Design and Operation of an Artificial Pit for the Fermentation of Chinese Liquor

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ABSTRACT

J. Inst. Brew. 113(4), 374–380, 2007

A laboratory-scale artificial pit for solid-substrate fermentation was designed and placed into operation to explore a new-style of distilled liquor. This product recaptures the quality of Chinese traditional liquor. The on-line parameters determined included temperature, gas production, and physicochemical and flavor characteristics of *Huangshui*. The quality of the liquor produced by the artificial pit was also monitored. Changes in the pit temperature reflected the fermentation conditions in the pit. The ratio between gas production and alcohol content of *Huangshui* was 1 : 2. On average, 6.24 litres per batch of the new-style distilled liquor was produced using this non-traditional pit and the basic quality was acceptable. Although the fermentation time was only 1 month, the yield ratio of alcohol from the artificial pit was 20.5% compared with a typical 3 month fermentation yield of 33% in the industry. This artificial pit was deemed feasible for fermentation research and for quality improvement for Chinese new-style distilled liquor.

Key words: Artificial pit, brewing, Chinese new-style distilled liquor, engineering design, on-line determination.

INTRODUCTION

From ancient times, Chinese traditional liquor has been very popular in China and because of its unique brewing technology and flavor, it holds a very special status in world brewing history. The traditional solid-substrate fermentation of Chinese liquor has the character of a hermetic brew and the product takes one to three months to produce. During the fermentation, various raw materials placed into the mud pit metabolize along the interfaces of the solid, liquid and gas phases of *Zaopei*. *Zaopei* is a mixture of fermented grains such as sorghum, corn, wheat and rice[9]. The metabolic reactions consist of an interaction of the microorganisms in the *Qu* environment, the pit mud, and numerous enzymes produced by the microbes. Chinese *Qu* is prepared by a natural inoculation of molds, bacteria and yeasts and their growth on grains. Included are amylases, proteinases, lipases and numerous other enzymes. To some extent, *Qu* is equivalent to malt and yeast for beer fermentation[9]. The decomposition and transformation of various macromolecular substances (mainly starches) is continuous and occurs while ethanol and numerous aromatic compounds, such as acids, aldehydes, alcohols and esters, are developing. As a result, the unique style and characteristics of Chinese traditional liquor produced by solid-substrate fermentation comes into being[10].

China possesses a unique brewing technology, but production and technology management depend mainly on experience and the intrinsic fermentation process is still mostly unrevealed. As for the methods to improve liquor quality, this has been restricted to the reconstruction and protection of the mud pit from changing shape[3] and primarily optimizing the compound formulation of the artificial pit mud[4]. This has retarded not only scientific research of the traditional liquor, but also the further development of brewing technology.

New-style distilled liquor is not the same as new technology liquor. New technology liquor is now the most popular distilled liquor in China besides the traditional liquor. New technology liquor is made mainly by blending a high quality solid-state fermented liquor and edible alcohol. It is blended according to the relative ratios of trace ingredients in the original famous liquor. New-style distilled liquor is fermented from grains using solid-state fermentation and moreover its brewing can take place anytime and anywhere. Also its flavour is relatively close to traditional liquor. New-style distilled liquor is the direction of the Chinese liquor industry in the future, so it is very important to study the metabolic regulation and the main metabolic pathways of the functional microbes during the solid-substrate fermentation process in the pit. Since the usual plant-scale inventory rating contains 10 tons of material and the liquor quality is influenced by experience, weather and geographical environment, a traditional mud pit is not a suitable vehicle for scientific research and the production of new-style distilled liquor. The aim of this research was to design and operate a moveable automatic brewing system for solid-substrate fermentation of Chinese liquor. Thus brewing would not be restrained by environment, weather or other geographical factors. At the same time, the pit could be made available for all kinds of fundamental research on distilled liquor and on the small-scale production of new-style liquor.

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Publication no. G-2007-0128-503
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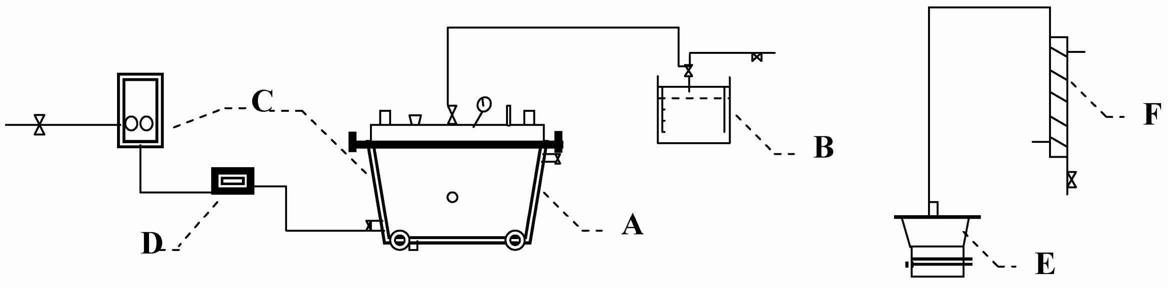

Fig. 1. Schematic diagram of the moveable automatic brewing system for solid-substrate fermentation of Chinese liquor. A: artificial pit, B: gas-collection-equipment, C: heating and cooling equipment using circulating water, D: temperature control equipment, E: distillating still for stewing grains and *Zaopei*, F: receiving device for alcohol condensation.

MATERIALS AND METHODS

Design of the artificial pit for solid-substrate fermentation

The moveable automatic brewing system for solid-substrate fermentation of Chinese liquor (Fig. 1) was designed according to the equipment used for Chinese traditional liquor production. All of the equipment was stainless steel. The artificial pit is where the fermentation takes place. The pressure and temperature were controlled by the gas collection equipment and the heating and cooling equipment. The main function of the gas collection equipment was for collecting instantaneous and interim gas respectively for gas analyses. Furthermore, it can be used as an air balance for the whole system and thus allows control of the inside pressure. The heating and cooling equipment used circulating water. The water temperature was adjusted by temperature control equipment. Temperature control was automated, replacing the heat preservation by ground temperature used in a traditional mud pit, and thus was more reliable. The artificial brewing pit could ferment about 100 kg of grains and *Zaopei* in one batch. After fermentation, the *Zaopei* with grains was distilled with a distillating still for the next batch, while the raw liquor was collected by a receiving device.

The artificial pit (250 L of cubage) was divided into two parts, the pit cover and pit body. The pit cover is connected to the pit body hermetically, by hold-down bolts (Fig. 2). The bifurcated vent on the pit cover is connected to the gas-collection-equipment. There is an interlayer, infall, outlet and temperature probe on the pit body, respectively connected with the heating and cooling equipment. The vent for sampling and draining at the bottom of pit body was used to sample the *Huangshui,* which was produced during the fermentation, both instantaneously and intermittently. Analytical results of *Huangshui* could help to explain the metabolism occurring in the pit. The vent can also be used to clean the pit after brewing. The *Huangshui* is a slightly thick brown liquid that deposits on the bottom of the pit after a *Zaopei* fermentation. It consists of a plethora of compounds and microorganisms. The microbes are mainly bacteria, while chemical compounds include unfermented reducing sugars, non-soluble starches and nitrogenous compounds from *Zaopei*, as well as the aromatic compounds produced by fermentation such as ethanol, higher alcohols, aldehydes and esters, abundant organic acids, as well as some tannins and pigments from the raw materials[1].

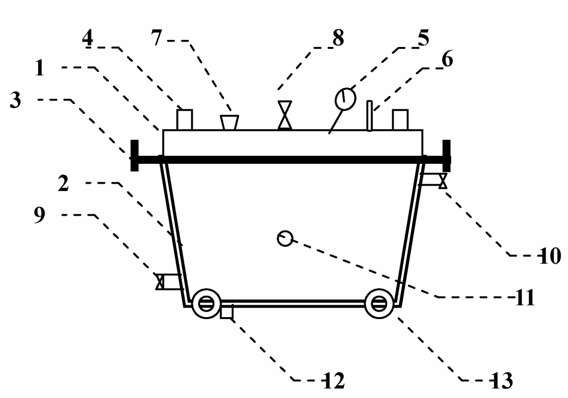

Fig. 2. Schematic diagram of the artificial pit. (1) pit cover, (2) pit body (including an interlayer), (3) hold-down bolt, (4) handle, (5) manometer, (6) thermometer, (7) sampling filler, (8) bifurcated vent, (9) infall, (10) outlet, (11) temperature probe, (12) vent for sampling and draining, (13) pulley.

Raw materials for fermentation

Grains (mainly sorghum), auxiliary raw materials (mainly chaff), *Zaopei* (from the last round of brewing, but not used in the first round), tap water, and *Qu* (supplied by a Sichuan Province distillery) were the raw materials.

Method of operation

The prepared raw materials were fermented for about one month in the pit after several procedures (Fig. 3), including material mixing and grain stewing. Then the materials were loaded into the pit and fermented. During fermentation, parameters of the pit, such as temperature changes and gas production, were determined daily. Physicochemical characters of *Huangshui* were determined after periodic sampling.

Temperature changes in the pit were determined using an on-line thermometer. Gas collection equipment was filled with NaCl saturated solution, using drainage-collecting gas methods to collect the gas and recorded. The pH value of the *Huangshui* samples was determined using a pH-meter (PHS-25C precise pH-Meter, Kangyi Instrument Ltd., Shanghai). Total acid was determined by the neu-

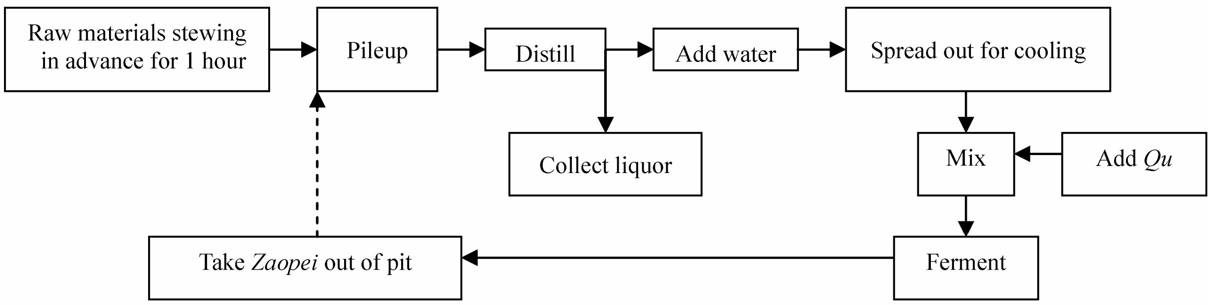

Fig. 3. Schematic diagram of the fermentation working procedures of the artificial pit.

tralization method[1]. Reducing sugar was determined at 530 nm by 3,5-dinitrosalicylic acid (DNS)-spectrophotometry (721 Visible spectrophotometer, Shanghai Analytical Instrument Company)[6]. Alcohol content was determined at 600 nm by potassium dichromate-spectrophotometry[7].

After brewing, as much as possible of the *Huangshui* was drained out of the pit for 3 to 6 hours, then the fermented grains were taken out and a distillation performed. The alcohol in the distilled liquor was determined directly using an alcoholometer (Liminju Glass Instrument Company, Hejian city, Hebei Province) and a conversion table of temperature and concentration. The aromatic compounds were determined using a GC-960 Gas Chromatograph (Haixin Chromatographic Instrument Ltd., Shanghai) equipped with a FID detector and DNP filled column. The column was a stainless helix column (2m×Φ3mm, filled with 6201 red supporter with 80~100 holes and a mixed stationary liquid of 15% dinonyl phthalate and 6% Tween-80). The injector, detector and column temperatures were set at 125°C, 120°C and 90°C, respectively. The carrier gas was N_2, and N_2, H_2 and air flow-rates were set at 20 mL/min, 20 mL/min and 230 mL/min, respectively[2,5,8]. The chromatogram was analyzed using a N2010 workstation (Zhida Information Engineering Ltd., Chekiang University).

Evaluation of the artificial system for liquor production

Percent conversion of starch (ξ, %) and alcohol yield ratio (η, %) were used to evaluate the fermentation effect of the artificial pit. Percent conversion of starch is the ratio of actual liquor yield (m_p, kg) and theoretical liquor yield (m_t, kg) from starch consumption (Eq. 1). Alcohol yield ratio is the ratio of actual liquor yield and total amount of starch (m_{ts}, kg) (Eq. 2).

$$\xi = \frac{m_p}{m_t} \times 100\% \qquad \text{(Eq. 1)}$$

$$\eta = \frac{m_p}{m_{ts}} \times 100\% \qquad \text{(Eq. 2)}$$

RESULTS AND DISCUSSION

Temperature changes in the artificial pit

A suitable temperature favorable for the growth and fermentation of microbes is important and temperature changes can indirectly reflect fermentation conditions in the pit. Because the air temperature changed from day to night, the temperature was determined manually three times a day to examine the influence of air temperature change on the fermentation. The extent of this change could guide the control of the temperature equipment. The operation of the pit appeared regular at the third round, after testing two rounds. The temperature rose rapidly to 28.2°C during the first stage of fermentation (i.e., first 4 days), then began to decline and was stable between 18.5°C and 21.5°C during the latter stage of fermentation (i.e, after 22 days) (Fig. 4). Aerobic microbes such as fungi grew and reproduced in large amounts during the first stage and the temperature inside of the pit increased rapidly. Later, with an increase in anaerobiosis, the temperature decreased to a suitable degree for fermentation. The temperature fluctuations arose from the changes of the microbes' metabolism during the fermentation and then reached homeostasis. Temperature control equipment on the artificial pit replaced the ground temperature control, and provided heat compensation for the pit at the later stage, to maintain a normal and continuous fermentation. The equipment set point was 25°C during this period.

Gas production and alcohol content in *Huangshui* in the artificial pit

Daily gas production rose sharply during the first stage (Fig. 5) and reached a peak of 550 litres per day. It declined from the 5th day. A total of 1317 litres of gas was produced by the 7th day. From the 8th day, daily gas production fluctuated and declined gradually. From the 8th day to the end of brewing, 438 litres of gas were produced.

The alcohol content of the *Huangshui* samples increased slowly from the 9th day (except the first sample) and the final alcohol content was about 15mL/100mL. If the fermentation proceeded according to the EMP metabolic pathway, the gas produced was CO_2, and from Eq. 3 there would be about 2.05 g alcohol produced for each litre of gas produced.

$$C_6H_{12}O_6 \xrightarrow{ferment} 2C_2H_5OH + 2CO_2 \qquad \text{(Eq. 3)}$$

Draining from the brewing grains, *Huangshui* reflects the substrate metabolism of the fermentation from other aspects. The higher alcohol content in the first sample of

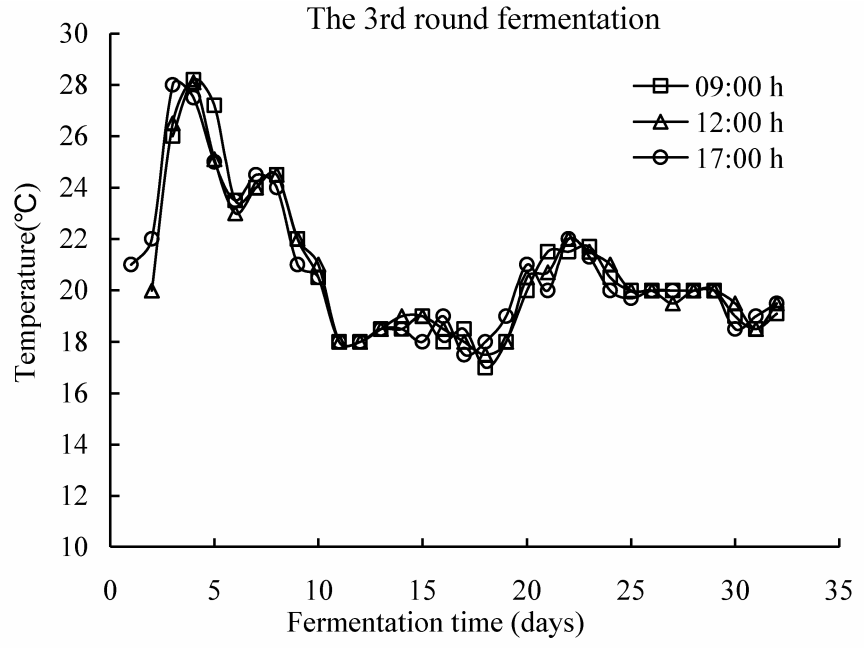

Fig. 4. Temperature changes in the artificial pit during fermentation.

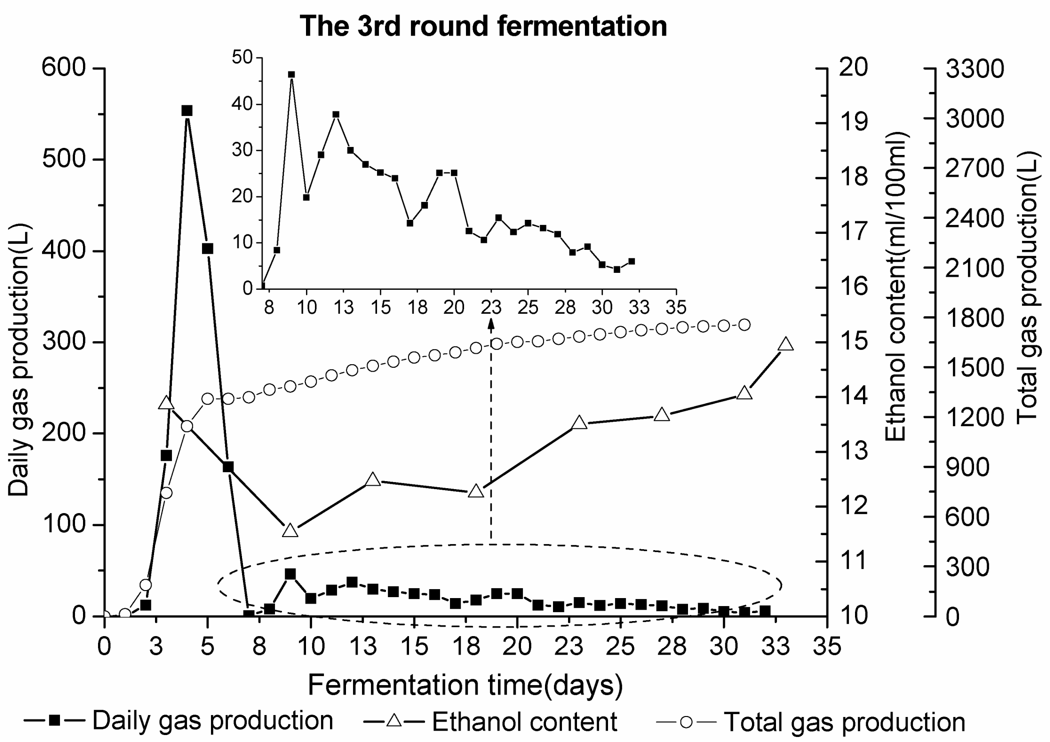

Fig. 5. Gas production and alcohol content in *Huangshui* from the artificial pit during fermentation.

Huangshui compared to the second, is because some *Zaopei* from the last round and the last distilled liquor was added into the raw materials for fermentation, to make the pit slightly alcoholic. Nutrition was abundant during the early stage of fermentation and microbes grew and quickly metabolized the substrate to produced abundant respiratory gas. The ethanol and micromolecular substances, decomposed from the macromolecular substances in the raw materials, were consumed by the microbes for nutrition. Thus, the alcohol content in the second sample of *Huangshui* was lower. During the middle stage of the fermentation (from the 6th day to the 21st day), microbes such as yeast began to transform the substances (mainly glucose) into alcohols and the gas production (mainly CO_2) and alcohol content slowly increased. The ratio of the daily yield was about 1:2. When the acid production period commenced, the environment of pit became too acidic and was unfavorable for yeast alcoholic fermen-

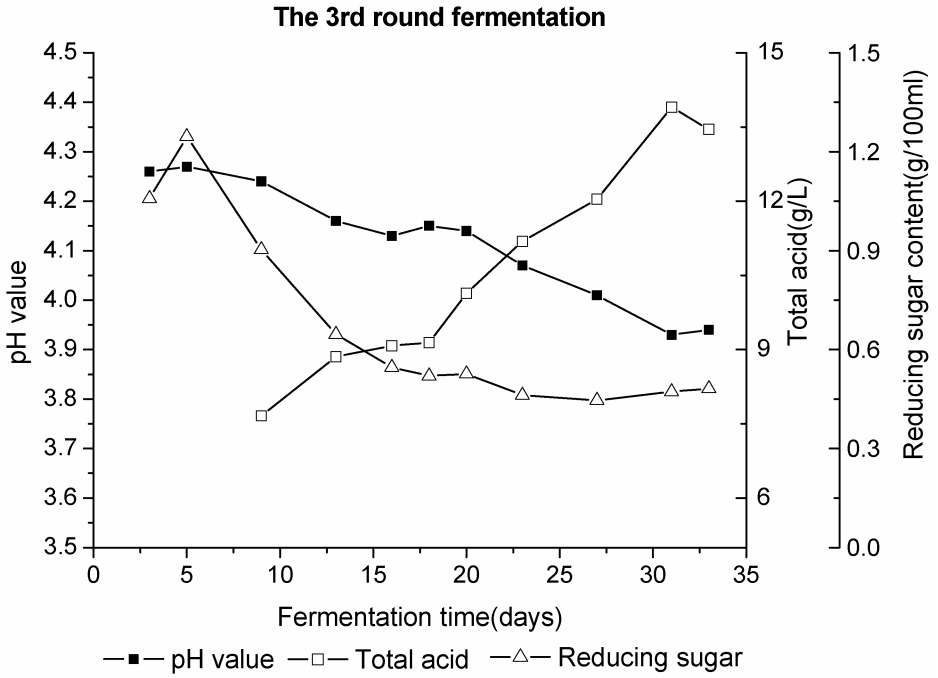

Fig. 6. Changes of the pH value, total acid and reducing sugar in *Huangshui* of the artificial pit during fermentation.

tation and esterification dominated the process. As some of the alcohols were used to synthesize acids and esters, daily gas production gradually approached zero. There was no relevant data about alcohol content in *Huangshui* available in the literature, only about *Zaopei*, from which the *Huangshui* drained and it is reported that the alcohol content in *Zaopei* of traditional liquor production reaches its highest value on the 21st day, and remains at that level until the end of the process[11]. Thus the alcoholic fermentation could be finished in one month, leaving time for other reactions such as esterification.

Changes in the acidity and reducing sugar content in *Huangshui*

Changes in the pH value and total acid in *Huangshui* can be used to judge the progress of the alcoholic fermentation. The change in reducing sugar content in *Huangshui* directly reflected the speed of the fermentation. The pH value in the pit during fermentation was maintained at pH 3.9~4.3, and the content of total acid increased from 7 g/L to 14 g/L (Fig. 6). The content of reducing sugar reached 1.25 g/100 mL during the first stage and then declined. It was between 0.45 g/100 mL and 0.5 g/100 mL during the middle and later stages of fermentation. At the first stage, owing to the presence of *Qu* which brought with it various hydrolytic enzymes such as amylases, saccharifying enzymes and proteinases, and the growing function of aerobic microbes such as mildew, as well as substances including starch and protein in raw materials, which were decomposed and transformed into oligosaccharides, monosaccharides, amino acids and other micromolecular substances. Thus, the content of the reducing sugar increased, while the pH value and total acid remained stable. At the middle stage, micromolecular substances were used to produce alcohols and the content of reducing sugar declined. The alcohols, mainly ethanol, dissolved in the liquid diffused into the interspaces of the *Zaopei*. The acidity of the environment began to change and this led to changes in the acidity of the *Huangshui*. During the last stage of fermentation, with the metabolism of the yeasts and fungi restrained by the alcoholic concentration in the pit, the content of reducing sugar appeared stable. Various kinds of acids produced by microbes, such as bacteria, increased in concentration. The pH value declined and total acid increased. When the alcohols and acids reacted to produce esters, the total acid declined slightly. Also compared to a traditional fermentation, the changes in the range of acidity and reducing sugar content in *Zaopei* are wide during the first one month, whereas in the last two months the changes are small[11].

Quality of the liquor produced in an artificial pit

The raw liquor from the artificial pit did not include the first distilled liquor or the last distilled liquor. This raw liquor belonged to high degree liquor of 60% (v/v) but was lower than *Quanxing* raw liquor (70%(v/v)). The pH value of the pit liquor was 4.55. The aromatic compounds in the liquor from the artificial pit were compared with those of *Quanxing* raw liquor (Table I). Acetic acid content was similar, 36.22 mg/100 mL and 38.94 mg/100 mL respectively. Corresponding ethyl acetate content was also similar, 71.69 mg/100 mL and 64.29 mg/100 mL respectively. Caproic acid and ethyl caproate were not detected, as no caproic acid bacteria were added during the fermentation. The concentration of ethyl lactate was twice that found in *Quanxing* liquor, making the pit liquor much sweeter in terms of taste. There were some shortcomings in the ratios among the other acids and esters. As a result, the pit liquor was deficient in liquor body and flavor compared with that of the finished products of famous Chinese liquor. Generally speaking, the distilled liquor of the artificial pit was distinctive. There were some differences in the aromatic profile between new and traditional liquor, but the body flavor was harmonious, and deemed successful for a liquor making process using an artificial pit, and was good for producing new-style liquor. High quality new-style liquors of different tastes were made after carrying out appropriate post-treatments on this raw liquor. The treated new-style liquor had a soft and mellow body, as well as an enjoyable aroma and flavor.

Evaluation of the artificial pit

Assuming that the consumed starchy materials were totally transformed into glucose, and all of the glucose was transformed into ethanol, the percent conversion of starch and alcohol yield ratio of the three rounds could be calculated from Equation 1 and 2. The percent conversion of starch and alcohol yield ratio of the artificial pit was 69.21±3.63% and 20.51±1.77% respectively (Table II). While the alcohol yield ratio of traditional liquor in the industry is from 33% to 35%, the artificial pit was fermented for only 35 days, compared with 3 months in the industry. Therefore, it is feasible for the distillation yield to develop further and it was concluded that the performance of the artificial pit was acceptable and that the new-style distilled liquor could be produced in different places, in a shorter time span, with this advancement in technology.

CONCLUSIONS

The operation of the artificial pit and the quality of the liquor indicated that the system was successful owing to the following reasons. Firstly, the system was designed to ferment in the same sealed environment as the traditional mud pit, and thus it ensured the integrality and infrangibility of the micro-ecological environment of the system. Secondly, the upside of a traditional mud pit is covered with a plastic film, which is used to keep a certain amount of gas inside while sealed, and to maintain the pressure during the fermentation. Similarly, there is gas collection equipment in the system which can not only observe the on-line gas production changes, but also can maintain the pressure inside the pit. The heating and cooling equipment using circulating water, replaced the uncontrollable natural ground temperature system, making the temperature controllable and the liquor brewing more independent of weather influences. Consequently, the system can be regarded as a copy of the traditional brewing system, but one that is better for research and for the production of new-style liquor. Compared with other solid-state fermentation systems of traditional products, this system is better supervised and controlled.

Table I. Comparison of the content of aromatic compounds in the raw liquor in the artificial pit and *Quanxing*[1].

Aromatic compound	Raw liquor artificial pit[2] (mg/100 mL)	Raw liquor *Quanxing* (mg/100 mL)	Aromatic compound	Raw liquor artificial pit (mg/100 mL)	Raw liquor *Quanxing* (mg/100 mL)
Acetaldehyde	8.99	35.01	Formic acid	15.97	9.60
Methanol	n.d.[3]	39.00	Acetic acid	36.22	38.94
Ethyl acetate	71.69	64.29	Propanoic acid	1.77	2.08
Propyl alcohol	15.72	14.91	Isobutyric acid	0.16	1.22
Sec-butyl alcohol	3.31	7.051	Butyric acid	3.76	19.08
Acetal	2.15	79.13	Isovaleric acid	n.d.	1.17
Isobutyl alcohol	14.01	44.08	Valeric acid	n.d.	2.98
Normal butanol	1.91	5.95	Lactic acid	7.67	21.02
Ethyl butyrate	n.d.	20.20	Caproic acid	n.d.	57.86
Isoamyl alcohol	58.26	47.31	Ethyl caproate	n.d.	209.90
Ethyl valerate	0.29	6.82	n-hexyl alcohol	n.d.	0.46
Ethyl lactate	191.10	84.63			

[1] Data are average results of three analyses using the 3rd round of fermentation.

[2] Alcohol content in raw liquor of the artificial pit was 60% (v/v), Alcohol content in the raw liquor of *Quanxing was* 70% (v/v).

[3] n.d. = Not detected.

Table II. Evaluation of the artificial pit.

Fermentation parameters and evaluation indices		1st round	2nd round	3rd round
Total amount of material (kg)		100.15	100.20	100.15
Raw starch in the material (%)		30.95	30.63	29.82
Raw starch in the fermented grain (%)		17.00	14.54	12.08
Amount of glucose consumed (kg)		15.52	17.91	19.74
Theoretical liquor yield (kg)		7.93	9.15	10.09
Actual liquor yield (kg)		5.81	6.24	6.68
Percent conversion of starch (%)	Value of each round	73.23	68.21	66.18
	Average	69.21		
	SD	3.63		
	Statistical result	69.21±3.63		
Alcohol yield ratio (%)	Value of each round	18.74	20.34	22.36
	Average	20.51		
	SD	1.77		
	Statistical result	20.51±1.77		

According to the observations taken in the artificial pit, temperature changes approximately reflect fermentation metabolism inside the pit. It was shown that there was a regular proportion relationship between gas production of the pit and the alcohol content of *Huangshui*, of 1:2 and this made fermentation control easier and more efficient. The flavor of the distilled liquor from the artificial pit was stronger and less mellow. Both starch conversion and alcohol yield ratio were correspondingly high. Along with the continuous improvement of brewing technology and the post-treatment technology, the new-style liquor produced by using the artificial pit will contribute to the body of research knowledge in the development of Chinese liquor.

ACKNOWLEDGEMENTS

This project was financially supported by the Project (No. 30270035) of the Natural Science Foundation of China (NSFC).

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(Manuscript accepted for publication December 2007)