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Research Article

Analysis on Flavor Compounds of Jujube Brandy from Different Fermentation Heights by HS-SPME-GC/MS, E-nose and E-tongue

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Abstract

Background and Objective: Nutrients and flora structure of different fermentation layers are different, directly impacting on the quality and aroma of brandy, which is also the hot research in brandy industry. The aim of present study is to find the effect of fermentation space (height) on flavor compositions of jujube brandy by HS-SPME-GC/MS, GC-O, E-nose and E-tongue. **Materials and Methods:** Flavor compositions of jujube brandy from different fermentation layers were investigated using headspace-solid phase microextraction-gas chromatography-tandem mass spectrometry (HS-SPME-GC-MS), gas chromatography-olfactometry (GC-O), electronic-nose (E-nose) and electronic-tongue (E-tongue). The SPSS was used for finding significance difference. **Results:** There are 17 flavor compounds found as contributory odorants, composing 13 esters and 4 aldehydes. Ethyl esters of butanoate, hexanoate and octanoate were the most important aroma compounds identified by GC-O and OAV (odor active value) analysis. Significant differences appeared in the flavor compositions of jujube brandy in different fermentation layers. The 1st layer sample showed the highest flavor concentration and the 6th the least. Many unique odorants were formed in the low fermentation layers. Jujube brandies from high fermentation layers had higher concentration of esters, aldehydes and ketones. Alcohols and terpenoids were easier to be formed in the low fermentation layers. Flavor compositions from different fermentation layers could be discriminated well by principal component analysis (PCA) and linear discriminant analysis (LDA) with E-nose and E-tongue. **Conclusion:** Overall, jujube brandies from high fermentation layers are better than lower ones, the 1st layer sample far away from other samples.

Key words: Jujube brandy, flavor compounds, fermentation layers, GC-MS, E-nose, E-tongue

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chinese jujube (*Ziziphus jujuba* Mill.), is a rosaceous fruit endemic to China, belonging to the *Rhamnaceae* family, which contains 58 genera, including about 900 species, which are common in subtropical and tropical regions¹⁻³. Chinese jujube is an important nutrient source due it is particularly high vitamin C content and abundance of the minerals potassium and iron⁴⁻⁶. The fruits, have multiple uses, as a fresh or dried fruit for human combustion, in fermented products and also for therapeutic uses in traditional Chinese medicine⁷⁻¹². Thus, Chinese jujube is valued for its dietary and health properties in China.

Jujube brandy, a popular traditional fermented alcoholic beverage, is one of the oldest alcoholic drinks in China¹³⁻¹⁵. Due to its unique composition of ester and other aroma compounds, jujube brandy has an aroma, which sets it apart from other fermented beverages. Jujube brandy is produced by solid fermentation, followed by distillation and aging. Jujube brandy uses caused whole fruits as pomace as the starting material. Additionally rice husks are supplied as adjunct, which can produce gap in the process of fermentation and distillation, then would be good for heat dissipation¹⁶⁻¹⁸. The production, if jujube brandy is analogous to a hybrid of rice wine and brandy production. Currently jujube production is rapidly increasing due to the rise of discretionary spending in China. The demand for jujube brandy is an impinged by limitations in production methods and knowledge of key flavor chemistries. Improving the understanding of these processes will lead to an improved product and increased production².

Currently, knowledge of fermentation processes in regards to aromatic flavor compounds in jujube production is limited. The fermentation process is the key stage to produce high-quality brandy, which is determined by the presence of desirable volatile flavor compounds while minimizing undesirable aroma compounds¹⁹. Different fermentation parameters such as fermentation temperature, humidity, selection of yeast strains, bacteria presence, all affect the final product. Changes in these parameters result in modulation of alcoholic content, taste and flavor of the finished product. Improved understanding of the physical characteristics of these processes will lead to an improvement of quality and technical knowledge.

In order to study these processes, a HS-SPME-GC/MS analysis of jujube brandy flavor components was conducted²⁰. These methods led to the identification and important aroma components of jujube brandy. The experiment consisted of comparing three different brands of

jujube brandy by GC-O-MS. Results show that the primarily important aroma compounds are comprised of ethyl decylate, ethyl laurate and ethyl tetradecanoate^{21,22}. Furthermore, varying fermentations using different parameters were conducted and the resulting compounds were analyzed in a similar method^{23,24}. This resulted in ability to isolate key findings to improve fermentation processes.

Currently, jujube brandy consumption has rapidly increased, but research on flavor compounds of jujube brandy is still insufficient. Recent studies of jujube brandy have been limited to enzymatic processing of juice. Additional fermentation methods' impact on aroma is a key knowledge gap. Thus, the aim of present study is to find the effect of fermentation space (height) on flavor compositions of jujube brandy by HS-SPME-GC/MS, GC-O, E-nose and E-tongue.

MATERIALS AND METHODS

Jujube brandy: Seven wine samples (~50% ethanol, v/v), from the jujube variety Fuping were produced in Hebei China region in 2014 (latitude 38°9-39°7 N, longitude 113°45-114°31 E). Among these samples, one was a blend and the remaining six samples were fermented under varying conditions.

For the production of these samples the fermentation tank (2.7 m × 1.3 m × 1.9 m) utilized was divided into 6 parts vertically, from up to bottom in to different partitions, 1-6. In total 1800 kg of starting material, comprised of 1500 kg of jujube and 300 kg of rice husks, was fermented along with 1.5% by weight jujube brandy Daqu, at 20-25 over 15 days. This resulted in 300 kg of fermented products of each partition that were then individually distilled in 1.4 m diameter pot still using steam distillation. This resulted in the six distillation partitions used for further analysis.

HS-SPME-GC-MS parameters: After distillation, jujube brandy partitions were normalized to 10% ethanol using distilled water. Then, sodium chloride (1 g) was added to 7.5 mL of sample solution in a 20 mL sealed glass vial. The sample was extracted at 40 °C for 40 min using a 50/30 µm DVB/CAR/PDMS fiber, then used to GC-MS analysis¹⁹⁻²⁰.

Volatile compounds were analyzed using an Agilent 5975 Mass Spectrometer coupled to an Agilent 7890A Gas Chromatograph (Agilent, Santa Clara, USA). The A DB-WAX column (60 m × 0.25 mm ID and 0.25 µm film thickness) was used for separation. The working parameters were as follows: Injector temperature of 250 °C, EI source of 230 °C, MS Quad of 150 °C and transfer line of 250 °C. The initial temperature was 50 °C for 3 min, which was increased to 80 °C at a rate of

3°C min⁻¹. The temperature was further raised to 230°C at 5°C min⁻¹ and maintained at 230°C for 6 min. The carrier gas had a flow rate of 1.0 mL min⁻¹. Samples were injected using the splitless mode. A mass range of 50-550 m/z was recorded at one scan per second. Three replicates of each partition were analyzed by GC-MS. Quantitative data were obtained based on measurement of relative peak areas compared with the area of the 3-Octanol internal standard^{25,26}.

GC-O analysis of volatile flavor compounds: Characteristic flavor compounds of jujube brandy were specified by GC-O. The GC analysis of volatile compounds was carried out on a GC-7890A equipped with a flame ionization detector (FID) and sniffing port. The column and temperature program was identical to GC-MS analysis. The effluent from the capillary column was split 1:1 between the FID and sniffing port using a "Y" splitter. Sniffing was carried out using OSS-9000 sniffer (Brechtbuhler, Switzerland). Three panelists were used for the GC-O analysis and 3 replications were tested by GC-O.

Electronic nose system: Headspace analysis was performed with an E-nose (PEN3, Airsense Analytics, Germany). The PEN3 system consists of a sampling apparatus, a detector unit containing the array of sensors and pattern-recognition software for data recording and evaluation. The sensor array system is composed of 10 metal oxide semiconductors (MOS) of different chemical compositions and thicknesses to select for volatile compounds. Table 1 lists all used sensors

and their main applications. This table contains current known or specified reactions and their detection limits.

A 10 mL sample juice was taken into a 500 mL beaker and the beaker was sealed with plastic wrap for 30 min to allow for compounds to volatilize. Samples were detected by E-nose immediately following the 30 min incubation time. After 30 min for the headspace incubation, the temperature of samples for the E-nose detection was same as the lab environment (approximately 18°C). The measurement phase lasted for 60 sec, which ensured stable signal values. Conditions for the sampling were as follows; the sample gas was transferred into the sensor chamber at a flow rate of 300 mL min⁻¹ and after 60 sec data were collected. After each experiment, nitrogen gas was driven through the system for 100 sec to purge the system ensuring a normalized background. These tests were replicated for three samples of each partition.

Electronic tongue system: An E-tongue (isenso, Shanghai Ruifen International Trading Co, Ltd., China) was employed to classify and characterize the jujube brandies. This instrument consists of seven potentiometric chemical sensors (ZZ, BA, BB, CA, GA, HA and JB), a reference electrode of Ag/AgCl, data acquisition system and basic data analysis software. The cross-sensitivity and selectivity of the sensor array contribute to the detection of substances found in the liquid matrix, providing a global taste perception. Table 1 lists all the sensors and their thresholds for five basic tastes.

Table 1: Sensors used and their main applications in the E-nose and E-tongue

Sensors used in the E-nose

Number	Sensors	Performance description	Refer
1	W1C	aromatic	Toluene 10 mL m ⁻³
2	W5S	broad range	NO ₂ 1 mL m ⁻³
3	W3C	aromatic	Benzene 10 mL m ⁻³
4	W6S	hydrogen	H ₂ 100 mL m ⁻³
5	W5C	arom-aliph	Propane 1 mL m ⁻³
6	W1S	broad-methane	CH ₄ 100 mL m ⁻³
7	W1W	sulphur-organic	H ₂ S 1 mL m ⁻³
8	W2S	broad-alcohol	CO 100 mL m ⁻³
9	W2W	sulph-chlor	H ₂ S 1 mL m ⁻³
10	W3S	methane-aliph	CH ₄ 10 mL m ⁻³

Sensors used and their thresholds in E-tongue (unit: mol L⁻¹)

Basic taste taste substance	Sour citric acid	Salty NaCl	Sweet glucose	Bitter caffeine	Savory MSG
ZZ	10 ⁻⁷	10 ⁻⁶	10 ⁻⁷	10 ⁻⁵	10 ⁻⁵
BA	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴
BB	10 ⁻⁷	10 ⁻⁶	10 ⁻⁷	10 ⁻⁴	10 ⁻⁴
CA	10 ⁻⁷	10 ⁻⁶	10 ⁻⁷	10 ⁻⁵	10 ⁻⁴
GA	10 ⁻⁷	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴	10 ⁻⁵
HA	10 ⁻⁶	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴
JB	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴

Seven potentiometric chemical sensors (ZZ, BA, BB, CA, GA, HA and JB)

The experiment was carried out with filtered jujube brandy to avoid the impacts caused by solid particles. The amount of sample was 30 mL to ensure that the sensors were fully immersed. The measurement time was set to 120 sec for each sample, which permitted the sensors to reach stable signal values and the sensors were rinsed for 10 sec using deionized water to minimize and correct sensor drift. The temperature of samples for the E-tongue detection was $20^{\circ}\text{C} \pm 3$. The detection voltage varied between -1 to 1V, with an interpulse interval of 100 mV and sensitivity of $1:10^5$. Three replicates of each brandy partition were tested using this E-tongue method.

Statistical analysis: Principal component analysis (PCA) is a multivariate technique that analyzes a data table in which observations were described by several inter-correlated quantitative dependent variables²⁷. The PCA can be done by eigenvalue decomposition of a data covariance matrix or singular value decomposition of a data matrix. First principal component has the largest possible variance. When succeeding component is orthogonal to the preceding components, the former has the highest variance. The higher cumulative contribution rate is and the more original information will be reflected. The PCA was used to understand the major components of these brandy samples in order to understand their composition and the effects of processes on chemical compositions.

Linear discriminant analysis (LDA) explicitly models the difference between the classes of data and tries to maximize the variance between categories and minimize the variance within categories. Compared with PCA, the LDA method can notice the distribution of points in the same category and the distance between them²⁸⁻³⁰. It provides a classification model, characterized by a linear dependence of the classification scores with respect to the descriptors and the eigenvalues of LDA were determined to get more information on the relation of the factors in the model analyses. Values are significant at $p < 0.05$.

RESULTS AND DISCUSSION

GC-O analysis of flavor compounds: With the methods of GC-O-MS testing 48 flavors were detected, including 23 esters, 8 alcohols, 6 acids, 11 aldehydes and ketones. Esters were the most abundant compounds found in the samples which are major compounds comprising the aromas of fruits and flowers. These were followed by alcohols and terpenes with are described as having green, vegetal, or grass like aroma.

The following compounds were detected in methods and are thought to contribute to the majority of the flavor/aroma profile of jujube brandy. Ethyl acetate (orange), ethyl butyrate (fruit/apple), ethyl-3-methyl-butyrate (apple), ethyl hexanoate (apple or aniseed), ethyl nonanoate (chocolate), methyl laurate (cucumber/honey), especially ethyl laurate (red dates), ethyl phenylpropionate (red dates) and ethyl tetradecanoate (red dates) (Table 2). There may be other compounds not detected due to method limitations or sensitivity but these compounds are the most prevalent in this analysis.

The odor activity values (OAVs = concentration/threshold) of 17 flavor compounds were greater than 1, which can be regarded as contributory compounds. These compounds include 13 esters and 4 aldehydes, of which both classes are found in fermented beverages. The OAV of 5 compounds (1-Butanol 3-methyl-acetate, benzoic acid ethyl ester, benzenepropanoic acid ethyl ester, hexanal, octanal and nonanal) were between 10-100 indicating contributory compounds. The next 3 compounds (butanoic acid ethyl ester, pentanoic acid ethyl ester and decanoic acid ethyl ester) had an OAV between 100-500, suggesting very strong contributory compounds. The last group of 3 compounds (butanoic acid 3-methyl- ethyl ester, hexanoic acid ethyl ester and octanoic acid ethyl ester) had an OAV greater than 500, which should be regarded as the most important flavor compounds and evaluation index of jujube brandy.

GC-MS analysis of flavor compounds

Comparison on flavor compounds of different layers:

Significant differences appeared on the flavor profiles of jujube brandies from different fermentation layers ($p < 0.05$). The 1st fermentation layer showed the highest total aroma content (12.581 mg L^{-1}) and the 6th showed the least (3.565 mg L^{-1}). Meanwhile, 87 kinds of flavor compounds were detected in the jujube brandy from the 5th fermentation layer, while only 75 were found in the 1st. Although more compounds were detected in the 6th partition, the first layer had higher concentrations, this suggests that changing the ratios of distillates in the final product could be varied due to blending. The changes in compounds found in the different partitions can lead to evaluations of which compounds consumers prefer and at what level leading to an improvement in the distillation process and the final end product.

From all the compounds detected using present study methods, 44 were detected in every partition (Table 3) and 15 were unique to a single partition. Methyl benzoate and ethyl 9-octadecenoate were only detected in the 1st and 3rd layer,

Table 2: Tentatively identified compounds of jujube brandy volatile compounds

Numbers	Time/min	Flavor compounds	RI	RI (literature ³⁰⁻³¹)	MW	CAS	Odor ³²⁻³³
Esters							
1	4.90	Butanoic acid, ethyl ester			116.084	000105-54-4	Strawberry, apple
2	5.10	Butanoic acid, 2-methyl-, ethyl ester			130.099	007452-79-1	Apple, sweet
3	5.36	Butanoic acid, 3-methyl-, ethyl ester	1080	1072	130.099	000108-64-5	Banana, fruity, sweet
4	6.23	1-Butanol, 3-methyl-, acetate			130.099	000123-92-2	Fruity, apple
5	6.44	Pentanoic acid, ethyl ester			130.099	000539-82-2	Nf
6	7.96	Butanoic acid, butyl ester	1208	1221	144.115	000109-21-7	Fruity, green apple, brandy, wine-like
7	8.24	Hexanoic acid, ethyl ester	1238	1238 ^a	144.115	000123-66-0	
				1236			
8	8.83	Propanoic acid, 2-methyl-, 3-methylbutyl ester	1355	Nf	158.131	002050-01-3	
9	9.00	Acetic acid, hexyl ester	1281	12705	144.115	000142-92-7	Apple, cherry, pear, floral
10	10.10	Heptanoic acid, ethyl ester	1340	1336	158.131	000106-30-9	Fruity
11	10.44	Propanoic acid, 2-hydroxy-, ethyl ester	1339	1334	118.063	000097-64-3	Fruity, buttery
12	15.63	Butanedioic acid, diethyl ester			174.089	000123-25-1	
13	11.80	Octanoic acid, ethyl ester	1428	1424	172.146	000106-32-1	Anise, fruity, pear
14	12.18	Isopentylhexanoate			186.162	002198-61-0	Sweet fruity
15	13.47	Nonanoic acid, ethyl ester	1520	1526	186.162	000123-29-5	Floral, fruity
16	15.17	Decanoic acid, ethyl ester	1643	1638	200.178	000110-38-3	Fruity, grape
17	15.57	Benzoic acid, ethyl ester	1665	1664	150.068	000093-89-0	Fruity
18	16.45	Undecanoic acid, ethyl ester	1739	1732	214.193	000627-90-7	Nf
19	17.23	Benzeneacetic acid, ethyl ester	1783	Nf	164.084	000101-97-3	Flowery
20	17.68	Acetic acid, 2-phenylethyl ester	1821	1829	164.084	000103-45-7	Flowery, anise
21	17.92	Dodecanoic acid, ethyl ester	1856	1835	228.209	000106-33-2	Sweet, jujube
22	18.83	Benzenepropanoic acid, ethyl ester	1892	1872	178.099	002021-28-5	Jujube
23	22.36	Tetradecanoic acid, ethyl ester	2057	2065	256.24	000124-06-1	Jujube
Alcohols							
1		1-Propanol	1038	1036	60.058	000071-23-8	Alcohol, ripe fruit
2	6.05	1-Propanol, 2-methyl-	1098	1090	74.073	000078-83-1	Fusel, Alcohol
3		1-Butanol	1148	1149 ^a	74.073	000071-36-3	Medicinal
				1159			
4	8.09	1-Butanol, 3-methyl-	1200	1206 ^a	88.089	000123-51-3	Solvent
				1208			
5	9.32	4-Heptanol	1344	Nf	116.12	053535-33-4	Lemon, orange, copper
6	10.50	1-Hexanol	1375	1371 ^a	102.104	000111-27-3	Herbaceous, grass, woody
				1392			
7	13.80	1-Octanol	1557	1558	130.136	000111-87-5	
8	19.42	Phenylethyl Alcohol	1900	1898	122.073	000060-12-8	Rose, honey
Acids							
1	12.38	Acetic acid	1464	1451	60.021	000064-19-7	Pungent, vinegar
2	13.80	Butanoic acid	1638	1630 ^a	88.052	000107-92-6	Rancid, cheese
				1642			
3	18.24	Hexanoic acid	1871	1855 ^a			
				1863			
4	18.23	Heptanoic acid	1970	1950 ^a	116.084	000142-62-1	Cheese, fatty
				1955			
5	23.26	Octanoic Acid	1095	2092	130.099	000111-14-8	Sweaty, cheese
6	28.21	n-Decanoic acid	2266	2269	144.115	000124-07-2	Fatty, rancid
					172.146	000334-48-5	Fatty, rancid

Table 2: Continue

Numbers	Time/min	Flavor compounds	RI	RI (literature ³⁰⁻³¹)	MW	CAS	Odor ^{2,2,33}
Aldehydes and Ketones							
1	5.64	Hexanal	1104	1089 ^a 1092	100.089	000066-25-1	Sweet, orange
2	8.67	3-Octanone			128.12	000106-68-3	Herbal
3	9.3	Octanal	1290	1284 ^a 1270	128.12	000124-13-0	Soapy, citric, fatty
4	11.05	2-Nonanone			142.136	000821-55-6	Fruity
5	11.13	Nonanal	1388	1388 ^a 1390	142.136	000124-19-6	Citrusy, floral
6	12.49	Furfural			96.021	000098-01-1	
7	12.86	Decanal	1500	1494 ^a 1499	156.151	000112-31-2	Grassy, orange skin-like
8	13.41	Benzaldehyde	1506	1503	106.042	000100-52-7	Almond
9	14.19	2-Furancarboxaldehyde, 5-methyl-			110.037	000620-02-0	
10	14.43	2-Undecanone			170.167	000112-12-9	
11	17.44	2-Tridecanone			198.198	000593-08-8	

Table 3: Comparison of flavor compounds of jujube brandy from different fermentation layers

		Fermentation layer/relative concentration (mg L ⁻¹)						
Numbers	Time/min	Flavor compounds	1	2	3	4	5	6
Ethyl esters								
1	5.95	Ethyl acetate	0.474±0.018	0.441±0.021	0.117±0.008	0.373±0.015	0.100±0.007	0.364±0.020
2	9.30	Ethyl butyrate	0.199±0.006	0.281±0.008	0.169±0.005	0.470±0.022	0.250±0.010	2.615±0.048
3	10.12	Ethyl-3-methyl -butyrate	0.048±0.004	0.080±0.005	0.029±0.003	0.111±0.008	0.050±0.003	0.127±0.006
4	12.10	Ethyl pentanoate	0.182±0.007	0.363±0.009	0.111±0.004	0.429±0.008	0.213±0.008	0.552±0.010
6	15.74	Ethyl hexanoate	33.629±2.007	3.823±0.116	4.420±0.218	5.694±0.205	3.637±0.108	7.843±0.317
8	19.02	Ethyl heptanoate	1.814±0.008	2.548±0.011	2.088±0.028	-	1.933±0.015	4.026±0.030
13	22.44	Ethyl octanoate	8.938±0.031	11.296±0.058	8.671±0.030	11.098±0.055	9.454±0.046	19.506±0.075
19	25.30	Ethyl nonanoate	2.585±0.022	3.061±0.031	2.178±0.027	2.723±0.028	2.801±0.0226	4.975±0.035
21	27.67	2-Furancarboxylic acid, ethyl ester	0.020±0.001	0.019±0.001	0.047±0.002	0.027±0.001	0.029±0.001	0.011±0.001
22	28.34	Ethyl decanoate	3.981±0.031	4.625±0.037	5.096±0.038	4.644±0.036	3.901±0.035	7.305±0.052
25	28.91	Ethyl benzoate	18.062±1.031	26.000±2.085	28.948±2.047	24.960±2.096	18.507±1.029	0.460±0.025
27	30.35	Ethyl undecanoate	0.923±0.020	0.705±0.016	1.374±0.025	0.724±0.015	0.939±0.020	1.362±0.023
28	31.53	Ethyl phenylacetate	0.274±0.005	0.305±0.009	0.391±0.008	0.321±0.005	0.299±0.006	0.542±0.003
30	32.96	ethyl laurate	11.689±1.006	19.089±1.031	17.162±1.040	19.156±1.026	19.793±1.035	4.326±0.056
31	32.33	Ethyl 2-hydroxy benzoate	-	0.045±0.008	-	0.043±0.006	-	-
32	33.85	Ethyl phenylpropionate	1.196±0.025	1.711±0.024	1.165±0.027	1.769±0.031	0.865±0.020	3.090±0.054
33	34.80	Ethyl tridecanoate	-	0.067±0.005	0.065±0.004	0.065±0.005	0.063±0.006	0.093±0.008
34	36.89	Ethyl tetradecanoate	0.947±0.015	1.247±0.022	1.577±0.025	1.553±0.023	1.391±0.020	2.248±0.031
36	38.84	Ethyl pentadecanoate	-	0.033±0.001	0.060±0.003	0.053±0.002	0.050±0.001	0.087±0.003
37	40.72	Ethyl hexadecanoate	0.305±0.015	0.299±0.018	0.681±0.017	0.573±0.010	0.538±0.009	0.912±0.020
40	44.98	Ethyl oleate	-	-	0.028±0.003	-	-	0.009±0.000
Other esters								
1	11.72	1-Butanol, 3-methyl-, acetate	0.393±0.011	0.403±0.013	0.129±0.008	0.324±0.012	0.132±0.010	0.897±0.009
2	15.27	Butyl butanoate	-	-	-	-	-	0.444±0.008
7	30.66	Isobutyl caprate	0.059±0.005	0.093±0.007	0.336±0.016	0.136±0.012	0.121±0.010	0.183±0.011
9	34.40	1-Butanol, 3-methyl-, benzoate	0.140±0.007	0.183±0.009	0.212±0.010	0.178±0.011	0.194±0.010	0.330±0.012
10	35.06	Isobutyl laurate	0.031±0.002	0.047±0.002	0.057±0.003	0.052±0.002	0.048±0.001	0.072±0.002
3	22.91	Isopentylhexanoate	0.329±0.022	0.394±0.016	0.445±0.014	0.409±0.019	0.383±0.018	0.648±0.026
		Total esters	89.312 ^f	81.12 ^d	79.615 ^c	81.215 ^e	70.022 ^a	70.905 ^b
Alcohols								
1	11.21	Isobutanol	0.392±0.021	0.013±0.001	-	-	0.757±0.013	-
2	15.55	Isoamylol	0.598±0.020	0.909±0.023	-	1.064±0.025	0.021±0.001	-
3	19.94	1-Hexanol	0.028±0.001	-	0.029±0.001	-	-	-
4	21.33	2-Nonen-1-ol	-	0.094±0.003	-	0.097±0.001	0.093±0.001	-
5	22.80	1-Octen-3-ol	0.076±0.001	0.107±0.010	0.181±0.008	0.138±0.008	0.007±0.000	0.333±0.012
6	22.95	1-heptanol	-	-	-	0.055±0.001	-	-
7	25.72	1-Octanol	-	0.120±0.008	0.128±0.012	0.138±0.011	0.257±0.010	-
8	28.36	1-Nonanol	0.076±0.001	0.294±0.006	0.239±0.008	0.318±0.009	0.064±0.001	0.095±0.002
9	29.61	Borneol	0.048±0.001	0.080±0.003	0.035±0.001	0.083±0.002	-	0.079±0.002
10	33.58	Benzyl alcohol	-	-	0.198±0.003	-	0.100±0.001	-
11	34.33	Phenylethyl alcohol	0.096±0.001	0.107±0.005	0.169±0.007	0.111±0.010	0.029±0.001	0.174±0.008
		Total alcohols	1.335 ^c	1.79 ^d	1.02 ^b	2.06 ^e	2.106 ^f	0.729 ^a
Acids								
1	35.30	Heptylic acid	-	0.027±0.001	-	-	-	-
2	37.39	Octanoic acid	0.055±0.001	0.080±0.002	0.058±0.001	0.055±0.001	0.072±0.002	0.111±0.002
3	41.61	n-decanoic acid	0.138±0.004	0.134±0.003	0.245±0.006	0.207±0.002	0.207±0.003	0.016±0.001
4	45.44	Dodecanoic acid	0.261±0.008	0.201±0.001	0.361±0.002	0.263±0.001	0.236±0.002	0.380±0.003
		Total acids	0.454 ^b	0.674 ^f	0.525 ^d	0.515 ^c	0.527 ^e	-
Aldehydes and ketones								
1	15.62	2-Hexenal	-	0.013±0.001	-	0.018±0.000	0.015±0.001	-
2	16.46	3-Octanone	0.010±0.000	0.056±0.003	0.022±0.001	0.049±0.001	0.024±0.001	0.046±0.002
3	19.15	2-Nonanone	0.096±0.002	0.007±0.000	0.093±0.003	0.008±0.000	-	-
5	21.05	Nonanal	0.103±0.004	-	0.072±0.001	-	-	-
9	23.57	Furfural	0.158±0.011	0.201±0.009	0.280±0.007	0.207±0.010	0.250±0.008	0.301±0.008
10	24.30	Decanal	0.104±0.007	0.129±0.005	-	0.125±0.003	0.088±0.002	0.143±0.004
12	25.27	Benzaldehyde	2.585±0.027	3.061±0.032	1.875±0.018	2.723±0.020	-	-
13	26.98	2-Undecanone	0.144±0.008	0.174±0.009	0.239±0.014	0.138±0.006	0.129±0.007	0.143±0.010
15	29.38	Benzaldehyde, 2-hydroxy-	0.061±0.003	-	-	0.071±0.002	-	-
17	31.97	2-Tridecanone	-	0.045±0.002	0.054±0.001	0.044±0.001	0.054±0.002	0.066±0.003
		Total aldehydes and ketones	3.261 ^d	4.13 ^f	2.976 ^c	3.862 ^e	0.861 ^a	1.367 ^b

Table 3: Continue

		Fermentation layer/relative concentration (mg L ⁻¹)						
Numbers	Time/min	Flavor compounds	1	2	3	4	5	6
Terpenes								
1	14.34	D-Limonene	0.069±0.001	0.067±0.001	-	0.180±0.005	0.072±0.002	0.048±0.001
2	29.49	α-murolene	0.055±0.002	-	0.245±0.006	0.083±0.002	0.136±0.004	0.190±0.005
3	30.29	Eudesma-4(14),11-diene	-	0.241±0.005	0.606±0.008	0.304±0.006	0.743±0.011	0.143±0.005
4	31.00	δ-Cadinene	0.213±0.001	0.321±0.005	0.780±0.003	0.415±0.002	0.793±0.008	1.204±0.010
5	31.12	(+)-Epi-bicyclosquiphellandrene	0.021±0.001	-	-	0.069±0.003	0.093±0.005	0.063±0.004
6	34.62	α-Calacorene	-	0.481±0.005	0.839±0.011	-	0.900±0.008	1.363±0.009
7	38.35	δ-Selinene	-	-	0.012±0.001	0.014±0.001	0.014±0.000	0.032±0.002
		Total terpenes	0.358 ^a	1.11 ^c	2.482 ^d	1.065 ^b	2.751 ^e	3.043 ^f

Values are the Mean±Standard deviation (n = 3) letters (a, b, c, d etc.) means significance difference (p<0.05)

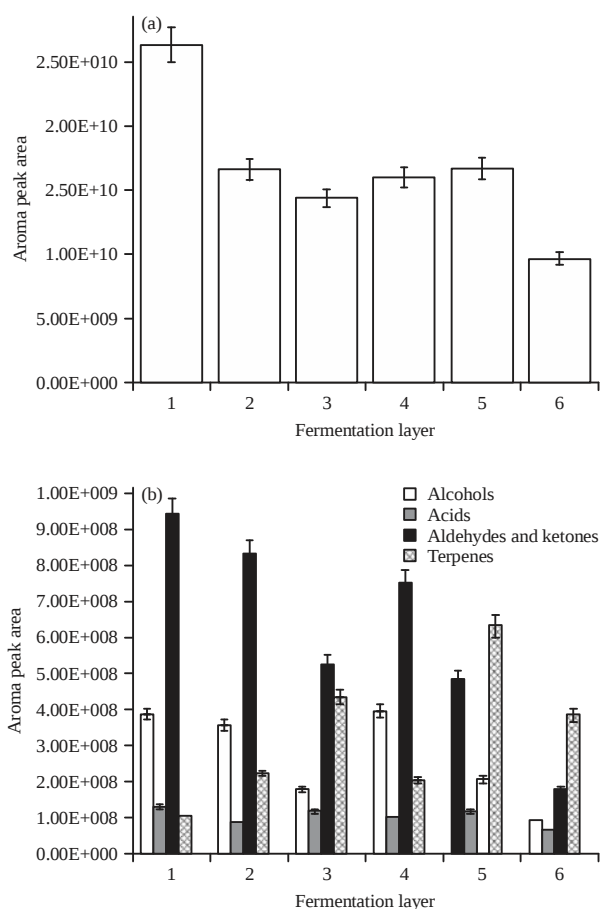


Fig. 1: Comparison on flavor compounds of jujube brandy, (a) Esters and (b) Alcohols, acids, aldehydes and ketones and terpenes from different fermentation layers
Values are the Mean±Standard deviation

respectively, suggesting some chemical reactions are produces in a decaying sine wave process. Another compound 3,4-Dihydro-2H-1-Benzopyran-2-one and heptylic acid were only found in the 2nd fermentation layer. Third, 4 and 4 flavor compounds are uniquely detected in the 4th, 5th, 6th

fermentation partitions, respectively. In conclusion, the most of the low concentration flavor compounds were formed in the early partitions of the distillation process. This may aid in focusing on consumer or scientifically interesting compounds in further studies.

Esters: On the basis of aroma concentration, esters have been shown to be one of the most important aroma classes in jujube brandy. These esters contributed to fruity, sweet and floral aromas and the flavors of apple and pineapple. Esters are formed by both fermentation and distillation and can be affected by yeast strain selection, fermentation temperature, ventilation and by sugar content. Significant differences appeared on total ester concentration of jujube brandies from different fermentation partitions (p<0.05) (Fig. 1). The 1st fermentation partition showed a negative linear relationship of esters and concentration in the partitions. As the highest ester concentration was found in the first partition and was due to the most intense esterification process and the last fermentation layer held the lowest concentration.

Ethyl esters: Ethyl esters were another dominant aroma compound class (Table 2). Ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl laurate and ethyl benzoate contribute significantly to jujube brandy due to their high aroma concentrations. Ethyl esters comprised of C4-C16 were all detected, within which unsaturated esters were formed from C6-C10. Only three branched-chain esters (Ethyl-3-methyl-butyrate, ethyl 2-methyl-octanoate and ethyl 2-hydroxy benzoate) were found exclusively in the 5th fermentation layer. Three aromatic esters (ethyl phenylacetate, ethyl-3 phenylpropionate and 2-hydroxy benzoate) were also detected. Lastly, 2-Furancarboxylic acid ethyl ester was also found with high concentration in the middle fermentation layer. Overall these ethyl esters contribute aromas and flavors, which are importance to jujube brandy quality.

Other esters: Methyl esters were formed from the esterification process between long chain fatty acids and methanol during fermentation. Methyl caprate and methyl laurate could be very important due to their high aroma concentration. Methyl caproate has been described several other foods and has a descriptor of fruity wine like. Methyl laureate is another aroma compound and is described as waxy or having a creamy property.

Butyl esters were formed from the esterification process between short chain and mid chain fatty acids and isobutanol during the fermentation. 1-Butanol, 3-Methyl-acetate and 1-Butanol 3-methyl-benzoate could be very important due to their high aroma concentration. These esters have common descriptors of banana.

Amyl esters were formed from the esterification process between mid chain fatty acids and isoamylol during the fermentation. Amyl butyrate was only found in the last fermentation layer, with the highest concentration among amyl esters. Isopentyl hexanoate was found in all fermentation layers and they are described as having apple and pineapple flavors.

Propyl esters were formed from the esterification process between mid chain fatty acids and 1-Propanol during fermentation. Their overall formation was favored in the early and mid partitions and decreased in the latter partitions.

The remaining three esters (phenyl ethyl tiglate, hexyl hexanoate, decylformate) were detected in first through 3rd fermentation layers.

Alcohols: The 4th and 5th fermentation layers were found where alcohols have highest concentration and the 6th the least ($p < 0.05$). As most alcohols are formed during fermentation, the distillation process serves to concentrate the alcohols and exclude water. Isoamylol, isobutanol, 1-Octen-3-ol and nonanol held the highest concentrations, which play an important role in aroma, taste and other characteristics of jujube brandy^{31,32}. Among the identified compounds, 1-Octen-3-ol gives a mushroom odor, whereas, benzyl alcohol gives more floral, sweet and fruity odors. Phenethyl alcohol which is also found in wine, gives rose and honey odors.

Acids: The identified acids overall had low concentration in jujube brandy. Among the different partitions the 2nd and 6th showed similar flavor concentration, which were different from other layers ($p < 0.05$). The major acid components are decanoic and dodecanoic acid due to their high

concentrations. Dodecanoic acid is particularly important as it can be described as giving cheese odors. Heptanoic acid can only be detected in the 2nd fermentation layer and 9,12-Octadecadienoic acid can only be found in the middle and low fermentation layers.

Aldehydes and ketones: High fermentation layer samples showed higher concentration of aldehydes and ketones than the low samples ($p < 0.05$). Benzaldehyde, furfural, 2-Undecanone and decanal were observed having high concentrations. These compounds could be contributors due to their functional structure. Benzaldehyde had the highest concentration among aldehydes, which was found in the middle and low fermentation layers, lending bitter almond notes, while furfural gives sweet odors. The unsaturated aldehydes, 2,4-Decadienal, 2,4-Hexadienal, 2-Nonenal were detected in the low fermentation layers. However, some aldehydes can only be found in specific fermentation partitions. For instance, 1-[4-(1-methyl-2-propenyl)phenyl]-ethanone can only be detected in the lowest fermentation layer and 3,4-dihydro-2H-1-Benzopyran-2-one only the 2nd fermentation layer, while some higher molecular weight aldehydes are absent in the first fermentation layer. These observations give indications of importance of these functional group compounds for further analysis and understanding in their significance in jujube brandy.

Terpenoids and other compounds: Terpenoids showed higher concentration in the middle and low fermentation layers ($p < 0.05$). The α -calacorene, δ -cadinene and Eudesma-4(14),11-Diene were observed to be highly abundant and contribute to the aroma profile. The furans 2-(2-propenyl)-furan and 2-pentyl-furan, were also detected in jujube brandy. This compound has also been shown to contribute to fermented products through these characteristics green flavor.

PCA analysis: In order to understand the relationships of the observed compounds, PCA was utilized to visually render the variation in the samples. Figure 2 provides a bi-plot showing the score plots as well as the loadings plots of the first two principal components (PC1 and PC2, respectively). PC1(78.099%) and PC2(14.370%) accounted for 92.469% of the total variability for jujube brandy samples. The PCA of present study explains most of the variation in the samples suggesting present study method is robust and captures the variability present in experimental design.

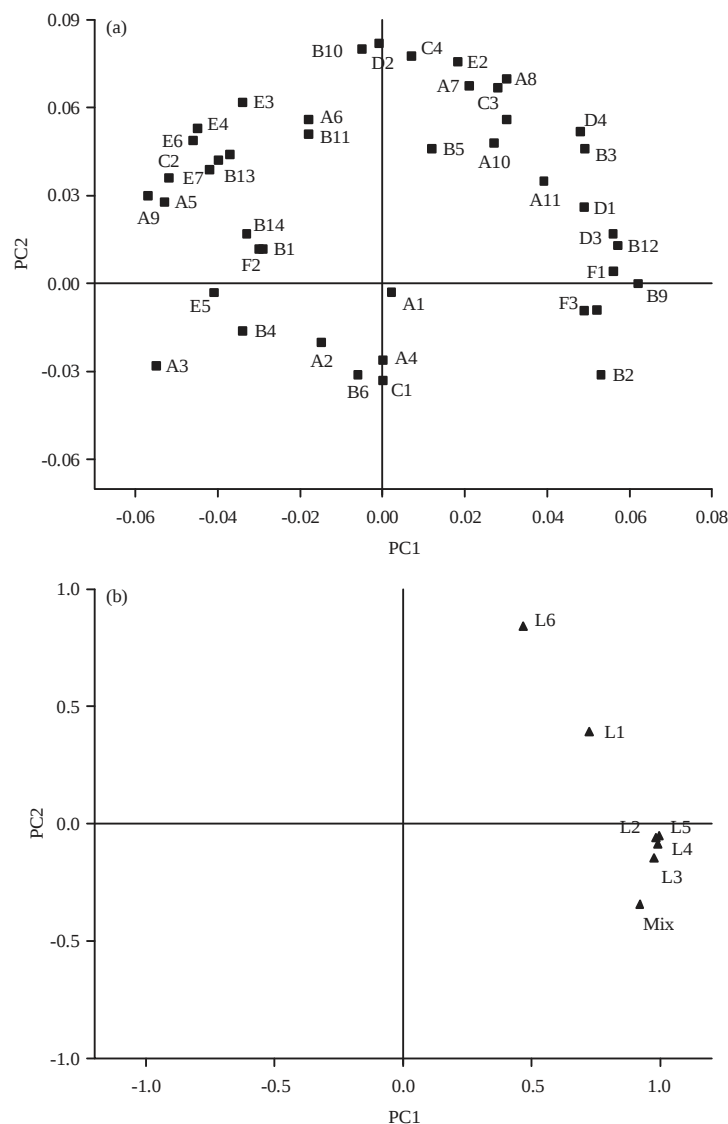


Fig. 2(a-b): PCA analysis of jujube brandy of different layers

The distribution of the samples in PCA showed four separate groups. The sample from partitions 2, 3, 4, 5 gathered closely, which represent they were similar. Samples of 1st, 6th layer and mixed sample were segregated outside this group. In the case of the mixed samples, the primary compounds that led their segregation were due to alcohols and two acids. The alcohols found in the 6th sample were isoamylol, along with non-2-en-1-ol, ζ -murolene and the acids benzoic acid ethyl ester, decanoic acid ethyl ester and decanoic acid. The variance that was explained in the PCA regarding the 1st sample could be traced to seven compounds (1-Octene-3-ol, nonanol, lauric acid ethyl ester, ethyl 3-phenylpropionate, 1-Hexanol, 2-Nonanone, 2-Undecanone). The four samples

that grouped closely had similar flavor compounds consisting of borneol, limonene, 1,1-2 Ethoxy-3-methyl-butane, 2-amyl furan.

E-nose analysis of flavor compounds

Classification results of jujube brandy by E-nose: Figure 3 shows the result of PCA analysis and shows clear differences in the studied samples. In the correlation matrix mode, the classification contribution rate of the first component is 99.921% and the sum of classification contribution rate of the second principal component reached 99.992%. The two principal components represent the significant majority of the data in the samples. In regards to individual partitions, the 4th

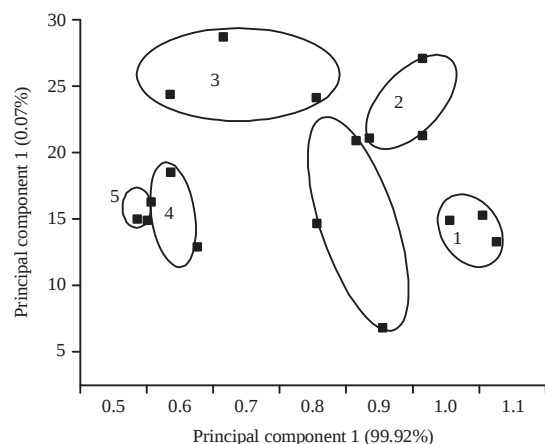


Fig. 3: PCA analysis of jujube brandy by E-nose

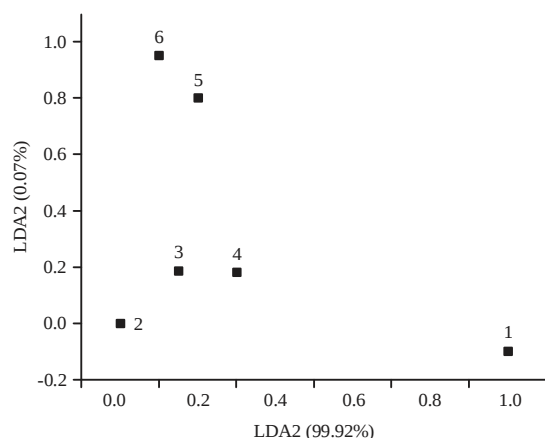


Fig. 4: Loading analysis of jujube brandy from different fermentation layers

and 5th fermentation layer samples group together showing similarity amount the compounds found. The 1st and 2nd samples show a relationship but are distinctly resolved from the 4th sample. In regards to the 6th sample, it was significantly resolved compared to the 5th while showing a similarity with remaining samples. The variation showed was not consistent over the course of the sampling.

Figure 4 shows the result of loading analysis. In this study, loadings algorithm was aimed at sensor analysis so as to confirm the contribution of each sensor to a distinct sample in specific conditions and then uncover which compounds play a main role in classifying samples. As it can be seen from the correlation matrix model chart of PCA, No. 2 sensor (W5S) showed the highest contribution rate on the first principal component. No. 7 sensor (W1W) contributed to the largest variance in the second principal component. So, oxynitride may be the main flavor compounds because of their high contribution rate.

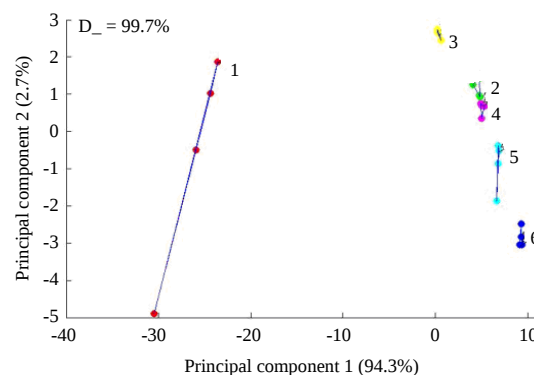


Fig. 5 PCA analysis of jujube brandy by E-tongue

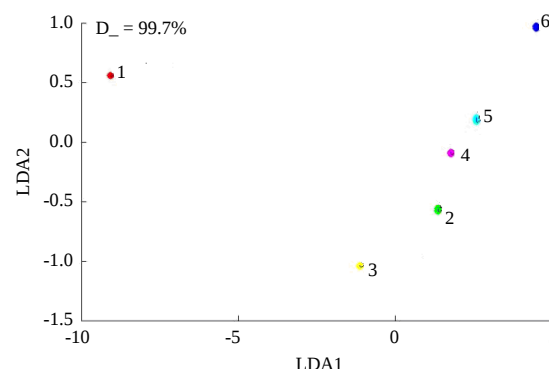


Fig. 6: Loading analysis of jujube brandy by E-tongue

Therefore, PEN3 electronic nose of Germany AIRSENSE Company can distinguish these 6 kinds of jujube brandies from different fermentation layers well. Unknown samples can be determined by the establishment of template files of different fermentation layers.

E-tongue analysis of flavor compounds: Sensor combination of S2_100HZS4_10HZS5_10HZS6_10HZ was used in this test, which showed good effect and frequency. It can be seen from the Fig. 5 and 6 that DI is 99.7%, resulting in a significant differentiation of the samples using PCA analysis. The total contribution rate of the main composition 1 and main component 2 was 97.0%, demonstrations that these two principal components can fully represent the vast majority of the sample signal. The experiments were repeatable and resulted in resolution all samples using principal component analysis (PCA).

In addition, there was no significant difference among 2nd to 6th fermentation layer samples. It can be seen from the chart that the 1st was clearly resolved, showing a differential chemical composition from the remaining samples resulting in this topology.

Comparison on aroma components of jujube and jujube brandy:

Jujube brandy contains more abundant aroma compounds than fermentation product or the aged fermentation product. This could be due to the concentration of aroma compounds during distillation and formation of compounds during distillation. Esters are major aroma compounds found in jujube brandy studied. This class of compounds contained 42 ethyl, 6 methyl, 11 butyl, 4 pentyl and 4 propyl esters in the jujube brandy. The amount and concentration of flavor compounds in jujube brandy were clearly higher than the fermented un-distilled jujube samples. Among the differences jujube brandy had high concentrations of ethyl hexanoate, ethyl benzoate and ethyl laurate, which were also found in the undistilled jujube samples.

Concentration of acids of jujube brandy was largely lower than jujube. Acetic, propionic, butanoic and hexanoic acid were found higher concentrations in the undistilled samples but were undetected in the jujube brandy. This is because esterification reactions occur between acids and alcohols during fermentation and aging and are removed during distillation.

Some ethers, namely, 3-Methyl butyl ether and ethyl butyl ether, were undetected in all samples. This situation also found in some plant biology components, such as phytol, tert-hexadecanethiol, N-methoxy formamide, N-methoxy and so on according to Galindo *et al.*³³ and Guo *et al.*³⁴.

CONCLUSION AND FUTURE RECOMMENDATIONS

Ethyl laurate, ethyl phenylpropionate and ethyl tetradecanoate butanoic acid 3-methyl-ethyl ester, hexanoic acid ethyl ester, octanoic acid ethyl ester were the most important flavor compounds by GC-O and OAV analysis. Flavor compounds of 2nd, 3rd, 4th, 5th segregated together whereas the 1st, 6th and mixed sample were all separate as shown by PCA analysis. The 1st fermentation layer showed the highest total peak area and the 6th contained the least. The 4th and 5th fermentation partitions contained the highest concentrations of alcohols, however acids showed little fluctuation between different fermentation layers. High fermentation layers showed higher concentrations of aldehydes and ketones than the low ones and terpenoids showed higher concentration in the middle and low fermentation layers. The E-nose and E-tongue results showed that flavor compositions from different fermentation layers could be well discriminated by PCA and LDA.

In comparison to classical techniques, this simultaneous utilization of the E-nose and the E-tongue represents a faster and cheaper recognition tool for industry. These results open

the path to the possibility of using E-nose and E-tongue to discriminate jujube brandies from different fermentation layers qualitatively and to predict jujube brandy quality. This method is novel to jujube brandy and shows clear improvement is possible using this method. In the near future, qualification and quantization of jujube brandy based on E-nose and E-tongue should be improved.

Knowing the primary organoleptic traits of this brandy will lead to further scientific understanding and quality improvements. In particular the systemic differences in the origination of the compounds in different partitions is not fully understood. More in-depth determination of their origin and how their formation will lead to a richer understanding of jujube brandy production and lead to quality improvements.

SIGNIFICANCE STATEMENTS

This study discovers the flavor compositions of jujube brandy from different fermentation layers that can be beneficial for improvement of brandy quality. This study will help the researcher to uncover the critical area of brandy in different fermentation layers that many researchers were not able to explore. Thus, a new theory on flavor compositions and possibly nutrients and flora of brandy from different fermentation layers, may be arrived at.

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- Study on the flavor character and its formation mechanism of Chinese date brandy(Founding No. 31371815)

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