Advance Journal of Food Science and Technology 8(9): 612-621, 2015
DOI:10.19026/ajfst.8.1575
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: December 10, 2014 Accepted: January 21, 2015 Published: July 05, 2015

Research Article

Analysis on Flavor Compounds of Jujube Brandy during Distillation by HS-SPME-GC/MS, E-nose and E-tongue

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Abstract: In order to find the change of flavor compounds in jujube brandy during distillation, HS-SPME-GC-MS, E-Nose and E-tongue were used to analyze flavor compositions during the distillation of jujube brandy. The results of GC-MS were as follows: total esters content dropped significantly when alcoholicity under 66.5% vol and the content of monoester decreased in the early stage of distillation; Alcohols and most higher alcohols decreased, while phenylethyl alcohol increased in the distillation; The number of acids added, acetals dropped and aldehydes, furfural and benzaldehyde presented fluctuation during distillation. E-Nose and E-tongue results showed that flavor compositions during the distillation could well be discriminated by PCA and LDA, aromatic compounds and oxynitride were the main flavor compounds because of high contribution rate and foreshot and after-run had a big difference with other samples.

Keywords: Aroma, distillation, E-nose, E-tongue, GC-MS, jujube brandy

INTRODUCTION

Brandy, one of the world's six distilled wines, mostly uses grape as a raw material. Jujube brandy, a unique brandy product in China, has a long history. Jujube brandy is produced by solid fermentation, distillation and aging using jujube as a raw material. The sensory characteristics of jujube brandy are heavily influenced by its volatile flavor components. Distillation is the key stage to brew a high-quality brandy, which need to collect volatile flavor compounds as much as possible in the fermented materials (Yang and Peppard, 1994). Therefore, the volatile flavor components of jujube brandy during the distillation should be analyzed.

Qualitative and quantitative characterizations of volatile compounds in wine are usually performed by GC-MS, one of the most sensitive techniques for the analysis of aroma in different samples. Solid-Phase Micro-Extraction (SPME) is a relatively new and simple adsorption technique for the isolation of headspace flavor compounds (Arthur and Pawliszyn, 1990). Headspace SPME sampling requires neither solvent extraction and purification steps nor a complicated purge-and-trap apparatus (Arthur *et al*., 1992). This technique shows high repeatability and possibility of carrying out simultaneous extractions, which is one of its advantages over other solvent-free techniques (Zhang and Pawliszyn, 1993).

Electronic nose (E-nose) and Electronic tongue (E-tongue), which are actually simulations of human nose and taste respectively (Escuder-Gilabert and Peris, 2010), have proven to be good alternatives for traditional techniques in odor and taste analysis of food (Pan *et al*., 2014; Longobardi *et al*., 2015). E-nose and tongue are fast analytical systems that provide global information about the sample instead of information on particular components (Rodriguez-Mendeza *et al*., 2014). At present, electronic nose and electronic tongue applied to jujube brandy testing is still in the preliminary stage in China, the corresponding study has not been reported (Zhang *et al*., 2014; Xiong *et al*., 2014).

In this research, HS-SPME-GC/MS was employed to analyze flavor compositions during the distillation of jujube brandy. Meanwhile, E-nose and E-tongue were used to distinguish these different-quality jujube brandies.

Practical application:

- Change rules on flavor compounds of jujube brandy during distillation were analyzed by GC-MS so as to get high-quality liquor.
- E-nose and E-tongue discriminated nine types of jujube brandies during distillation well, which can be taken into practice.

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

Number	Sensors	Performance description	Reference
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_2S 1 mL/m^3
8	W2S	Broad-alcohol	CO 100 mL/m^3
9	W2W	Sulph-chlor	H_2S 1 mL/m^3
10	W3S	Methane-aliph	CH_4 10 mL/m^3

MATERIALS AND METHODS

Jujube brandy: Samples are fresh jujube brandies (Hebei, Fuping) during distillation (The average alcohol is 50%).

Sampling method: Sampling 100 mL every other 10 L jujube brandies, amount to 9 samples, which are foreshot, 10, 20, 30, 4, 50, 60 and 70 L, respectively after-run.

HS-SPME parameters: Jujube brandy was diluted by distilled water (10% alcohol content). Sodium chloride (1 g) was added to 7.5 mL of sample solution in a 20 mL sealed glass vial. The sample was exacted at 40°C for 40 min with 50/30 μm DVB/CAR/PDMS fiber, then used to GC-MS analysis.

GC-MS parameters: Flavor compounds of jujube brandy were detected by GC-MS. The contents of flavor compounds were quantified using an internal standard (3-octanol, 99%, Sigma-Aldrich). Wine volatile compounds were analyzed using an Agilent 5975 Mass Spectrometer coupled to an Agilent 7890A Gas Chromatograph (Agilent, Santa Clara, USA). 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. The temperature was further raised to 230°C at 5°C/min and maintained at 230°C for 6 min. The carrier gas had a flow rate of 1.0 mL/min. Samples were injected using the splitless mode. A mass range of 50-550 m/z was recorded at one scan per second.

Electronic nose system: The 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 elaboration. The sensor array system is composed of 10 Metal Oxide Semiconductors (MOS) of different chemical compositions and thicknesses to provide selectivity toward volatile compound. Table 1 lists all used sensors and their main applications. This table contains current known or specified reaction; the detection limit values are also given.

A 10 mL sample juice was taken into a 500 mL beaker and the beaker was sealed by plastic warp for a headspace generation time of 30 min to ensure that the headspace volatiles could be enough for detection. Samples were detected by E-nose immediately. The measurement phase lasted for 60 sec, which was long enough for the sensors to reach stable signal values. After each experiment, cleaning gas (nitrogen) was pumped into the sample gas path for 100 sec, which was long enough to normalize sensor signals. During the sampling process, the sample gas was transferred into the sensor chamber at a flow rate of 300 mL/min and per 60 sec a signal was collected. After 30 min for the headspace gerneration, the temperature of samples for the E-nose detection was same as the environment.

Electronic tongue system: An E-tongue (isenso, Shanghai Ruifen International Trading Co., Ltd., China) was employed to classify and characterise the jujube brandies. This instrument mainly 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 most substances found in the liquid matrix, providing a global liquid and taste perception. Table 2 lists all the sensors and their thresholds for five basic tastes.

Experiment was carried out with filtrated strawberry juice to avoid the influence caused by solid particles. The amount of sample was 30 mL to ensure that the sensors could be fully immersed in the liquid. The measurement time was set to 120 sec for each sample, which was long enough for the sensors to reach stable signal values and the sensors were rinsed for 10 sec using deionized water to minimize and correct the drift of sensors. The temperature of samples for the E-tongue detection was 20±3°C. The detection voltage is between -1V-1V, with interpulse interval 100 mV and sensitivity 10^5.

Statistical analysis: Principal Component Analysis (PCA) is a multivariate technique that analyzes a data table in which observations are described by several

Table 2: Sensors used and their thresholds in electronic tongue (unit: mol/L)

Basic taste	Taste substance	ZZ	BA	BB	CA	GA	HA	JB
Sour	Citric acid	10^{-7}	10^{-6}	10^{-7}	10^{-7}	10^{-7}	10^{-6}	10^{-6}
Salty	NaCl	10^{-6}	10^{-5}	10^{-6}	10^{-6}	10^{-4}	10^{-4}	10^{-5}
Sweet	Glucose	10^{-7}	10^{-4}	10^{-7}	10^{-7}	10^{-4}	10^{-4}	10^{-4}
Bitter	Caffeine	10^{-5}	10^{-4}	10^{-4}	10^{-5}	10^{-4}	10^{-4}	10^{-4}
Savory	MSG	10^{-5}	10^{-4}	10^{-4}	10^{-4}	10^{-5}	10^{-4}	10^{-4}

inter-correlated quantitative dependent variables (Aaby *et al*., 2012). 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 and each succeeding component in turn has the highest variance possible under the constraint that it be orthogonal to the preceding components. The higher cumulative contribution rate is and the more original information will be reflected.

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 them (Hong *et al*., 2012). 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.

RESULTS AND DISCUSSION

GC-MS analysis of flavor compounds: Table 3 was the comparison of main flavor compounds of jujube brandy during distillation.

Change rules of esters: Figure 1 showed that total esters of jujube brandy changed little from foreshot to 30 L and dropped dramatically when alcohol content is less than 66.5% vol. Boiling point lower than ethanol, ethyl acetate is the main flavor of wine, its fragrance is pineapple alike, which is one of the main technical requirements of distilled wine and compound wine (Li and Li, 2010); The content of ethyl acetate reach the highest levels in foreshot, then reduced gradually with the distillation. With boiling point of other esters higher than ethanol, ethyl butyrate, ethyl caproate and ethyl caprylate, which boiling point is similar, changing trends are also similar. The contents of ethyl benzoate, decanoic acid ethyl ester, ethyl laurate showed similar trends, slowly rose from foreshot to 30 L, then began to drop dramatically. It is visible that in the distillation of jujube brandy, the contents of esters have a certain relationship with its boiling point, which was on the decline as a whole.

Change rules of alcohols: Figure 2 showed the change rules of alcohols of jujube brandy during distillation. The highest three contents of alcohols were isoamylol, 1-Octen-3-ol and phenethanol, which play an important role in the formation of aroma, taste and other characteristics of jujube brandy (Yan *et al*., 2008). The total alcohol content is on the decline during distillation. Most alcohols showed the highest contents between the foreshot and early stage of distillation, such as isobutanol, isoamylol, 1-Octen-3-ol, 1-dodecanol, 10-dodecanol, then declined gradually and reached the lowest in after-run, some of which even cannot be found, like isobutanol, 1-dodecanol, 10-

Table 3: Comparison of main flavor compounds of jujube brandy during distillation

Time /min	Compounds	Sampling/content (mg/L) Foreshot	10 L	20 L	30 L	40 L	50 L	60 L	70 L	After-run
	Ethanol (50%)	73.0%	72.5%	70.5%	66.5%	59.0%	49.5%	36.5%	22.0%	15.0%
	Esters									
5.95	Ethyl acetate	0.115	0.053	0.050	0.036	0.035	0.002	0.019	0.004	0.007
9.30	Butanoic acid, ethyl ester	0.349	0.151	0.087	0.075	0.025	0.018	0.016	0.010	0.014
15.74	Hexanoic acid, ethyl ester	3.901	2.083	1.403	1.232	0.541	0.448	0.217	0.078	0.104
22.44	Octanoic acid, ethyl ester	7.872	4.668	3.688	3.925	1.641	0.918	0.247	0.098	0.151
28.91	Benzoic acid, ethyl ester	1.156	1.120	1.288	1.562	1.101	0.799	0.422	0.184	0.002
28.34	Decanoic acid, ethyl ester	2.310	7.345	7.177	8.210	6.591	2.316	0.457	0.229	0.296
32.96	Dodecanoic acid, ethyl ester	8.786	7.560	8.328	8.866	4.635	2.134	0.365	0.220	0.124
	Acohols									
11.21	Isobutanol		0.009	0.003	0.001					
15.55	Isoamylol	0.393	0.365	0.357	0.356	0.220	0.013	0.097	0.051	0.029
22.80	1-octen-3-ol	0.079	0.159	0.066	0.060	0.035	0.024	0.016	0.012	0.010
22.95	1-heptanol					0.009	0.009	0.011	0.010	0.006
28.36	1-nonanol					0.072	0.055	0.048	0.023	0.017
34.33	Phenethanol	0.010	0.010	0.014	0.019	0.018	0.028	0.026	0.034	0.038
35.15	1-dodecanol	0.006	0.010	0.009	0.010	0.007	0.008	0.005	0.003	
39.68	10-dodecanol	0.005	0.003	0.003	0.003	0.003	0.004	0.003		
	Acids									
32.94	Hexanoic acid									0.015
35.30	Heptanoic acid							0.004	0.006	0.006
37.39	Octanoic acid							0.010	0.013	0.014
41.61	Decanoic acid	0.081	0.009	0.002	0.009	0.039	0.002	0.019	0.065	0.016
45.44	Lauric acid	0.080	0.026	0.034	0.032	0.025	0.040	0.055	0.020	0.009
	Aldehydes									
10.35	1, 1-ethyoxyl-3-methyl-butane	0.019	0.007	0.004	0.004	0.003	0.002	0.001		
18.77	1, 1-ethyoxyl-heptane	0.010	0.007	0.007	0.006					
23.57	Furfural		0.018	0.044	0.054	0.046	0.042	0.030	0.026	0.030
25.27	Benzaldehyde	0.184	0.204	0.266	0.343	0.288	0.218	0.125	0.086	0.082

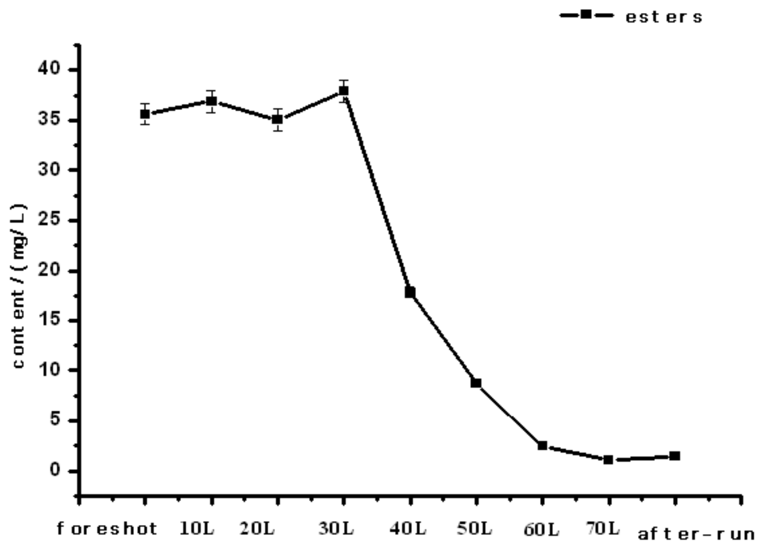


Fig. 1: Change rules of esters of jujube brandy during distillation

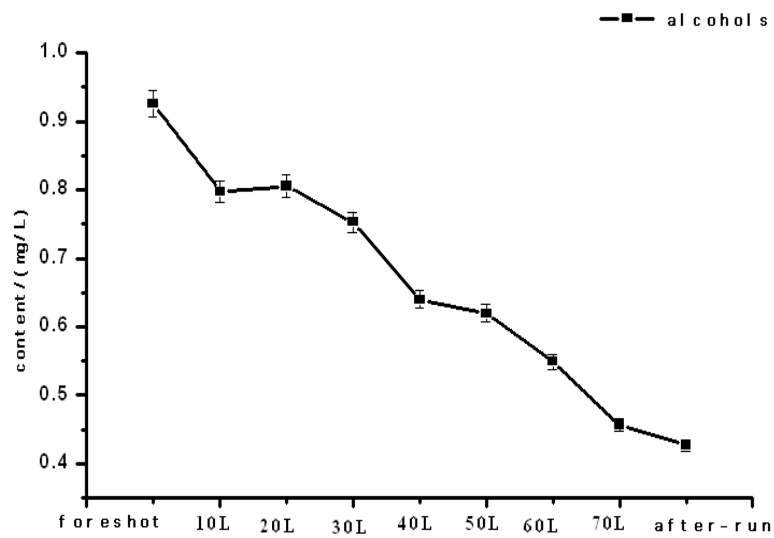


Fig. 2: Change rules of alcohols of jujube brandy during distillation

dodecanol. 1-heptanol, 1-nonanol, cannot be detected in the foreshot and appeared in the middle stage of the distillation, which showed a downward trend. In contrast, phenethanol increased gradually in the process of distillation and reach the highest contents at the after-run.

Change rules of acids: Content of total acids in jujube brandy showed fluctuation during the distillation, reached the lowest at 10 and 50 L. Types of acids increased in the process of distillation, reached most in the after-run. Caproic acid and heptanoic acid can only be detected in the later stage of distillation and showed a rise trend. Decanoic acid showed a similar trend with the total acids. Contents of lauric acid also showed fluctuation during the distillation, reached the maximum in the foreshot, minimum in the after-run.

Change rules of aldehydes: Figure 3 and 4 showed the change rules of total aldehydes, furfural and benzaldehyde during distillation. During the distillation, total contents of aldehydes of jujube brandy rise first, reached the highest levels at 30 L, then decreased and remain stable in the after-run. Furfural and benzaldehyde followed a similar change trend with the total aldehyde, furfural cannot be detected in the foreshot. Unlike them, acetal compounds lke 1, 1-ethoxy-3-methyl-butane and 1, 1-ethoxy-heptane had the highest contents in the foreshot, then reduced gradually, even cannot be found in the later stage of distillation.

E-nose analysis of flavor compounds:

Characteristics response of E-nose to flavor compounds: Sensor response to flavor compounds can

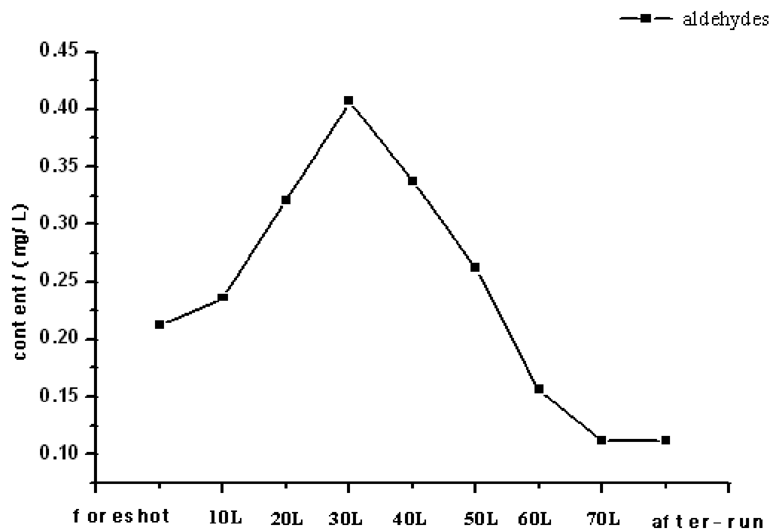


Fig. 3: Change rules of aldehydes of jujube brandy during distillation

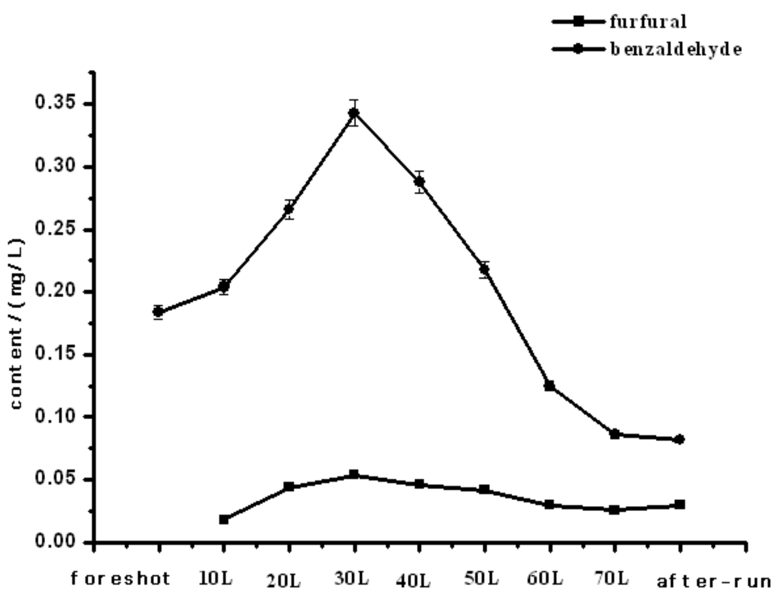
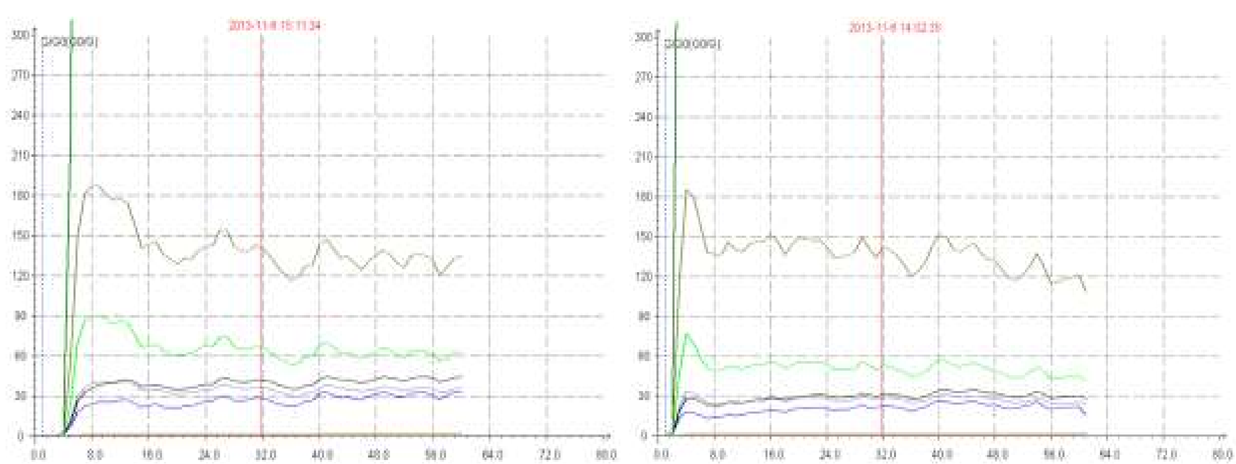


Fig. 4: Change rules of furfural and benzaldehyde during distillation



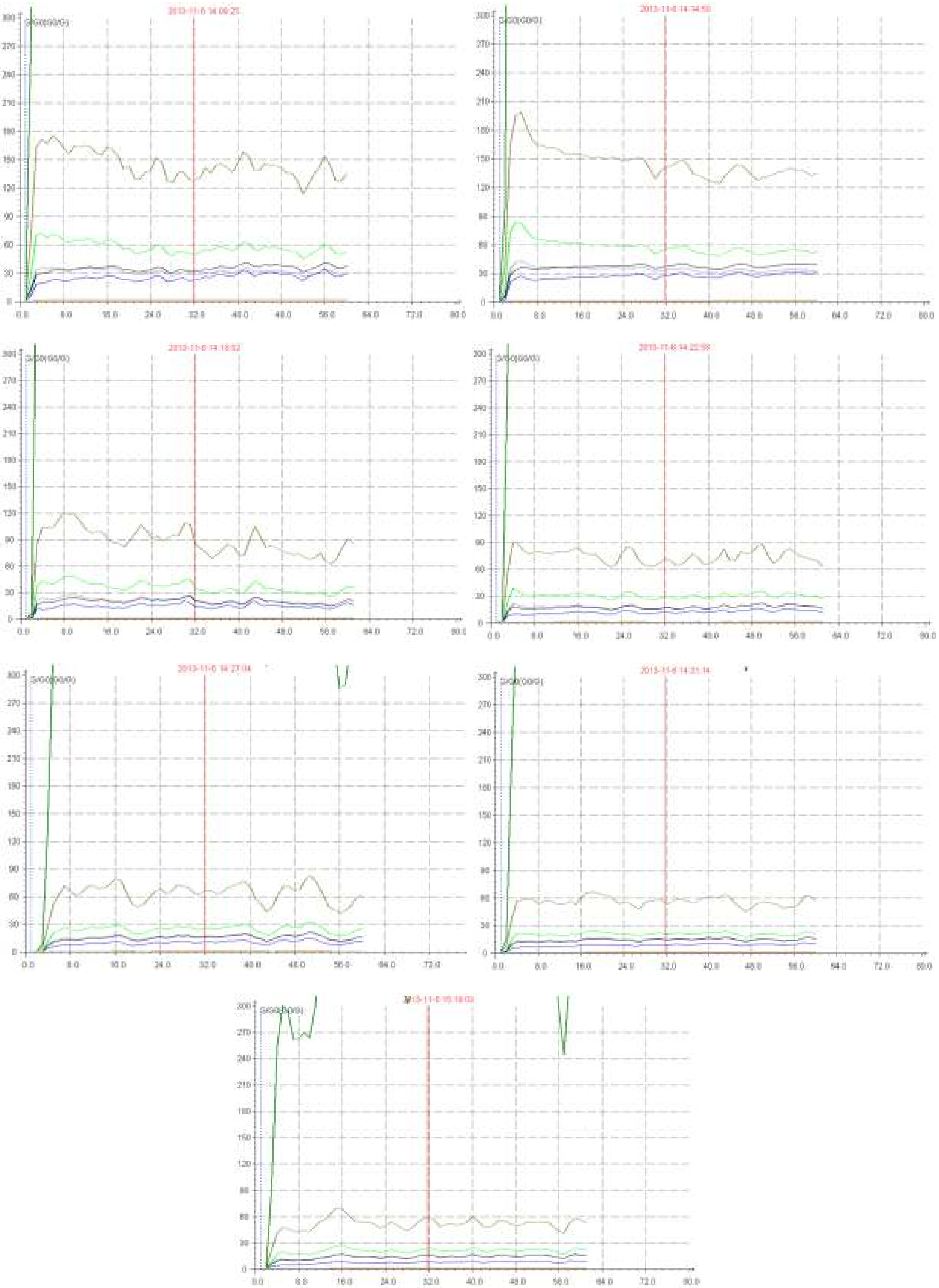


Fig. 5: Characteristics radar charts of distillate (foreshot, 10, 20, 30, 40, 50, 60 and 70 L and after-run, respectively)

be seen clearly by the change curve of each sensor response signal, the signal value of each point in time and star radar chart or columnar fingerprint. Response in different numbers of sensors can also be seen by sensor selection set. Each sensor was exclusive to specific flavor compounds and response heavily, so which kind of characteristic flavor compounds in sample can be determined.

Figure 5 were the characteristics radar charts of distillate, also called response curves of 10 sensors to volatiles, including corresponding G/G0 (or G0/G) values of each sensor. It can be seen from the figures that, response intensity of sensors tend to decrease during distillation.

Classification results of jujube brandy by E-nose: Figure 6 and 7 showed the result of PCA analysis. After-run sample was not chosen because of low parallelism. In the Correlation matrix mode, the classification contribution rate of the first main part is 99.926%. The sum of classification contribution rate of the two principal component reached 99.987%, so the two principal components can basically represent the main characteristics information of the sample. (10, 20,

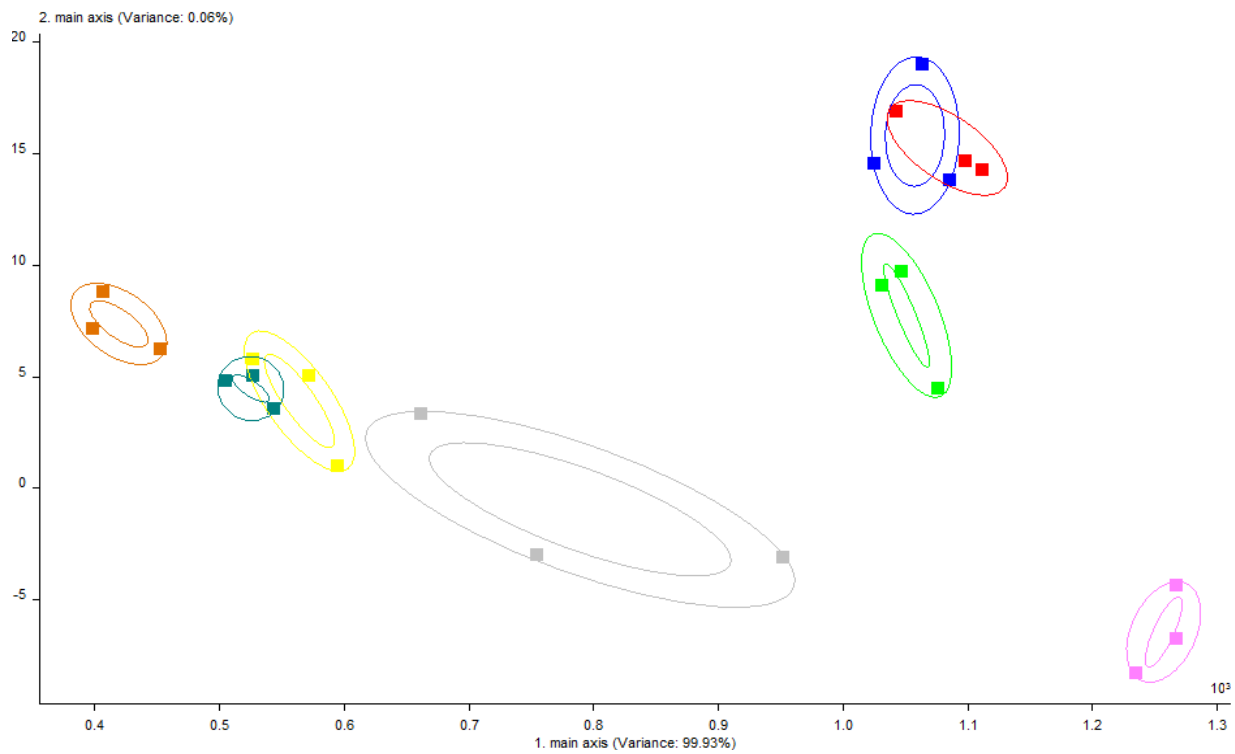


Fig. 6: PCA analysis of jujube brandy by E-nose

PCA-Analysis

normalization : PCA :
Matrix : Correlation-M.
Algorithm: PCA
Variance: : 99.987 %
1. main axis: 99.926 %
2. main axis: 6.1096e-002 %
Pattern File:
Discrimination power:

	10L	20L	30L	40L	50L	60L	70L	酒头
10L		0.145	0.227	0.704	0.989	0.994	0.994	0.960
20L	0.145		0.363	0.696	0.990	0.996	0.995	0.974
30L	0.227	0.363		0.738	0.988	0.993	0.993	0.932
40L	0.704	0.696	0.738		0.620	0.701	0.818	0.880
50L	0.989	0.990	0.988	0.620		0.432	0.885	0.996
60L	0.994	0.996	0.993	0.701	0.432		0.871	0.998
70L	0.994	0.995	0.993	0.818	0.885	0.871		0.998
酒头	0.960	0.974	0.932	0.880	0.996	0.998	0.998	

Fig. 7: PCA classification analysis of jujube brandy by E-nose

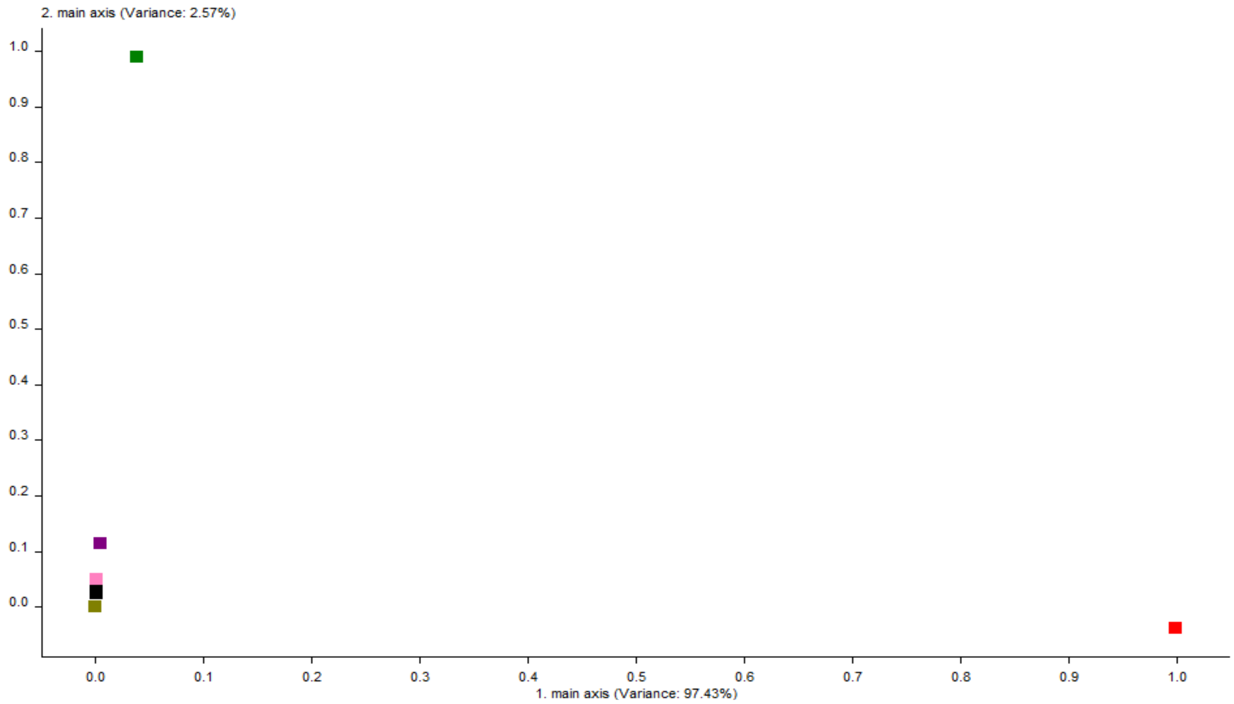


Fig. 8: Loading analysis of jujube brandy during distillation

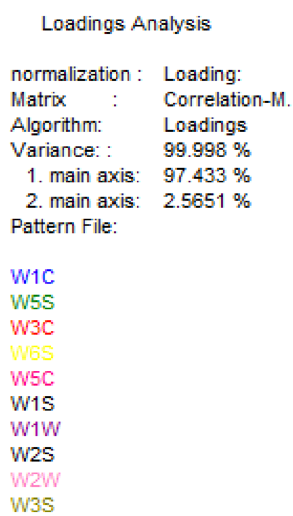


Fig. 9: Contribution rates of sensors to jujube brandy during distillation

and 30 L, respectively had a similarity with each other and different with other samples. Sample information of 40, 50, 60 and 70 L, respectively was similar. There was a big difference between foreshot and other samples, because most components of foreshot were low-boiling substance.

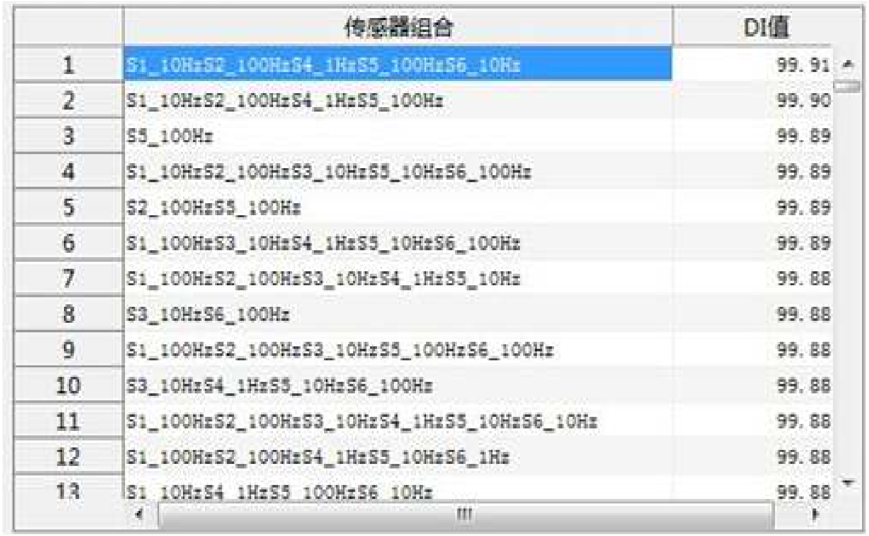
Figure 8 and 9 showed the result of Loading analysis. In this study, Loadings algorithm was aimed at sensor analysis so as to confirm the contribution of each sensor to the distinction different samples in specific conditions, then conform which kind of flavor compounds play a main role in classifying samples. As it can be seen from the Loading analysis, PCA on the correlation matrix model chart, No. 3 Sensor (W5S) showed the highest contribution rate on classify the first principal component and No. 2 sensor the largest contribution to distinguish the second principal components. So aromatic compounds and oxynitride were main flavor compounds because of their high contribution rate.

Therefore, PEN3 electronic nose of Germany AIRSENSE Company can distinguish these 9 kinds of jujube brandies during distillation well. Unknown samples can be determined by the establishment of template files of different distillates.

E-tongue analysis of flavor compounds:

Sensor optimization of electronic tongue: Figure 10 showed the sensor combination list of E-tongue It can be seen clearly from the figure that the sensor combination of S1_10HZS2_100HZS4_1HZS5_100HZS6_10HZ showed the best effect and frequency.

Classification results of jujube brandy by E-tongue: It can be seen from the Fig. 11 and 12 that DI is 99.9%, means PCA analysis and Loadings of the overall classification showed great effects. The total contribution rate of the main composition 1 and main component 2 was 98.2%, means these two principal components can fully represent the entire information of the sample. The experiments had good repeatability, samples occupied different areas of the figure, there was no overlap between each other, so Principal



	传感器组合	DI值
1	S1_10HzS2_100HzS4_1HzS5_100HzS6_10Hz	99.91
2	S1_10HzS2_100HzS4_1HzS5_100Hz	99.90
3	S5_100Hz	99.89
4	S1_10HzS2_100HzS3_10HzS5_10HzS6_100Hz	99.89
5	S2_100HzS5_100Hz	99.89
6	S1_100HzS3_10HzS4_1HzS5_10HzS6_100Hz	99.89
7	S1_100HzS2_100HzS3_10HzS4_1HzS5_10Hz	99.88
8	S3_10HzS6_100Hz	99.88
9	S1_100HzS2_100HzS3_10HzS5_100HzS6_100Hz	99.88
10	S3_10HzS4_1HzS5_10HzS6_100Hz	99.88
11	S1_100HzS2_100HzS3_10HzS4_1HzS5_10HzS6_10Hz	99.88
12	S1_100HzS2_100HzS4_1HzS5_10HzS6_1Hz	99.88
13	S1_10HzS4_1HzS5_100HzS6_10Hz	99.88

Fig. 10: Sensor combination list of E-tongue

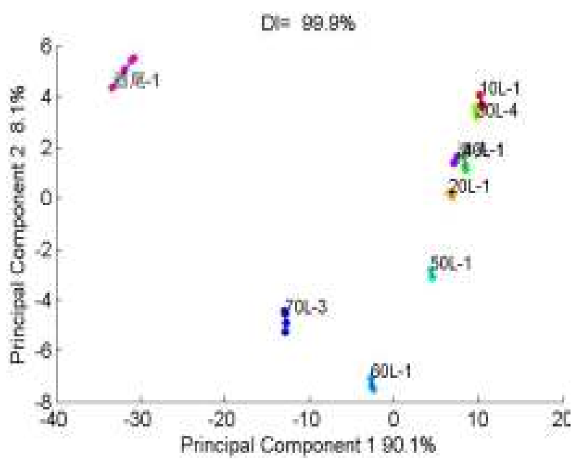


Fig. 11: PCA analysis of jujube brandy by E-tongue

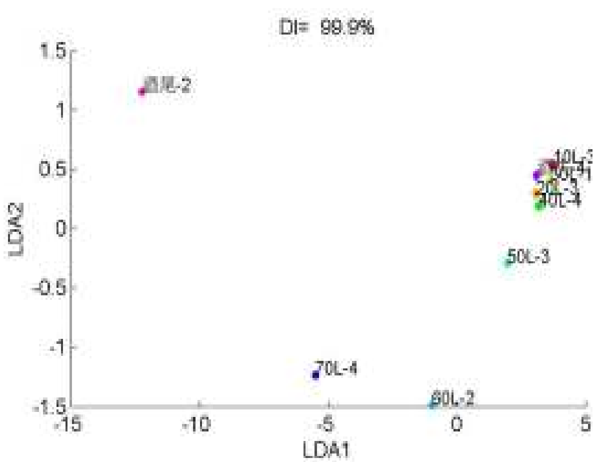


Fig. 12: Loading analysis of jujube brandy by E-tongue

Component Analysis (PCA) can distinguish the nine jujube brandies samples well.

In addition, the sample information of foreshot, 10, 20, 30 and 40 L, respectively is very similar and different from that of 50, 60 and 70 L, respectively which also had some difference between each other. It is the most obvious that after-run sample was far from other samples, means after-run and other samples exist certain differences on the taste.

CONCLUSION

HS-SPME-GC-MS, E-Nose and E-tongue were used to analyze flavor compositions during the distillation of jujube brandy. The results of GC-MS were as follows: total esters content dropped significantly when alcoholicity under 66.5% vol and the content of monoester decreased in the early stage of distillation; Alcohols and most higher alcohols decreased, while phenylethyl alcohol increased in the distillation; The number of acids added, acetals dropped and aldehydes, furfural and benzaldehyde presented fluctuation during distillation.

E-Nose and E-tongue results showed that flavor compositions during the distillation could well be discriminated by PCA and LDA, aromatic compounds and oxynitride were the main flavor compounds because of high contribution rate and foreshot and after-run had a big difference with other samples.

In comparison to classical techniques, this simultaneous utilization of the E-nose and the E-tongue represents a faster and cheaper recognition tool for liqure industry. These results open the path to the possibility of using E-nose and E-tongue to discriminate jujube brandies during distillation qualitatively and to predict jujube brandy well. In the near future, qualification and quantization of jujube brandy based on E-nose and E-tongue should be improved.

ACKNOWLEDGMENT

This research was supported by:

- The National Natural Science Foundation of China, the Project named The Research of Methanol and Fusel Oil Formation Mechanism and Control Measures in Traditional Chinese Jujube Brandy (Founding No. 31171725/2011).
- Study on the flavor character and its formation mechanism of Chinese date brandy (Founding No. 31371815).

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