Research on the identification method of Chinese liquor brewing technology types

Wenzheng Liu¹, Hongxia Li², Mingyu Zhao¹, Junjie Zhang¹*, Min Zhi²*

¹ Collage of Chemical Engineering, College of Life Science, Library, North China University of Science and Technology, Thangshan, Hebei, 063210, China
² Hebei Zhide Inspection and Testing Co., Ltd, Shijiazhuang, Hebei, 050000, China
*Corresponding author’s e-mail: Junjie Zhang, 1418537665@qq.com. Min Zhi, huanlezhimin@126.com

Abstract. The brewing technology types of Chinese liquor include: solid method, liquid method and solid-liquid mixture method. Due to the difference of technology and raw materials, the price of three types of liquor is quite different. The outlaw often passes off the liquor brewed by the low-grade liquid brewing method as the liquor brewed by the solid brewing method. In this paper, according to the difference of amino acid content in liquor of different brewing technology types, two methods of preliminary identification of Chinese liquor methods were discussed. One method is the qualitative analysis for the colour reaction of amino acids with ninhydrin reagents, and the other method is the classification and quantitative analysis of amino acids in samples by GC. Seven kinds of liquor purchased from different channels are identified and the results of the two methods are consistent. The results of the experiment will give an instructive suggestion to guide the consumers to identify the adulteration of Chinese liquor.

1. Introduction
Chinese liquor is a special kind of food belonging to the material, but at the same time integrates into people's spiritual life, and it is a cultural symbol. Since ancient times, from princes and ministers to the common people, all have a special preference for Chinese liquor. The brewing technology of Chinese liquor includes solid state method, liquid state method and solid-liquid mix method. Solid-state liquor, also known as pure grain solid-state fermented liquor, is a traditional Chinese liquor making process [1, 2]. The making process of Chinese liquor is that the grinding grain added with koji fermented naturally for a certain time in the mud pool or pottery tank, then distilled at high temperature, and finally blending into the appropriate degree liquor. Solid state brewing process is extremely complex. Owing to the exquisite materials, the very high cost of equipment, time and labor, the liquor brewed by solid state method has high selling price. Liquor made by solid fermentation of pure grain has the characteristics of full taste, rich aroma. Moreover, it contains all kinds of amino acids and minerals beneficial to human health [3]. Liquid liquor is a new process of liquor, which usually takes tubers rich in starch, molasses rich in sugar as raw materials, through liquid fermentation, distillation, and then liquid fermentation of the formation of the liquor base as the basis (or use edible alcohol directly), through skewering, flavour, finally blending out of liquor. The liquor brewed by this new technology is often called "alcoholic liquor". Compared with traditional solid pure grain fermented liquor, the process liquid liquor is simple, highly mechanized, and can be produced in large quantities, and all
kinds of nutrients in this kind of liquor are greatly reduced or even absent, and the richness of flavour substances is far from enough. Solid-liquid mixed liquor is blending by at least 30% solid liquor and liquid liquor, but some solid-liquid mix liquor made by liquor enterprises adding less than 30% solid liquor.

With the opening of liquor market, the safety and authenticity of liquor are becoming more and more serious. In the face of staggering windfall profits, the incidents of "black-hearted businessmen selling fake wine" are common. One kind of fake liquor is to label the low-cost and nutrient-deficient liquid process liquor artificially adding some spices and food additives for improving the taste as the traditional solid-fermented liquor with pure grain. Even seasoned wine tasters can't easily distinguish this kind of fake liquor from the traditional solid-fermented liquor made from pure grains. Therefore, some lawbreakers are given an opportunity to sell their goods at a high price and illegally obtain a windfall. Another type of fake liquor is made from industrial alcohol by adding a variety of spices and labelled with solid-fermented liquor. In order to identify fake liquor, various detection methods have been developed. Some detection methods are based on the spectroscopy of compounds in liquor, such as near-infrared spectroscopy [3], attenuated total reflectance (ATR) – Fourier transform infrared (FT-IR) spectroscopy [4], mass spectrometry [5]. The most methods are based on the identification of volatile components in liquor, such as gas–chromatography–flash electronic nose technique [6], colorimetric artificial nose [7], electronic nose recognized based on the chaotic Back Propagation Neural Network [8], chemo-sensor based on fluorogen [9] and metal oxide gas sensor [10, 11].

In view of the raw material of solid fermentation liquor such as sorghum, barley, wheat and corn contains rich in amino acids, and the use of koji in the process of brewing also rich in amino acids [12], in this paper, two methods of the ninhydrin Color qualitative detection of amino acids and gas chromatography of amino acid were adopted to preliminary judge the brewing types of liquor, especially the former is suitable for family to preliminary judge of liquor category and for guiding consumers to effectively identify fake liquor.

2. Experimental

2.1. Reagents and instruments
Amino acid standard (sigma–aldrich, USA), acetonitrile and methanol (chromatographic pure), ninhydrin, acetic anhydride and other reagents are all analytical pure. The Chinese liquor samples in the experiment come from different purchasing channels, such as large supermarkets, Jingdong mall, Taobao, Liquor special stores, Free market bulk, etc. In order to avoid disputes over rights and interests, they are respectively represented by codes 1-7 in this paper.

Gas chromatograph (GC-2014c, Shimazu Co. LTD.)

The mixed standard samples of 19 kinds of amino acids is prepared at the concentration of 1000nmol/L for each amino acid and is refrigerated at 4°C.

Preparation of ninhydrin color reagent: 0.75g ninhydrin is dissolved in 50ml n-butanol, and then 1.5mL acetic acid is added.

Preparation of HCl-n-butanol solution: ammonium chloride is put into a flask with three mouths and concentrated sulfuric acid is added slowly. The hydrogen chloride gas generated is dried through concentrated sulfuric acid and then led into 10mL n-butanol solution. HCl-n-butanol solution with a concentration of 4mol/L is obtained by calibration with NaOH of 3mol/L.

2.2. Qualitative determination of amino acids in liquor samples
Put 10mL liquor sample in a small beaker and dried under 80°C air blast in a drying oven or under 85°C constant temperature in a water bath. When the liquid is steamed to 1-2ml, concentrate the liquor in a corner of the beaker and continue to steam dry. Add 2 drops of ninhydrin colorant on the dried sample, and then placed the beaker in a drying oven for drying, and then observe the color changed in the bottom of the beaker after evaporation. The reagent blank test is down at the same time. The amino acids will react with ninhydrin to form blue-purple compounds under heating and weak acid
conditions. If a blue-purple stain appears at the bottom of the beaker, it means the liquor contains amino acids. On the contrary, if the color stamp of sample is the same as the blank of ninhydrin’s, it indicates that there are not amino acids in the liquor sample.

2.3. Quantitative analysis of amino acids

2.3.1. Quantitative analysis of amino acid standard

(1) Amino acid derivation method

The 0.5ml mixed amino acids standard in a test tube is dried in a vacuum at 60℃. After drying, add 1mL HCl-n-butanol solution in the tube, ultrasonic for 5min, then sealed and esterification for 40min in a water bath at 95℃. Take out the tube and cool it slightly. Dry in a 70℃ oil bath with helium gas passed. After dried, 1mL ethyl acetate and 1mL acetic anhydride are added in the tube for acylation at 100℃ for 20min. Then, it is cooled to room temperature and vacuum dry at 50℃. After drying, it is dissolved with 0.3 mL acetonitrile.

(2) Determination of mixed amino acids standard by gas chromatography

The acylated amino acids standard is detected by GC in analysis conditions as follows: HP-5 (30m×0.25mm×0.25μm) capillary column, carrier gas: N2, shunting ratio in 1:4, injection volume of sample: 1μl, injection port temperature: 260℃, detector temperature: 280℃. The heating procedure is as: the initial temperature is 70℃ and kept for 1min, then, the temperature raised in 10℃/min speed to 270℃ and kept for 15min.

2.3.2. Quantitative analysis of amino acids in liquor samples

Put 45mL liquor sample in a small beaker and condense them to 1-2ml in a drying oven with air blast at 80℃ or in a constant temperature water bath at 85℃. Use 5ml liquor sample for transfer the concentrated solution to the test tube in several times, and evaporate to dry in the tube. The following steps of esterification, acylation and GC analysis are the same as those of mixed amino acids standard.

3. Results and discussion

3.1 Qualitative determination of amino acids in liquor samples

Qualitative test by ninhydrin is carried out for 7 liquor samples purchased from different channels and in different brewing types. The test results are shown in Figure 1 and Table 1.

![Figure 1. Color development of liquor samples with ninhydrin.](image)

A: blank of liquor, B: blank of ninhydrin, No.1 - No.7: liquor + ninhydrin.

The results of qualitative determination by ninhydrin indicate that the amino acids in liquor can develop obvious blue-purple mark. As can be seen from Figure 1 and Table 1, the liquor samples of No. 2, No.6 and No.7 contain amino acids, which brewing types are consistent with the advertised on the packaging. The results show that the advertised brewing technology of No. 1 and No. 4 liquor samples are not consistent with the brewing technology indicated by the test results, while the
advertised brewing technology for other liquor samples are consistent with the test results. The samples of No.1 and No.4 liquor are found to be fake liquor.

Table 1. Basic information of liquor samples and qualitative test results of ninhydrin.

| No. | Alcohol Degree (°C) | Advertised brewing type | Color | Amino acids (Yes/No) | Consistency (Yes/No) |
|-----|---------------------|-------------------------|-------|----------------------|---------------------|
| 1   | 52                  | Solid                   | No    | No                   | No                  |
| 2   | 52                  | Solid                   | blue-purple | Yes              | Yes                  |
| 3   | 52                  | Liquid                  | No    | No                   | Yes                  |
| 4   | 52                  | Solid                   | No    | No                   | No                  |
| 5   | 52                  | Liquid                  | No    | No                   | Yes                  |
| 6   | 52                  | Solid                   | blue-purple | Yes              | Yes                  |
| 7   | 45                  | Solid-liquid            | blue-purple | Yes              | Yes                  |

3.2. Quantitative analysis of amino acids in liquor

3.2.1. Anti-slagging and anti-corrosion measures. The single amino acid standard and the mixed standard of 19 amino acids are acylated and detect by GC, respectively. The retention time and response factors of various amino acids are obtained in Table 2.

Table 2. Retention time and response factor of mixed standard of 19 amino acids.

| No. | Name      | Short name | M  | Retention time | Peak area | Response factor (×10^4) |
|-----|-----------|------------|----|----------------|-----------|------------------------|
| 1   | Alanine   | Ala        | 71 | 7.415          | 3794      | 7.7974                 |
| 2   | Cysteine  | Cys        | 103| 7.541          | 5433      | 7.8993                 |
| 3   | Glycine   | Gly        | 57 | 11.831         | 5497      | 4.3205                 |
| 4   | Valine    | Val        | 99 | 12.761         | 4010      | 10.2868                |
| 5   | Leucine   | Leu        | 113| 13.506         | 8002      | 5.8839                 |
| 6   | Serine    | Ser        | 87 | 14.086         | 7444      | 4.8697                 |
| 7   | Isoleucine| Ile        | 113| 14.358         | 2922      | 16.1134                |
| 8   | Arginine  | Arg        | 157| 14.866         | 2955      | 22.1376                |
| 9   | Threonine | Thr        | 101| 15.228         | 2007      | 20.9683                |
| 10  | Proline   | Pro        | 97 | 15.998         | 6603      | 6.1210                 |
| 11  | Methionine| Met        | 149| 16.933         | 7891      | 7.8676                 |
| 12  | Asparagine| Asn        | 114| 17.162         | 1981      | 23.9778                |
| 13  | Aspartate | Asp        | 114| 17.408         | 16386     | 2.8988                 |
| 14  | Phenylalanine | Phe   | 147| 19.166         | 13014     | 4.7065                 |
| 15  | Glutamate | Glu        | 128| 20.187         | 10459     | 5.0993                 |
| 16  | Histidine | His        | 137| 21.188         | 3366      | 16.9588                |
| 17  | Lysine    | Lys        | 129| 21.817         | 11667     | 4.6070                 |
| 18  | Tryptophan| Trp        | 186| 22.336         | 8420      | 9.2043                 |
| 19  | Tyrosine  | Tyr        | 163| 24.035         | 10008     | 6.7862                 |

3.2.2. Determination of amino acids in liquor by GC. The derivatization and GC analysis of liquor samples 1-7 are carried out under the same conditions as those of standard samples. The GC maps of no. 2, 6 and 7 liquor samples are shown in Figure 2 and the contents of amino acids see Table 3, while the other liquor samples show basically no amino acids.

The results show that No. 2 and No. 6 liquor samples contain abundant amino acids, which are consistent with the advertised solid brewing process. The sample of No.7 liquor contain a small amount of amino acids, which are in line with the advertised solid-liquid combination brewing process. No amino acids are detected in other liquor samples. The brewing types of No. 1 and No. 4 liquor samples are advertised for solid brewing method. Therefore, they are fake liquors for the test results and propaganda of brewing technology do not conform to. The test results for liquor samples of No. 3
and No. 5 are consistent with the propaganda with liquid brewing technology. The results obtained by GC are consistent with those obtained by qualitative determination by ninhydrin.

![Figure 2. GC maps of liquor sample No. 2, 6 and 7.](image)

**Table 3. Content of amino acids in liquor samples (mg/L).**

| No. | Short name | Retention time | No. 2 Peak area | No. 2 content | No. 6 Peak area | No. 6 content | No. 7 Peak area | No. 7 content |
|-----|------------|----------------|-----------------|---------------|----------------|---------------|----------------|---------------|
| 1   | Ala        | 7.415          | 46761           | 8.751         | 32562          | 6.094         | 15372          | 2.877         |
| 2   | Cys        | 7.511          | 112314          | 21.293        | 53816          | 10.203        | 22167          | 4.202         |
| 3   | Gly        | 11.831         | 10133           | 1.051         | 16182          | 1.678         | 16732          | 1.735         |
| 4   | Val        | 12.761         | 29817           | 7.361         | 63920          | 15.781        | 2113           | 0.522         |
| 5   | Leu        | 13.506         | 39622           | 5.595         | 12653          | 1.787         | 1677           | 0.237         |
| 6   | Ser        | 14.086         | 22372           | 2.615         | 16583          | 1.938         | -              |              |
| 7   | Ile        | 14.358         | 8422            | 3.257         | 30252          | 11.699        | 1786           | 0.691         |
| 8   | Arg        | 14.866         | 57566           | 30.585        | 12143          | 6.452         | -              |              |
| 9   | Thr        | 15.228         | 25529           | 12.847        | 60369          | 30.380        | 6984           | 3.515         |
| 10  | Pro        | 15.998         | 39754           | 5.840         | 3877           | 0.570         | 6873           | 1.010         |
| 11  | Met        | 16.933         | 77428           | 14.620        | 15380          | 2.904         | -              |              |
| 12  | Asn        | 17.162         | 1108            | 0.638         | 12606          | 7.254         | -              |              |
| 13  | Asp        | 17.408         | 7100            | 0.494         | 91464          | 6.363         | 3972           | 0.276         |
| 14  | Phe        | 19.166         | 22131           | 2.500         | 22053          | 2.491         | 16025          | 1.810         |
| 15  | Glu        | 20.187         | 2002            | 0.245         | 12801          | 1.567         | 1979           | 0.242         |
| 16  | His        | 21.188         | 1214            | 0.494         | 14099          | 5.738         | -              |              |
| 17  | Lys        | 21.817         | 12615           | 1.395         | 38085          | 4.211         | 2022           | 0.224         |
| 18  | Trp        | 22.336         | 1530            | 0.338         | 36813          | 8.132         | -              |              |
| 19  | Tyr        | 24.035         | 1200            | 0.195         | 32757          | 5.335         | -              |              |

Total content of amino acids: 120.113 mg/L for No. 2, 130.576 mg/L for No. 6, and 17.340 mg/L for No. 7.
4. Conclusion

For 7 liquor samples from different sources, the results of determination of amino acids by ninhydrin are consistent with the results of amino acids by GC. Therefore, it is feasible to use these two methods to identify liquor brewing types. The quantitative determination of amino acid in liquor by GC can not only the total amount of amino acid be detected, but also the different contents of various amino acids can be analyzed. The qualitative determination of amino acid in concentrated liquor by ninhydrin is simple and more practical. With a small amount of ninhydrin reagent, consumers can do a preliminary test of the brewing category at home and give the basic judgments for brewing types of liquors.

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