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Research Article

Analysis on Main Aromatic Components of Chinese Traditional Fen-flavor Sichuan Xiaoqu Liquor by GC with Direct Injection

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Abstract: Commercially available Sichuan Xiaoqu Liquor of Chongqing Jiangjin, Chongqing Yongchuan, Sichuan Kaijiang, Sichuan Zigong were studied in this experiment. Gas Chromatography (GC) with direct injection was used for analysis on main aromatic components i.g., fusel oil (advanced alcohol), ethyl acetate, etc. The results showed that chromatogram with the similar peaks and kinds of aromatic components in four samples. And the highest among all aromatic components in Xiaoqu Liquor samples of Chongqing Jiangjin, Chongqing Yongchuan, Sichuan Kaijiang were isoamyl alcohol. The concentrations in these saples were 99.53 mg/100 mL, 117.01 mg/100 mL, 77.21 mg/100 mL, respectively. But the highest in the liquor of Sichuan Zigong was the ethyl acetate, mass concentration was up to 208.08 mg/100 mL. Isoamyl alcohol and normal propyl alcohol had close quantity relative ratio relationships in four samples, the ratio respectively were 1.0, 1.2, 1.1, 1.5, isoamyl alcohol and isobutyl alcohol ratio were 2.5, 1.8, 2.2, 2.4, respectively. Special ratio relationships among fusel oil can be used as a sign of Sichuan Xiaoqu Liquor. The main aromatic components of recovery test results was 82.09-116.53%, Relative Standard Deviation (RSD) was 1.23-6.88%. The method had fine accuracy and precision and suitable for quantitative analysis of aromatic constituents of Sichuan Xiaoqu Liquor.

Keywords: Gas chromatography, quantitative analysis, sichuan xiaoqu liquor

INTRODUCTION

According to the classification of odor type, Chinese liquor can be divided as follows: Luzhou-flavour, Maotai-flavour, Fen-flavor, Rice-flavor, Xifeng-flavor liquor and others (Soybean-flavor, Sesame-flavor, Site-flavour liquor, etc.) (Shen, 2007). And Fen-flavor liquor attracts an increasing attention from consumers because of its unique flavor and taste among numerous liquors. With the development of the international co-operation and communication, people's habits of drinking are transiting to light and elegant flavor. Xiaoqu Liquor's taste and style exactly conforms to the developing tendency (Wu *et al*., 2014; Zhong *et al*., 2010). A large number of Xiaoqu liquor were produced in Sichuan province, which was often called Sichuan Xiaoqu Liquor (Li, 2006). The so-called Sichuan Xiaoqu Liquor was the representative of this kind of liquor technology. Chinese Sichuan Xiaoqu Liquor by Solid Fermentation had a long history with its typical and unique taste. Its annual output was about 200-300 thousand KL. It experienced multiple Koji-making and summarized the experience of advanced technology and improved the technology in multi-aspect. Finally, its product odor type was identified as Xiaoqu Fen-flavor. Now, there is a set of mature technology, which promotes the development of the production. Some main features are as follows: Using the whole grain of raw materials and pure *rhizopus* and *yeast*, with less Koji (about 0.3-0.4% of raw materials) and short fermentation period (about 7 days), high liquor yield (liquor degree is not less than 65%vol) and flavor is soft, sweet and pure, simple devices and low-cost. For these enterprises, they adapted the needs of the development of township and formed the situation of steady development (Wang *et al*., 2011). The Xiaoqu liquor of Chongqing, Dazhou and Zigong city of China were the representative of Fen-Flavor Sichuan Xiaoqu liquor and so four kinds of commercially available liquor from three areas in Sichuan were choose as the object of this study with certain representativeness in experiment. Analysis of the mass concentration and the quantity relationship of fusel oil (normal propyl alcohol, isobutyl alcohol, isoamyl alcohol, etc.) and the liquor common esters (ethyl acetate, ethyl caproate and ethyl lactate, ethyl butyrate, etc.) in Sichuan Xiaoqu liquor was conducive to clarify the forming law of aromatic components (Xu *et al*., 2012; Zhang *et al*., 2007).

Refer to the research reports of aromatic components quantitative in liquor. GC and the quantitative analysis of internal standard method were

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adopted to analyze the main aromatic components (isoamyl alcohol, normal propyl alcohol, ethyl acetate, etc.) of Sichuan Xiaoqu liquor (Meng *et al*., 2011; Tang *et al*., 2010; Guo and Kang, 2008; Zhang and Liu, 2002). This study is for the purpose of finding the formation rule of aromatic components in Sichuan Xiaoqu Liquor and providing a reference for later.

MATERIALS AND METHODS

Samples: Four kinds of commercially available Fen-flavor Sichuan Xiaoqu liquor samples were obtained from four main productive Sichuan Xiaoqu liquor production base (Jiangjin district and Yongchuan district of Chongqing city; Kaijiang County and Zigong city of Sichuan province; China). The alcohol content respectively were 50, 63, 52, 62% vol, respectively. Samples were stored in refrigerator at 4°C until used.

Instrument and reagent: GC-7860 (Shanghai appropriate electronic technology co., LTD, China) and GX-300A/500A (Beijing zte huili technology development co., LTD, China) used the DB-WAX capillary column (60 m×250 μm×0.25 μm, Nanjing chromatography technology co., LTD, China). The oven temperature was held at 40°C for 5 min, then programmed from 40 to 80°C at the rate of 5°C/min and held at 80°C for 2 min. And programmed from 80 to 230°C at the rate of 10°C/min and held at 230°C for 5 min. Injector and detector temperature were both kept at 230°C. Carrier gas flow and pressure respectively were 500 mL/min and 0.08 MPa. And the ratio of nitrogen and hydrogen was 80 to 20. Micro-syringe (Shanghai Anting trace sampler works, China) was used directly and the sample size was 1 μL.

Authentic standards were obtained from commercial sources. Ethyl acetate, ethyl caproate and ethyl butyrate, ethyl lactate, amyl acetate (internal standard), isoamyl alcohol, normal propyl alcohol and isobutyl alcohol were purchased from Tianjin fine chemical industry (Tianjin, China). All of the chromatographically pure substances above were GC quality, with at least 97% purity. Analytically pure alcohol from Kelon chemical reagent factory (Chengdu, China) was freshly redistilled before use.

Standard solution preparation: Dissolve and weigh (0.0001 g) 2 mL of the chromatographically pure standards in volumetric flask and dilute with alcohol of 60%vol to 100 mL. Standard solution of 2% volume fraction was made in this way and mass concentration unit was mg/100 mL. Then each standard solution of 2% volume fraction was taken for 3 mL in 25 mL volumetric flask and diluted with alcohol of 60%vol to 25 mL. Thus, mixed standard solution was prepared to establish system template and calculate the correction factor. Finally, 1 mL internal standard (amyl acetate) of 2% volume fraction and 1 mL mixed standard solution were respectively added in 10 mL volumetric flask and dilute with each sample to 10 mL, in order to quantify and calculate sample recovery.

RESULTS AND DISCUSSION

Establish system template and calculate the correction factor: According to the various substances in the same column and the same instrument conditions

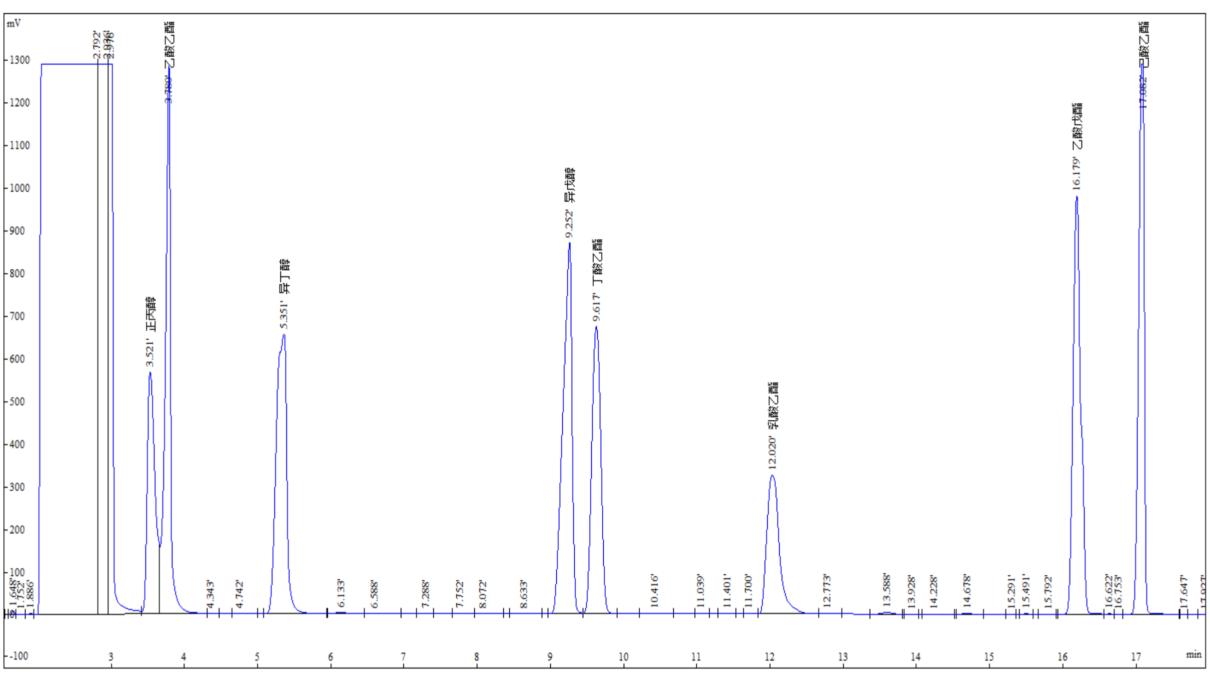


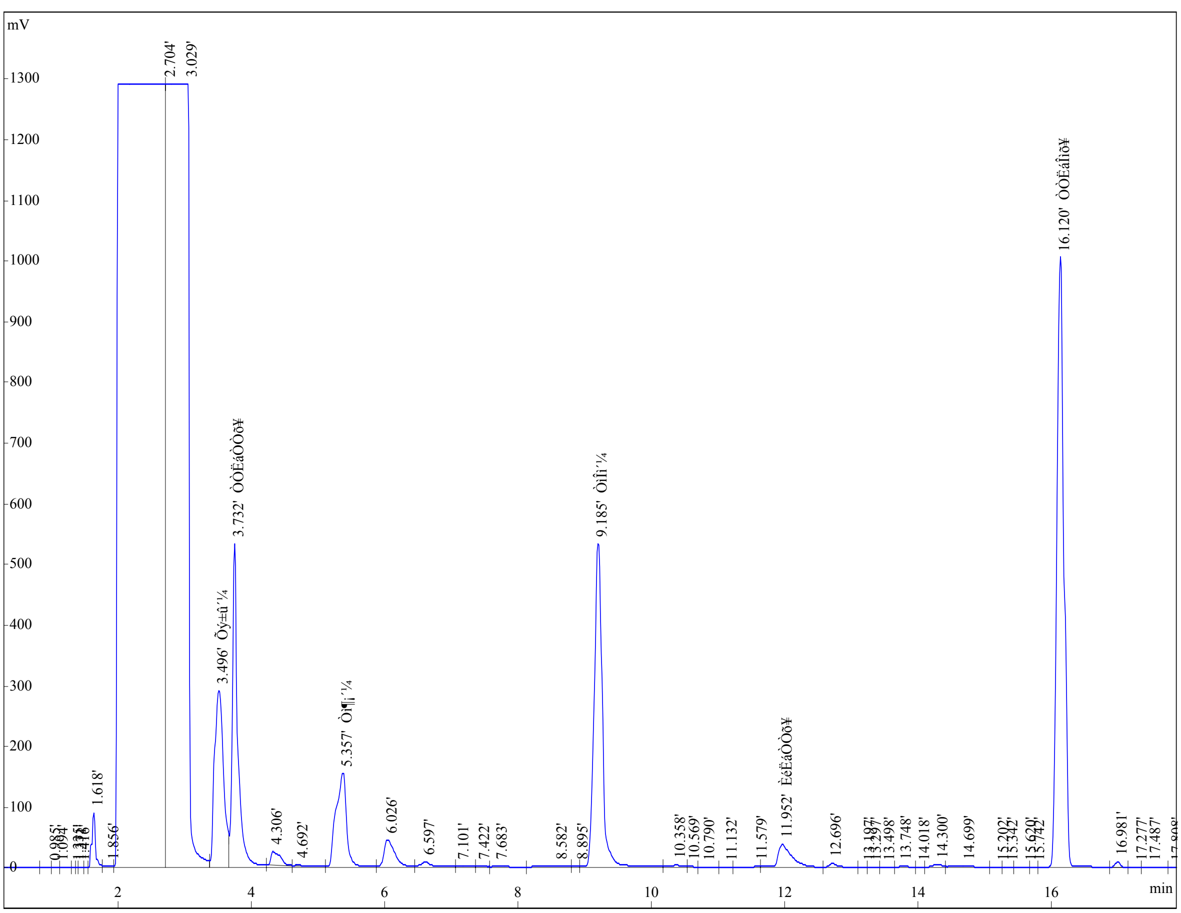
Fig. 1: Total ion current chromatogram of mixed standard solution

Table 1: The correction factor of mixed standard solution

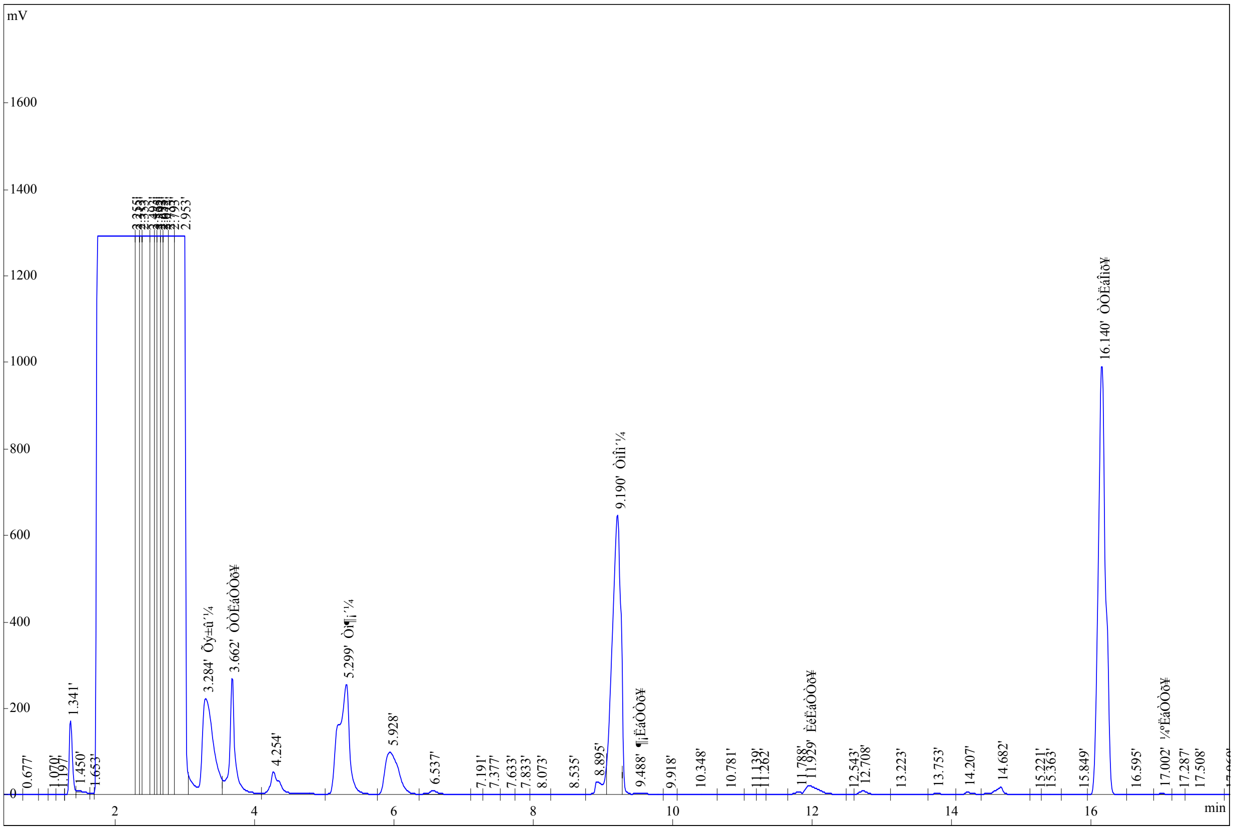
Number	Component	Mass concentration (mg/100 mL)	Correction factor 1	Correction factor 2
1	Propanol	184.656	1.3659	1.4290
2	Ethyl acetate	218.844	1.2634	1.1938
3	Isobutyl alcohol	178.932	0.9226	0.8965
4	Isoamyl alcohol	193.38	0.9078	0.8538
5	Ethyl butyrate	210.204	1.2289	1.2413
6	Ethyl lactate	227.892	2.0529	1.8138
7	Amyl acetate	208.596	1.0000	1.0000
8	Ethyl caproate	207.516	1.1869	1.0651

Number	Correction factor 3	Correction factor 4	Average correction factor	RSD (%)
1	1.4492	1.4879	1.4330	3.56
2	1.2400	1.2573	1.2386	2.54
3	0.9142	0.9410	0.9186	2.01
4	0.8814	0.9070	0.8875	2.88
5	1.2359	1.3048	1.2527	2.80
6	1.9454	1.9567	1.9422	5.06
7	1.0000	1.0000	1.0000	0.00
8	1.0679	1.2022	1.1305	6.56

had determined the same retention value and so every components in mixed standard solution can be qualitative and determined the peak order. Mixed standard solution was injected by 4 times. It can be clearly seen from the Fig. 1 that chromatographic separation effect is good. And the peaks sequence individually were propyl alcohol, ethyl acetate, isobutyl alcohol, isoamyl alcohol, ethyl butyrate, ethyl lactate, amyl acetate and ethyl caproate. Correction factor was calculated by peak area and mass concentration of mixed standard solution. Correction factor = peak area of internal standard * mass concentration of standard solution/ (mass concentration of internal standard * peak area of standard solution). From the Table 1, it can be seen that the RSD scope of 7 components in mixed standard solution was 2.01-6.56%. And so it was suitable for the quantitative analysis of aroma components because of its relatively small amount.



(a)



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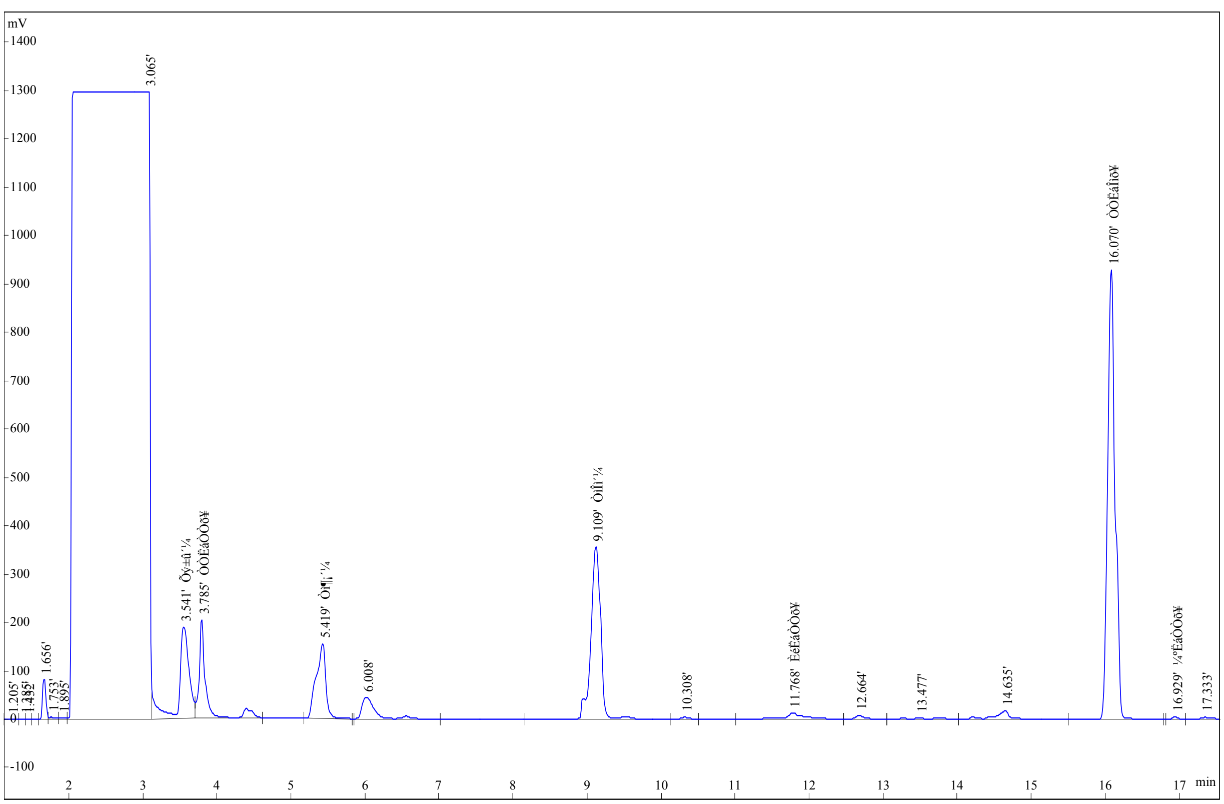
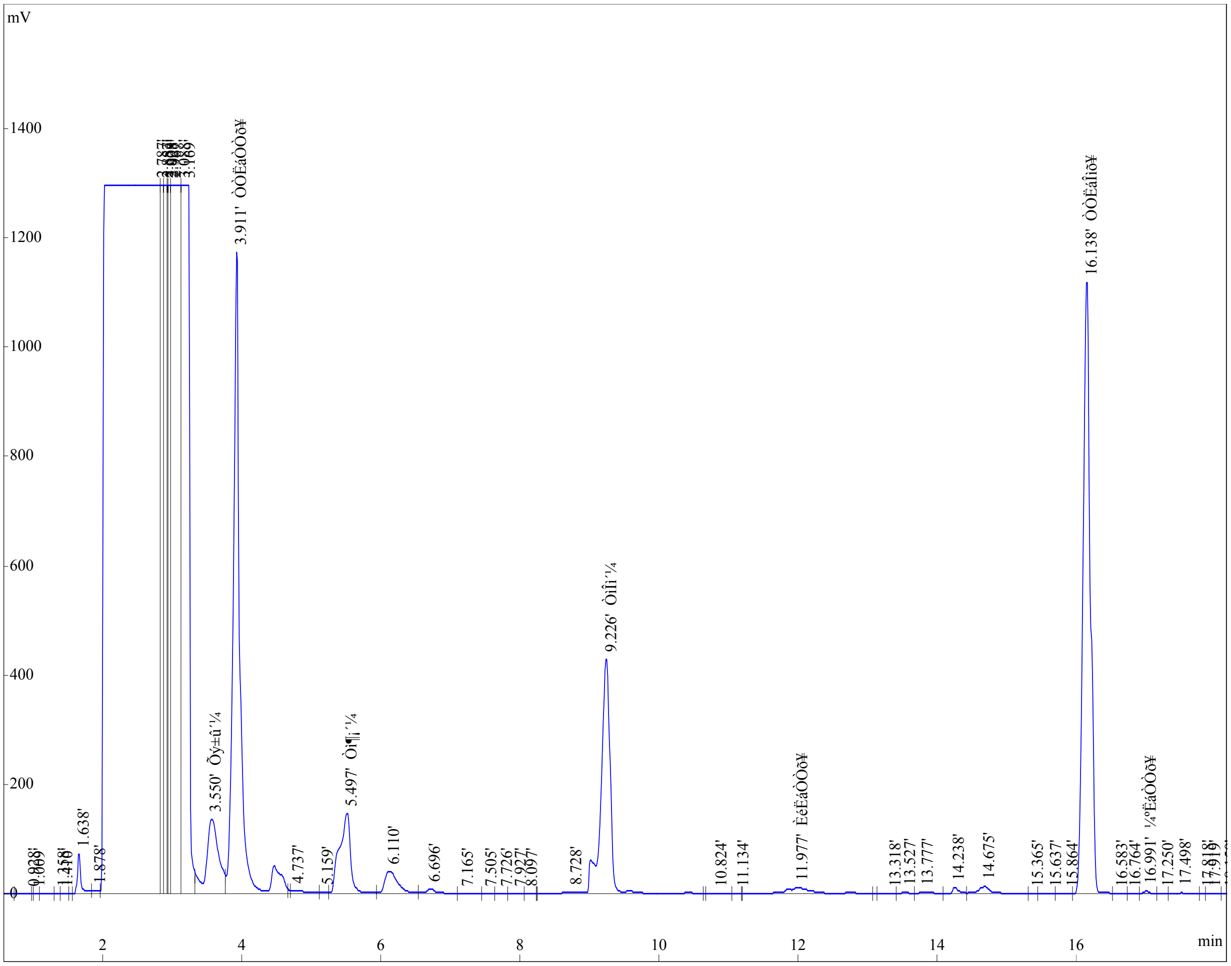


(c)



(d)

Fig. 2: Total ion current chromatogram of four kinds of Xiaoqu Liquor; (a): Total ion current chromatogram of Jiangjin Xiaoqu Liquor; (b): Total ion current chromatogram of Yongchuan Xiaoqu Liquor; (c): Total ion current chromatogram of Kaijiang Xiaoqu Liquor; (d): Total ion current chromatogram of Zigong Xiaoqu Liquor

Chromatogram of samples and qualitative analysis: From the Fig. 2, chromatographic separation effect of four samples were good. The template established was used to identify the aromatic component by specific retention time. So the peak sequence of each sample individually were propyl alcohol, ethyl acetate, isobutyl alcohol, isoamyl alcohol, ethyl butyrate, ethyl lactate, amyl acetate and ethyl caproate. Chromatogram of four different district samples were basically the same and all detected the propyl alcohol, ethyl acetate, isobutyl alcohol, isoamyl alcohol, ethyl lactate and amyl acetate (internal standard). Thus, although the producing area were different, the main aromatic components were the same from the chromatogram.

Quantitative analysis: Internal standard method was used to quantify the aromatic components in four samples. Mass concentration = correction factor *peak area of aromatic components * mass concentration/peak area of internal standard. The results were shown in Table 2. Fusel oil mainly refers to the isoamyl alcohol and isobutanol in general. As shown in the Table 2, the ratio of isoamyl alcohol and isobutanol in four samples respectively were 2.5, 1.8, 2.2 and 2.4. According to the foreign wine research showed that the ratio can distinguish different types of wine and this scope (1.8-2.5) belonged to sake's category (Yang *et al*., 2001). In view of the flavor of sake was quietly elegant and isoamyl alcohol content was also relatively high, in addition, Sichuan Xiaoqu liquor belonged to Fen-flavor. So the flavor of between both had similar place indeed. When the ratio in liquor was between 2 and 2.5, the liquor smell was less obviously. And this echoed sample's pure fragrance, sweet soft, natural harmony and aftertaste.

In addition, the ratio of isoamyl alcohol and propyl alcohol in four samples respectively were 1.0, 1.2, 1.1 and 1.5. Thus it showed that there was a close ratio between isoamyl alcohol and propyl alcohol in Sichuan Xiaoqu liquor. It can be seen from the data measured

Table 2: The main aromatic components concentration of four kinds of Xiaoqu Liquor mass concentration unit: mg/100 mL

Number	Component	Average correction factor	Jiangjin Average mass concentration	Jiangjin RSD (%)	Yongchuan Average mass concentration	Yongchuan RSD (%)
1	Propanol	1.4330	97.29	2.36	96.43	1.25
2	Ethyl acetate	1.2386	95.93	3.48	41.7	3.43
3	Isobutyl alcohol	0.9186	40.46	3.14	63.71	2.09
4	Isoamyl alcohol	0.8875	99.53	2.83	117.01	1.23
5	Ethyl butyrate	1.2527	-	0.00	-	0.00
6	Ethyl lactate	1.9422	28.09	5.28	18.63	1.78
7	Ethyl caproate	1.1305	-	0.00	-	0.00
8	Total	-	361.3	-	337.48	-

Number	Kaijiang Average mass concentration	Kaijiang RSD (%)	Zigong Average mass concentration	Zigong RSD (%)
1	71.49	2.34	54.16	1.37
2	42.03	4.01	208.08	1.42
3	34.68	1.57	33.27	0.83
4	77.21	1.85	80.01	1.59
5	-	0.00	-	0.00
6	11.15	3.69	20.8	4.75
7	-	0.00	-	0.00
8	236.56	-	396.32	-

Table 3: Recovery test of four kinds of Xiaoqu Liquor

Number	Component	Adding standard (mg/100 mL)	Jiangjin Average recovery (%)	Yongchuan Average recovery (%)	Kaijiang Average recovery (%)	Zigong Average recovery (%)
1	Propanol	18.4656	91.24	110.64	95.92	103.82
2	Ethyl acetate	21.8844	88.87	116.53	90.38	86.22
3	Isobutyl alcohol	17.8932	90.65	102.41	85.05	98.19
4	Isoamyl alcohol	19.3380	82.19	99.80	94.52	100.50
5	Ethyl butyrate	21.0204	95.66	97.91	96.38	103.79
6	Ethyl lactate	22.7892	89.87	97.76	96.76	89.66
7	Ethyl caproate	20.7516	98.33	96.87	100.44	103.06

that three district sample's total mass concentration was more than 300 mg/100 mL except Kaijiang district and this conformed with the characteristic of Fen-flavor Xiaoqu liquor. However, the mass concentration of ethyl acetate in Zigong district sample was more than 200 mg/100 mL and higher than the other three areas. It was reported that the mass concentration of ethyl acetate in Fen-flavor Xiaoqu liquor was rarely more than 150 mg/100 mL (Fan and Xu, 2014). And the ethyl lactate was less amount, it could show that this data was connected with different brewing process in local enterprises. After all, Xiaoqu liquor brewing technology with thousands of year heritage in different areas, local water quality and raw material it existed difference between each other.

In one word, the content of fusel oil and ethyl acetate belonged to the normal "Xiaoqu liquor" scope. Analysis of the several kinds of aromatic components (Gao *et al*., 2014). Propyl alcohol was fruity and the smell of the grass and the threshold value was 150 mg/L in alcohol of 10%vol. Ethyl acetate was fruity, fragrance of a flower and pleasant aroma and the threshold value was 7.5 mg/L in alcohol of 10%vol. Isobutyl alcohol was herb tea aroma and the threshold value was 40 mg/L in alcohol of 10%vol. Isoamyl alcohol was cheese fragrance, rancid, spicy and bitter almond scent and the threshold value was 30 mg/L in alcohol of 10% vol. Ethyl lactate was not sweet and the threshold value was more than 2000 mg/L. The threshold value of five components was different. In liquor system, a certain amount of the ratio relationship between fusel oil and esters formed the flavor of Sichuan Xiaoqu liquor all together.

Some researches (Yang *et al*., 2001) had shown that the aromatic components in Fen-flavor Xiaoqu liquor was the complex aroma of ethyl acetate and ethyl lactate. This study showed fusel oil was a big proportion except ethyl acetate and indicated the importance of fusel oil in Xiaoqu liquor. Furthermore, in the reports of Daqu liquor and Bran koji liquor, it said that the aroma was the result of combined action by a number of components and terpene chemicals (such as β-DMST) (Xu *et al*., 2012; Gao *et al*., 2014) had been playing an important role in forming the flavor of Fen-flavor liquor. So, more work was needed to see that the important key aromatic components and its ratio in Sichuan Xiaoqu liquor and whether contained β-DMST terpene compounds, etc.

Sample recovery test: Take four kinds of liquor samples, 2 of each sample, 1 to add the standard of known concentration, 1 don't add the standard, four times sample analysis, respectively, in the same conditions. Sample recovery = (adding-not adding) /addling standard *100%. The sample average recovery from 82.19 to 116.53% satisfied the requirements of quantitative analysis (Table 3). It was reliable and suitable for the quantitative analysis of the main aromatic components of Sichuan Xiaoqu liquor. It did not detect the ethyl butyrate and ethyl caproate in the four samples, but that didn't mean that must not exist in the sample. Maybe it was less or others. However, the recoveries of both were high and more than 95%, it also suggested that the result of recovery test was feasible to evaluate the method.

CONCLUSION

In this study, four kinds of commercially available Sichuan Xiaoqu Liquor were the object. Quantitative analysis of the main aromatic components was carried out by GC with direct injection, finally, five kinds of aromatic components were detected accurately (see in Table 2 detailed). Chromatogram of four different district samples were basically the same and all detected the propyl alcohol, ethyl acetate, isobutyl alcohol, isoamyl alcohol and ethyl lactate. Although the producing area was different, the main aromatic components were the same from the chromatogram. There into, Isoamyl alcohol and normal propyl alcohol had close quantity relative ratio relationships in four samples, the ratio respectively were 1.0, 1.2, 1.1, 1.5, isoamyl alcohol and isobutyl alcohol' ratio respectively were 2.5, 1.8, 2.2, 2.4. Special ratio relationships among fusel oil can be used as a sign of Sichuan Xiaoqu Liquor. There was a big ratio of fusel oil in Sichuan Xiaoqu liquor. And fusel oil with ethyl acetate and ethyl lactate worked together and formed the special flavor of Sichuan Xiaoqu liquor.

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