. We found a large quantity of aroma compounds in low-temperature rice-acid than high-temperature rice-acid. Ethyl acetate had the high contribution to the aromatic flavor and showed the significant correlation with ethanol. GABA improved the nutritional characteristics of rice-acid, especially in low-temperature fermentation rice-acid. In addition, correlation analysis results revealed that lactic acid bacteria and yeasts had the positive correlation with

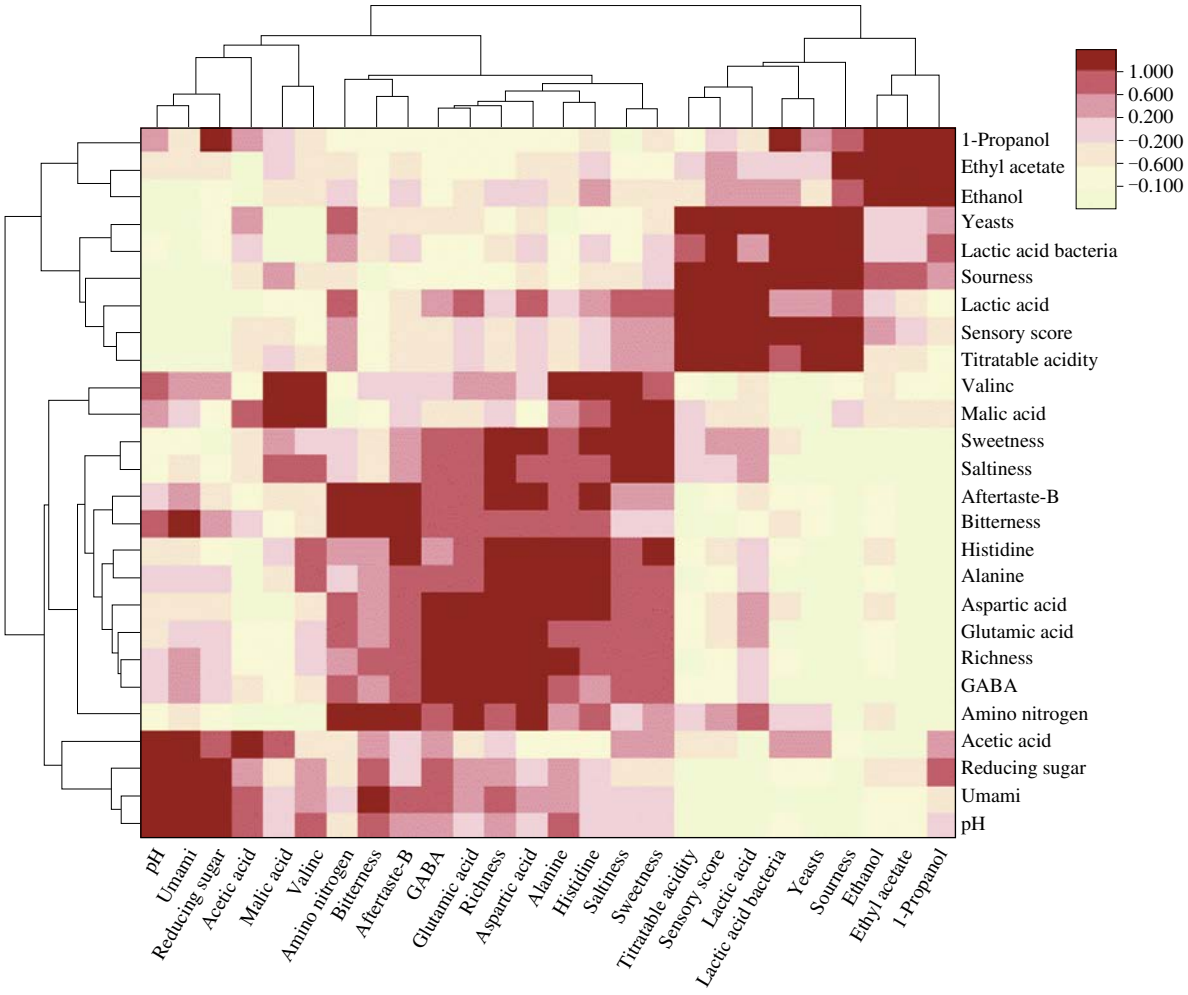

Fig. 5 The correlations between the key components (organic acids, amino acids, nutritional components, taste compounds and aroma compounds) and sensory evaluation scores are analyzed by Pearson's coefficient in different low-temperature rice-acid (H1, H2, H3, H4, L1, M1 and M2) and high-temperature rice-acid sample (D1, D2 and D3).

sensory score, lactic acid and titratable acidity. This study could help rice-acid producers and researchers to better monitor the quality of this traditional product and define a standard production protocol. In the future, we will explore the variations of chemical–physical indicators and taste and aromatic compounds with the production process parameters. This study will largely promote the development of industrial-scale Chinese fermented rice-acid.

Declaration of Competing Interest

The authors declare that they have no competing financial interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://doi.org/10.1016/j.fshw.2021.11.019.

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