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Article

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Research on the Resource Utilization of Fuzao by Semi-solid Fermentation

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Abstract: Research on the resource utilization of Fuzao. The degradation of Fuzao's residual starch was carried out using a horizontal rectangular pressure steam sterilizer. Under the optimized orthogonal design conditions of a pressure of 0.13 MPa, a temperature of 124.5 °C, a pH of 1.0, and a time ranging from 30 to 50 minutes. Glucoamylase was added at a dosage of 100 $u \cdot g^{-1}$ for saccharification, and yeast koji was inoculated at a rate of 1% for anaerobic fermentation. The wine yield was 35.49 $g \cdot kg^{-1}$. The total production time was curtailed from 361.5 hours to 76 hours. In order to make full use of another waste from the winery, the bottom pot water, it is esterified under the action of the screened esterase strain, and after mixing, the content of the new liquor flavor substance ethyl lactate is increased to improve its quality.

Keywords: Fuzao; Resource utilization; fermentation of semi-solid; Residual Starch; New liquor

Introduction

Strong-flavor Daqu (A saccharifying starter for Chinese wine) liquor holds a prominent position among the renowned traditional distilled wines in China. Fuzao (Solid waste from the second round of grain free fermentation of Chinese strong aroma Daqu wine mash) represents the principal solid waste in the production process of strong-flavor Daqu liquor. The fermented grains of strong-flavor Daqu liquor are involved in a cyclic fermentation process, with grains being continuously incorporated during the cycle. When certain fermented grains are slated for disposal, they enter the fermentation stage without the addition of grains. The fermented grains following distillation are termed Diuzao (Solid waste from the first round of grain free fermentation of Chinese strong aroma Daqu liquor mash). Subsequently, when Diuzao enters the second round of fermentation without grain addition and the resulting fermented grains after distillation are identified as Fuzao. The waste distiller's grains of strong-flavor Daqu liquor primarily consist of Diuzao and Fuzao. Diuzao is replete with nutrients such as 10% - 13% of unutilized starch, concentrated proteins, amino acids, vitamins, mineral elements, nitrogen-free extracts, and microbial residues (Wang, Q, et al., 2020). In contrast, Fuzao exhibits a lower residual starch content and poses greater challenges in terms of utilization.

A multitude of scholars have been engaged in exploring methods for the resource utilization of the starch present in waste distiller's grains. Current techniques encompass the production of fuel ethanol (Li, T, et al., 2014; Lv, JW, et al., 2019), dietary fiber

(Chen, TT, et al., 2024), cultivation of edible fungi (Ren, Y, et al., 2017), production of xylo-oligosaccharides (Zhao, XX, et al., 2016), extraction of proteins (Chen, JX, et al., 2018), among others. However, for liquor production enterprises, the utilization of waste distiller's grains for wine production holds the potential to generate greater economic value (Huang, MY, et al., 2023; Li, M, et al., 2018; Li, ZS, et al., 2019). Strong-flavor Daqu distilleries typically sell their waste distiller's grains directly to feed mills and fertilizer factories. The market prices for fresh Diuzao and fresh Fuzao are approximately 200 ¥/ton and 150 ¥/ton, respectively. Evidently, wine represents a product with a considerably higher added value.

The utilization of the special substances within waste distiller's grains exerts a certain impact on wine quality (Hu, ZM, 2024). The prevalent processes for the re-production of wine from waste distiller's grains involve the application of yeast, glucoamylase, cellulose (Gao, L, et al., 2022; Han, YC, et al., 2022; Ma, WP, et al., 2016; Yang, G, et al., 2021; Zhou, KX, & Ni, L, 2021), etc. Nevertheless, these technologies are associated with issues such as a lackluster aroma in the wine body (Su, XL, et al., 2019). Waste distiller's grains can be harnessed to produce Fuzao liquor by incorporating enhanced fermentation strains (He, G, et al., 2019) and complex enzymes (Wang, B, et al., 2020). Additionally, waste distiller's grains can undergo a stacking process (Zhao, D, et al., 2011 a; Zhao, D, et al., 2011 b) and a cross-steaming process (CN102127497A, 2011), accompanied by the addition of active aroma-producing yeast (CN105255644A, 2016), which serves to address the problem of relatively insufficient flavor substances.

Diuzao can enhance the quality of base wine through re-fermentation and optimization of process parameters (Zhang, YD, et al.,2012) .

In general, the existing literature reports on the re-fermentation of waste distiller's grains for wine production, both domestically and internationally, predominantly concentrate on the relatively straightforward second round of fermentation without grain addition, namely the stage of using Diuzao to produce Fuzao liquor. In contrast, there is a dearth of reports regarding the further re-fermentation of Fuzao to produce wine, corresponding to the third round of fermentation without grain addition. This study endeavors to employ the semi-solid fermentation method for the re-fermentation of Fuzao to produce wine, thereby filling this research gap.

Results

Starch Degradation Process Parameters Single-factor Experiments

In the determination of process parameters, the concentration of reducing sugar generated subsequent to the semi-solid degradation of Fuzao starch was adopted as the index for condition assessment. In the investigation of the impact of temperature on the degradation of Fuzao starch, 350 g of Fuzao was taken, and 700 ml of water was added. The samples were then treated at 40 °C, 80 °C, 120 °C, 160 °C, and 200 °C for 1 hour, followed by cooling and filtration. After saccharification of the filtrate, the concentration of reducing sugar was determined. The experimental results are presented in Fig. 1:

It is evident from Fig.1 that as the treatment temperature escalates, the amount of residual starch degradation in the sample experiences a significant increase, concurrently accompanied by a substantial rise in the reducing sugar content of the sample. The concentration of reducing sugar in the degradation solution initially exhibits a pronounced elevation with the augmentation of the treatment temperature. Different lowercase letters denote significant differences ($P < 0.05$). It attains its maximum value at 160 °C, thereby indicating that an increase in temperature is conducive to starch degradation. During the analysis and detection process, a peculiar phenomenon was observed. In the titration operation for quantifying the concentration of reducing sugar in the extract following treatment at 200 °C, the color change of the liquid transitioned from blue-green to orange-yellow, rather than the typical transformation from blue to the brick red color at the titration endpoint. This suggests that at excessively high temperatures, not all of the starch degradation products of Fuzao are converted into reducing sugars. Simultaneously, reducing sugars are prone to decomposition reactions under such elevated temperature conditions.

When examining the influence of treatment time on the concentration of reducing sugar, it was discovered that its effect pattern bore resemblance to that of temperature. In the study of the influence of pH on the concentration of reducing sugar, considering that the degradation solution containing reducing sugar necessitates neutralization and an upward adjustment of pH prior to the subsequent fermentation step, the lowest pH was set at 1.0. In the exploration of the impact of pressure conditions on the concentration

of reducing sugar, due to the constraints of the operational parameters of the horizontal rectangular pressure steam sterilizer, the treatment pressures were set at 0.02 Mpa, 0.05 Mpa, 0.07 Mpa, 0.11 Mpa, and 0.13 Mpa, respectively.

Orthogonal Experiments

During the semi-solid degradation treatment of Fuzao residual starch, there existed a substantial interaction among the factors influencing the production of reducing sugar in Fuzao. Through the design of orthogonal experiments, the reaction conditions conducive to maximizing the production of reducing sugar were identified.

Since a horizontal rectangular pressure steam sterilizer was utilized as a pressurization device for treating Fuzao, a certain correlation was established between pressure and temperature. When the treatment pressures were set at 0.02 Mpa, 0.05 Mpa, 0.07 Mpa, 0.11 Mpa, and 0.13 Mpa, the corresponding temperatures of the reaction systems rose to 104.5 °C, 111 °C, 115 °C, 121.5 °C, and 124.5 °C, respectively. Consequently, in the design of orthogonal experiments within the framework of the existing equipment conditions, only three factors, namely pressure (P), time (t), and pH, were taken into consideration.

Based on the outcomes of single-factor experiments concerning the production of reducing sugar in Fuzao, an orthogonal experiment was conducted to optimize the three factors of pH, t, and P. The specific analysis results are detailed in Table 1:

The optimization results of the orthogonal experiment pertaining to the three factors of pH, t, and P are presented in Table 5. The intuitive analysis reveals that RC > RA > RB,

145 signifying that the primary and secondary factor relationships influencing the
146 degradation of Fuzao starch are as follows: C (P) > A (pH) > B (t). The optimal process
147 condition combination was determined to be A3B3C3, corresponding to a pressure of
148 0.13 MPa, a pH of 1.0, and a time of 90 minutes. Under the optimized conditions, the
149 concentration of reducing sugar was 4.12 ± 0.13%, and the degradation rate of starch
150 was 35.16 ± 1.56%.
151 The aforementioned experiments entailed one-time degradation treatments of Fuzao
152 starch, with a solid-liquid ratio of Fuzao to water of 0.5. As a result, the volume of the
153 collected filtrate was relatively limited. When the amount of water was increased, the
154 starch degradation rate also increased. However, due to the dilution effect, the
155 concentration of reducing sugar decreased, which was unfavorable for the subsequent
156 fermentation. Through experimentation, it was ascertained that under the optimized
157 conditions of the orthogonal experiment, when multiple degradation treatments with
158 water addition were carried out until the solid-liquid ratio of Fuzao to water reached
159 0.83, the total amount of reducing sugar produced by the degradation of Fuzao starch
160 was relatively abundant, and the concentration of reducing sugar in the degradation
161 solution was also more suitable for the ensuing alcohol fermentation. In the orthogonal
162 experiment, the time factor did not exert a significant influence on the formation of
163 reducing sugar. Considering the time cost, the conditions for the intermittent
164 degradation of Fuzao starch were ultimately established as follows: the first treatment,
165 at a pressure of 0.13 MPa and a pH of 1, with a solid-liquid ratio of 0.5 for 30 minutes;

the second treatment, at a pressure of 0.13 MPa and a pH of 1, with a solid-liquid ratio of 1.0 for 40 minutes; the third treatment, at a pressure of 0.13 MPa and a pH of 1, with a solid-liquid ratio of 1.0 for 50 minutes. The total solid-liquid ratio of the three treatments was 0.83, the starch degradation rate was 40.52 ± 0.03%, and the concentration of reducing sugar was 3.91 ± 0.17%. Owing to the increase in the volume of the filtrate, the total content of reducing sugar was significantly higher than that of the one-time degradation treatment.

Saccharification and Fermentation

Following the degradation of Fuzao under semi-solid conditions, 100 $u \cdot g^{-1}$ of glucoamylase was added to the filtrate for saccharification, and yeast koji was inoculated at a rate of 1% for anaerobic fermentation and subsequent distillation. The wine yield was 35.49 ± 0.48% $g \cdot kg^{-1}$. In comparison with the third round of fermentation without adding grains of Fuzao using the traditional solid-state fermentation method in our prior experiment, the wine yield was merely 5.19 ± 0.16% $g \cdot kg^{-1}$. Hence, the semi-solid fermentation method demonstrated a remarkable effect. The comparison of the wine yield of Fuzao semi-solid fermentation and traditional solid-state fermentation is illustrated in Fig. 2:

Furthermore, the traditional solid-state fermentation method for liquor production typically requires 361.5 hours (approximately 15 days), whereas this process can be completed within 76 hours.

The comparison of the production cycle of semi-solid re-fermentation for wine

187 production and traditional solid-state fermentation is depicted in Fig. 3:

188 **Development of New Wine**

189 The bottom pot water generated during the production process of strong-flavor Daqu
190 wine is a type of wastewater that is rich in organic acids and aromatic constituents.
191 High-performance liquid chromatography analysis indicates that it contains a
192 substantial amount of lactic acid (3882 mg·100 mL^{-1}) and other organic acids. A
193 portion of the wine produced by the semi-solid fermentation of Fuzao and the bottom
194 pot wastewater were blended in a specific proportion, and esterifying enzyme was
195 incorporated to initiate an esterification reaction between alcohol and organic acids,
196 thereby yielding a high ethyl lactate flavor wine. The content of ethyl lactate was
197 determined to be 19.86 $g \cdot L^{-1}$. The flavor wine exhibited a milky and turbid appearance,
198 accompanied by a faint acidic ester aroma.
199 The wine produced by the semi-solid fermentation of Fuzao was blended and flavored
200 with the high ethyl lactate flavor wine to formulate a new wine. In accordance with the
201 analysis data of Guilin Sanhua Liquor by Shen Yifang (Shen, YF, 1997) and the
202 analysis data of Fen Liquor by Wang Yuantai (Wang, YT, 1997) , the components of
203 the experimental new wine were compared with those of these two liquors. The
204 experimental results are presented in Fig. 4:
205 The components responsible for imparting aroma and taste in liquor are highly complex.
206 In addition to ethanol, it encompasses hundreds of trace chemical constituents. Its main
207 components can be categorized into alcohols, esters, acids, and aldehydes and ketones.

By comparing the aforementioned rice-flavor liquor, light-flavor liquor, and the new wine developed in this study, it can be observed that, on the one hand, the contents of ethyl lactate in the two flavor liquors are 110.0 mg·100 mL^{-1} and 155.97 mg·100 mL^{-1}, respectively. In contrast, the content of ethyl lactate in the new wine is considerably higher, reaching 280.56 mg·100 mL^{-1}. On the other hand, the contents of other chromatographic components in the new wine are all relatively low and exhibit minimal interference with the chromatographic component contents of the aforementioned two flavor liquors. Different types of liquors possess distinct main aroma components. For instance, in Fen-flavor liquor, ethyl acetate is the predominant aroma component, while ethyl lactate also plays an important supplementary role. In rice-flavor liquor, the content of ethyl lactate is higher than that of ethyl acetate, endowing ethyl lactate with a more prominent position.

Since ethyl lactate is a crucial chromatographic skeleton component and a significant flavor ingredient in rice-flavor and light-flavor liquors, the new wine developed in this study can be utilized as a specialized base wine in the blending of rice-flavor and light-flavor liquors, particularly for augmenting the ethyl lactate content in the base wine during the production of these two flavor liquors.

discussion

Many Chinese liquor enterprises usually sell Fuzao to some feed mills, fertilizer factories or other dealers through low-price bidding. Obviously, the added value of Fuzao has not been fully exploited. This experiment attempts to find a way to increase

the added value of Fuzao by making wine again.

Regarding the resource utilization of residual starch in waste distiller's grains of strong-flavor Daqu wine for wine production, the current research at home and abroad is generally in two stages. That is, Hongzao (The mash of Chinese strong aroma Daqu Wine during the first round of non-grain fermentation) is fermented without adding grains for the first time to produce Diuzao wine, and then Diuzao is discharged. Diuzao is fermented without adding grains for the second time to produce Fuzao wine, and then Fuzao is discharged. However, there is currently no mature process for large-scale application in the re-fermentation of Fuzao to produce wine for the third time without adding grains.

In the previous experiment, when the traditional solid-state fermentation process was used to ferment Fuzao without adding grains for the third time by adding glucoamylase and yeast koji, the wine yield was only 5.19 $g \cdot kg^{-1}$. The reason for the insignificant effect was that it was difficult for the traditional solid-state fermentation method to utilize this part of residual starch. It can be seen that the traditional solid-state fermentation process has little significance for popularization when Fuzao is fermented without adding grains for the third time.

Using the semi-solid re-fermentation process in this study, the wine yield reached 35.49 $g \cdot kg^{-1}$. Calculated at 30 ¥ per kilogram of wine, the value of wine produced per ton of Fuzao is about 1040 ¥. The residual grains after wine making can enter the husk environmental protection boiler as fuel for steam production because the husk contains

a large amount of lignin (Zhang, P, et al., 2023) . The value generated per ton of Fuzao in this link is about 300 ¥. After deducting various costs of about 400 ¥ in the two links, this process can bring about 1000 ¥ of income for the enterprise, and its value is much higher than that of directly selling to feed mills and fertilizer factories.

In addition, this study has developed a new wine with ethyl lactate as the main chromatographic skeleton component by using the waste resources of the distillery such as the re-fermentation tank and the bottom pot wastewater of wine making. It has great economic value and environmental protection significance in the aspect of resource development and utilization.

Materials and Methods

Materials

Yeast koji: The yeast koji was prepared by screening strains on the Fuzao stacking site, using bran as the culture medium and adopting the rack fermentation method.

Glucoamylase: Produced by Angel Enzyme Preparation Company in Yichang, Hubei Province, China, with an enzyme activity of 100,000 $U \cdot g^{-1}$.

Esterifying enzyme: Refer to the method of screening esterifying enzyme strains by Yanbo Liu(Liu, YB, et al., 2021) et al. Using light-flavor Daqu as the screening source and bran Fuzao as the enzyme-producing culture medium, a Monascus strain was screened out to prepare a crude enzyme preparation, with an enzyme activity of 100,000 $U \cdot g^{-1}$.

Fuzao: Provided by a certain strong-flavor Daqu distillery in Sichuan Province, China.

Methods

Degradation: Take 350 g of Fuzao, add 700 ml of distilled water, and place it in a 1,000 ml beaker. Degrade it under certain conditions of temperature, pressure, time and pH (first conduct single-factor experiments, and then conduct orthogonal experiments), and then filter.

Saccharification: Place the filtrate in a 60 °C water bath, adjust the pH to 4.5, add 100 $U \cdot g^{-1}$ of glucoamylase (Zong, XY, et al., 2018), and the saccharification time is 60 minutes. Determination of reducing sugar: Direct titration method with Fehling's reagent (Determination of reducing sugar in food, 2016). Determination of starch: Acid hydrolysis method(Determination of starch in food, 2016).

Fermentation: Cool the saccharified filtrate to 30 °C, inoculate yeast koji at an inoculation rate of 1%, and conduct anaerobic fermentation at 32 °C for 72 hours.

Distillation: Distill the fermentation broth and collect the distillate in different grades. The determination of alcohol content is carried out by the potassium dichromate oxidation method. Draw a standard curve with alcohol concentration and absorbance. Determine the alcohol concentration (% by volume) on the standard curve.

Preparation of high ethyl lactate flavor wine: The pH value of the bottom pot wastewater of strong-flavor Daqu wine production is relatively low, and lactic acid is its main component. Mix a part of the wine distilled from the semi-solid fermentation of Fuzao with the bottom pot wastewater in a certain proportion to make the alcohol

291 content of the mixture 15% and the concentration of lactic acid 1.5%. Add 8% of
292 Monascus esterifying enzyme(SU, Xl, et al.,2019). After maintaining the esterification
293 reaction at 30 °C for 21 days(Wei, ZY, et al., 2019), concentrate the solution and distill
294 it to an alcohol content of 30% (v/v) to obtain the high ethyl lactate flavor wine.
295 **Preparation of new wine with ethyl lactate as the main skeleton:** Blend the wine
296 distilled from the semi-solid fermentation of Fuzao with the high ethyl lactate flavor
297 wine to obtain a new wine with ethyl lactate as the main chromatographic skeleton.
298 Conduct laboratory standard analysis on the physicochemical indexes such as methanol,
299 cyanide, mycotoxin and pollutants of the new wine.
300 **Treatment of waste residue:** The solid waste residue obtained by filtration can be sent
301 to the husk boiler to obtain thermal energy by burning.
302 **Calculation of wine yield:** The wine yield of Fuzao by semi-solid fermentation ($g \cdot kg^{-1}$)
303 is calculated according to Equation (1):
304 Note: 60 represents the liquor converted to 60°. a: The alcohol content of the distilled
305 liquid at 20 °C; b: The volume of the distilled liquid (mL); 0.90915 represents the
306 density of 60° alcohol at 20 °C; c: The mass (kg) of repeated grains used for semi-solid
307 fermentation.
308 **Statistical analysis of data:** Each experiment was repeated three times. The
309 experimental data were expressed as the average value ± standard deviation, and
310 analyzed by using *SPSS 26.0* software.
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https://zenodo.org/records/23659505

Effect of temperature on residual sugar content of complex distiller's grains | Zenodo.

https://zenodo.org/records/23660284

Content of chromatographic components in new liquors | Zenodo.

https://zenodo.org/records/23660381

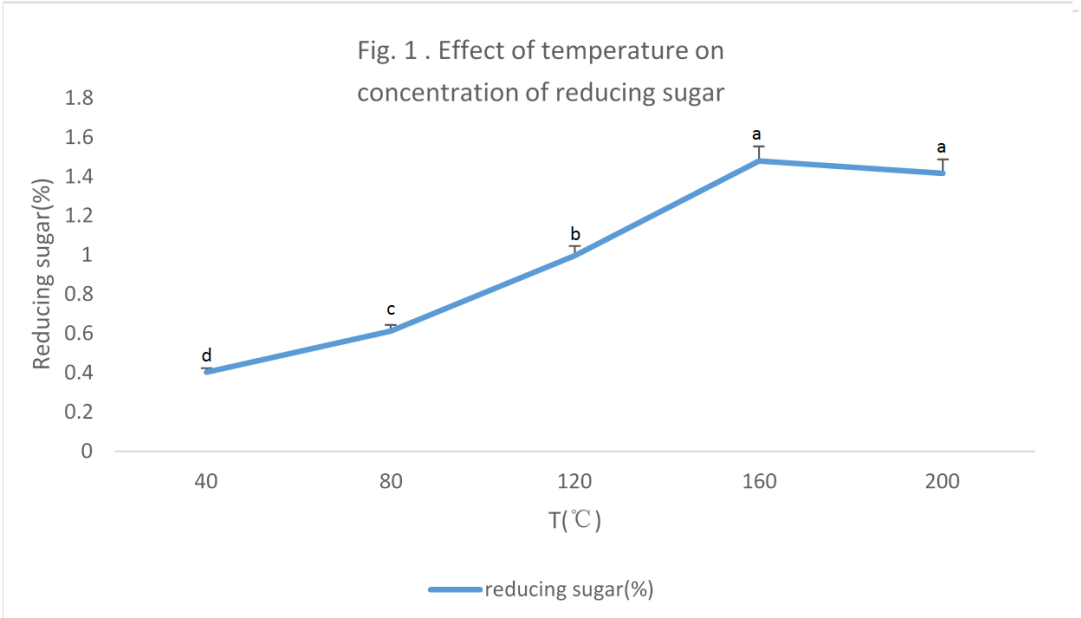
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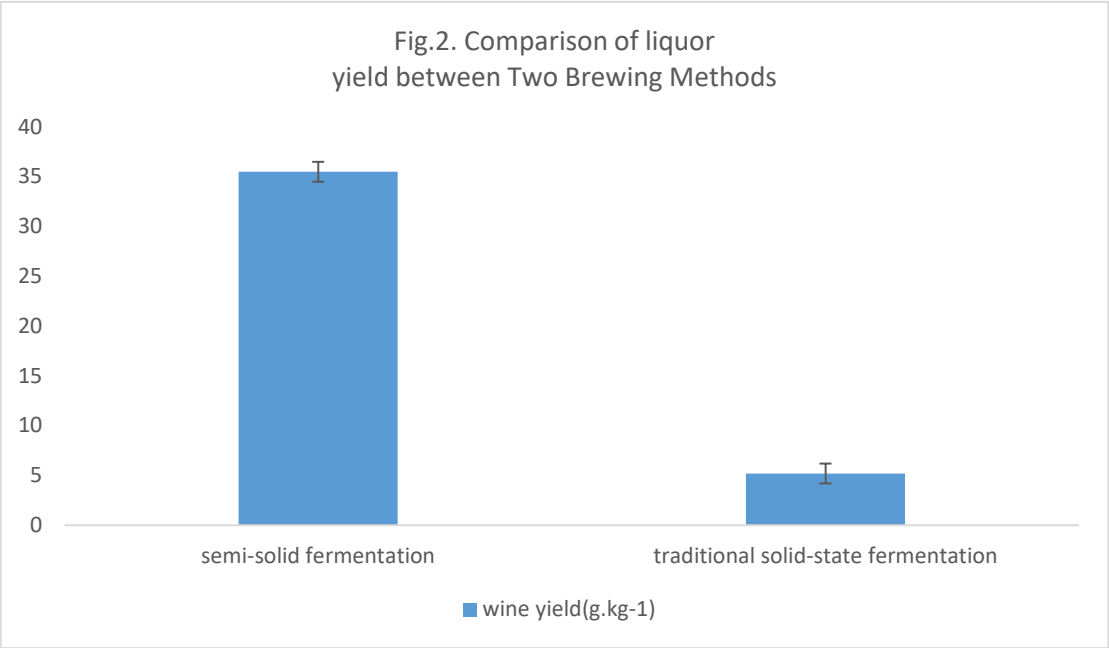
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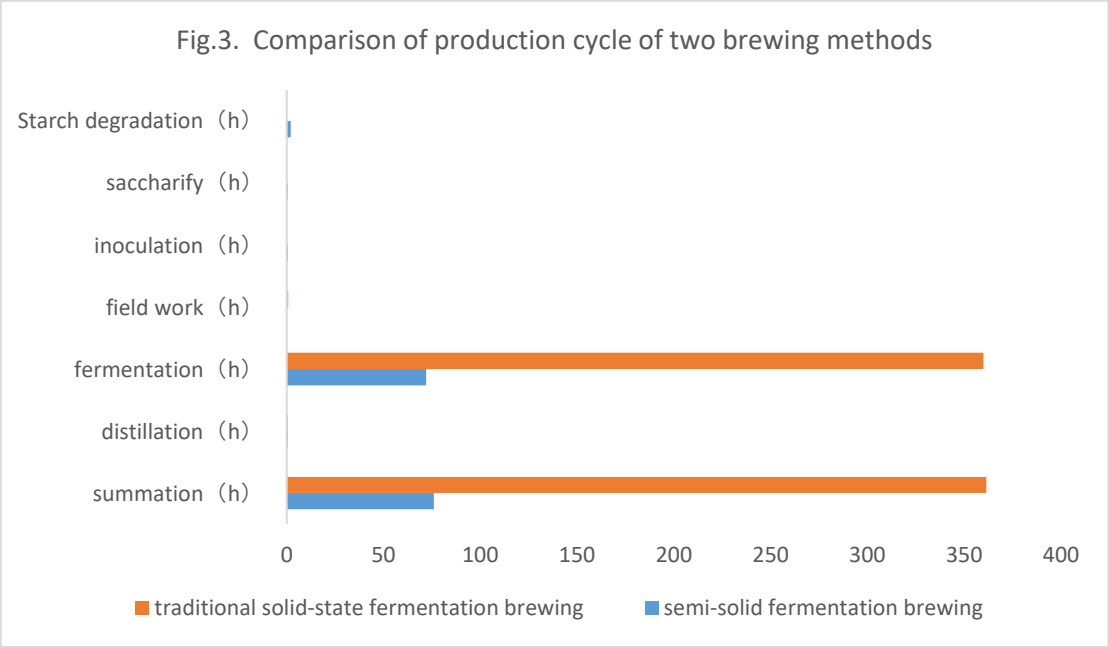
Supplementary Information

Figure legends



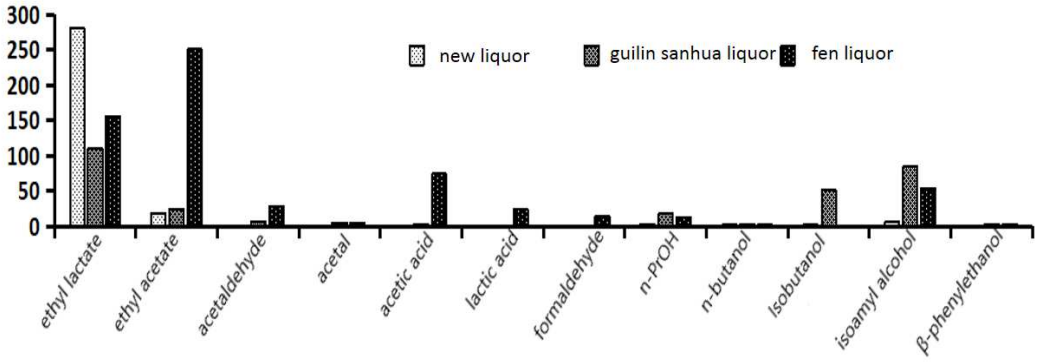

Fig.2. Comparison of liquor yield between Two Brewing Methods
40
35
30
25
20
15
10
5
0
semi-solid fermentation
traditional solid-state fermentation
wine yield(g.kg-1)

473


Fig.3. Comparison of production cycle of two brewing methods
Starch degradation（h）
saccharify（h）
inoculation（h）
field work（h）
fermentation（h）
distillation（h）
summation（h）
0
50
100
150
200
250
300
350
400
traditional solid-state fermentation brewing
semi-solid fermentation brewing

474

Fig.4. Composition comparison of three wines



475

476 **Tables**

Table 1 L_9 (3^4) orthogonal experiment

No	**（A）pH**	**（B）t （min）**	**（C）P （Mpa）**	reducing sugar（%）
1	1（3.0）	1（30）	1（0.02）	2.01
2	2（2.0）	2（60）	2（0.07）	2.92
3	3（1.0）	3（90）	3（0.13）	4.23
4	3（1.0）	2（60）	1（0.02）	2.50
5	1（3.0）	3（90）	2（0.07）	2.72
6	2（2.0）	1（30）	3（0.13）	3.24
7	2（2.0）	3（90）	1（0.02）	2.31
8	3（1.0）	1（30）	2（0.07）	2.98
9	1（3.0）	2（60）	3（0.13）	2.57

k_1	2.43a	2.74	2.27
k_2	2.82	2.66	2.87
k_3	3.24	3.09	3.35
R	0.81b	0.42	1.07

Note: [a] $k_i^A = \sum$ reducing sugar $A_i/3$; [b] $R_i^A = \max\{k_i^A\} - \min\{k_i^A\}$.

Equations

$$\text{liquor yield} = \frac{a \times b}{60} \times \frac{0.90915}{c} \quad (1)$$

Supplementary Files

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