Environmental Technology & Innovation 20 (2020) 101049

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Solid-state fermentation with pretreated rice husk: Green technology for the distilled spirit (Baijiu) production

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ARTICLE INFO

Article history:
Received 26 April 2020
Received in revised form 25 June 2020
Accepted 12 July 2020
Available online 15 July 2020

Keywords:
Baijiu
Rice husk
Solid-state fermentation
Gas chromatograph

ABSTRACT

Environmentally friendly chemistry is the development trend of Baijiu production. However, there are still many problems that need to be addressed urgently in the current Baijiu production process. First, the production of Baijiu produces a large amount of brown water (Huangshui, HS), which may cause pollution to the environment. Second, the solid-state fermentation produces low amounts of alcohol. Combining with the practical experience of Baijiu production, we have proposed an environmentally friendly method of producing Baijiu. The results confirmed that rice husk with pretreatment of HS significantly reduced the hardness of rice husk, enhanced the elasticity of the skeleton structure of rice husk, and improved its efficiency as an auxiliary material for solid-state fermentation. Therefore, this method can not only effectively utilize HS, but also improve the efficiency of solid-state fermentation and reduce the amount of food consumption, which is in line with the development concept of green chemistry.

1. Introduction

The alcoholic drinks have improved the daily diet habit of Chinese people and have a great influence on the catering culture in China (Liu and Sun, 2018; Wu et al., 2017). Baijiu, also named Chinese spirit, is a traditional alcoholic drink, which is typically obtained by the natural solid-state fermentation and distillation (Fan et al., 2019). It is one of the major fermented products in China with an production of 13 million metric tons, and approximately $ 8.8 billion in sales (Liu et al., 2017). The diverse aroma profiles of Baijiu results from their complexity in starting material and manufacturing technology (Du et al., 2011; Wu et al., 2015; Xiao et al., 2016). During the production, the starting material (e.g. sorghum, corn, rice) is firstly cooked and then mixed with active microbiota (also named *Jiuqu* powder) for the natural solid-state fermentation (Liu and Sun, 2018). After fermentation, Baijiu is obtained by distilling the fermented mixtures (Dong et al., 2019; Jiang et al., 2019).

Solid-state fermentation is defined as the growth of microorganisms without free-flowing aqueous solution. It has generated great interest in recent years as the microbes are considered to be optimally active under very low moisture conditions. It should be noted that a large amount of brown water (also named Huangshui, HS) is produced at the bottom

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https://doi.org/10.1016/j.eti.2020.101049
2352-1864/

of fermentation vessel (Zou et al., 2018). The medium-sized Baijiu enterprises (with an annual Baijiu output of 10,000 tons) approximately produce 10 tons of HS per day (Huo et al., 2020; Xia et al., 2014). HS is a brown viscous liquid and a by-product of liquor production. It is not only rich in alcohols, aldehydes, acids and esters, but also contains a large number of long-term domesticated brewing microorganisms (Zhang et al., 2015). However, during production, HS is normally tapped off, and a considerably large proportion of manpower and financial costs is allocated to deal with the potential environmental pollution caused by HS (sewage treatment). Therefore, the comprehensive utilization of HS is one of the urgent problems in liquor industry.

In addition, rice husks, an auxiliary material, are used in the production of Baijiu (Vegas et al., 2004; Xu et al., 2017). Rice husk is the husk that is taken off when rice is processed (Vegas et al., 2008). It is an appendage of rice grain that contains pectin, pentosan, and a small amount of nutrients. The auxiliary material is used as a filler for fermentation to improve the contact interface between microorganisms and grain in the solid-state fermentation process, and accelerate the propagation of microorganisms, so as to improve the efficiency of fermentation and increase the yield of Baijiu. However, the high hardness of rice husk and the insufficient elasticity of its skeleton structure limit its effectiveness as an auxiliary material to increase the efficiency of microbial fermentation (Wood et al., 2016).

Combined with the production experience of the winery, a method has been designed to solve both the above problems: before fermentation, the rice husks are soaked in HS, utilizing the microorganisms in HS to pretreat the rice husks, so as to reduce the hardness of rice husks and improve the elasticity of its skeleton structure. The experimental results showed that the physical and chemical properties of rice husks changed significantly after pretreatment. And a higher yield of Baijiu was observed. In other words, pretreatment of rice husks with HS can improve the fermentation efficiency. Under the premise of the same yield, HS has not only been effectively used, but also the use of grain has been reduced, thus conforming to the development concept of green chemistry.

2. Materials and methods

2.1. Materials

Different standards of alcohols, esters and acids, all of HPLC grade, were purchased from Dikma Technologies Inc., Beijing, China. Chemicals were used without further purification. All solutions were freshly prepared by ultrapure water, with a resistivity of 18.2 MΩ cm^{-1}. HS samples were collected from the manufacturer Hunan Xiangjiao Brewing Co., LTD, Shaoyang, China

2.2. Methods

The chroma of the rice husk was analyzed using a tristimulus colorimeter (CR-400, Nikon, Tokyo, Japan) and Spectra-Match software, set to L*, a*, b* mode (Cofrades et al., 2013). The moisture content of rice husks was analyzed according to the standard protocol (Chinese National Standards, GB5009.3-2016). The bulk density, hardness and elasticity of the rice husk were analyzed by an Texture Analyser (AMETEK, Inc, Shanghai, China).

The Baijiu product was analyzed by an Agilent 7890B gas chromatograph. The pressure of nitrogen gas was maintained between 0.35 and 0.50 MPa. Whether the septum and liner needed to be replaced were determined according to the sample analysis. The helium source pressure was set at 0.30–0.35 MPa; the air source pressure was set at 0.35–0.40 MPa. 4.9 ml Baijiu sample was added with 0.1 ml internal standard, Agilent CP-Wax 57 CB capillary column (0.25 mm × 50 m ×0.2 μm), carrier gas was N_2, flow rate was 1.0 mL/min, split ratio was 20:1, injection volume was 1 μL, equilibration time was 1 min, inlet temperature was 200 °C, detector temperature was 300 °C. Temperature program: initial temperature 40 °C for 5 min, increased to 50 °C by 3 °C/min and maintained for 6.5 min, then increased to 90 °C by 6 °C/min and maintained for 5 min, then increased to 130 °C by 10 °C/min and maintained for 2 min, then increased to 190 °C by 5 °C/min and maintained for 1.4 min, then the temperature was raised to 195 °C by 10 °C/min and maintained for 20 min. Gas flow rate: hydrogen was 30 ml/min, air was 400 ml/min, FID tail gas was 25 mL/min. The chemical compounds are identified by comparing the retention times with reference standards. 2-ethylbutyric acid (18.122 mg/100 ml) and n-pentyl acetate (17.316 mg/100 ml) were used as the internal standards. The acid and ester contents in Baijiu samples measured by gas chromatograph are shown in Table 1 and Table 2, respectively.

The Baijiu product was analyzed by an electronic tongue. The electronic tongue data were collected at room temperature. The electronic tongue tested each sample for 60 s, the parallel samples were cleaned for 60 s, and the cleaning time between samples was 120 s. Baijiu samples of different layers of fermented grains (upper layer, middle layer, and lower layer) were analyzed by principal component analysis (PCA).

2.3. Statistical analysis of experimental data

The reported result is the mean value of three parallel measurements (n = 3) and the data are expressed as the mean ± S.E.M. *$P < 0.05$ compared with the control, as estimated by One simple t Test. Statistical analysis and PCA were performed with Origin 2019 (OriginLab Co., Northampton, USA).

Table 1
Main acids in Baijiu samples performed by gas chromatography. The concentration unit: mg/100 mL.

Constituents	Control (without HS)	HS	Domesticated microorganisms
Acetic acid	67.71 ± 1.67	39.58 ± 1.52	49.64 ± 1.03
Propionic acid	1.97 ± 0.48	1.82 ± 0.34	2.51 ± 0.64
Isobutyric acid	1.71 ± 0.86	0.54 ± 0.16	1.35 ± 0.41
n-butyric acid	10.69 ± 1.82	4.09 ± 1.85	8.23 ± 0.94
Isovaleric acid	0.78 ± 0.13	0.62 ± 0.22	1.07 ± 0.27
n-pentanoic acid	1.06 ± 0.19	1.02 ± 0.18	1.16 ± 0.37
2-ethylbutyric acid	18.32 ± 0.33	17.95 ± 0.32	17.85 ± 0.08
n-hexylic acid	22.78 ± 1.25	12.6 ± 1.49	18.59 ± 1.55

Table 2
Main esters in Baijiu samples performed by gas chromatography. The concentration unit: mg/100 mL.

Constituents	Control (without HS)	HS	Domesticated microorganisms
Ethyl acetate	199.33 ± 6.97	163.3 ± 5.77	238.14 ± 5.92
Ethyl butyrate	11.55 ± 1.81	7.18 ± 1.15	9.05 ± 0.82
Ethyl valerate	2.78 ± 0.49	3.13 ± 0.36	3.08 ± 0.58
Ethyl caproate	103.01 ± 5.15	121.32 ± 1.92	114.02 ± 4.74
Ethyl lactate	313.26 ± 6.22	232.17 ± 6.03	340.78 ± 7.25
Ethyl palmitate	5.19 ± 0.47	3.68 ± 0.51	5.09 ± 0.57
Ethyl oleate	1.76 ± 0.18	1.05 ± 0.08	1.54 ± 0.44
Ethyl linoleate	4.51 ± 0.23	2.13 ± 0.21	3.82 ± 0.51

3. Results and discussion

As shown in the scheme (Fig. 1), the pretreatment process of rice husk includes mixing, soaking, washing, and drying. The grain materials were steamed and solid-state fermented with the pretreated rice husks and *Jiuqu* (Wang et al., 2019a). Then the solid-fermented grain were distilled together with the auxiliary material to obtain the steam, which was the original Baijiu. The aroma of the original Baijiu is pungent and acrid, thus it is aged for years (usually more than 3 years) to remove the pungent and volatile compounds such as allyl alcohol, hydrogen sulfide, and acraldehyde. After that, the aged base of Baijiu are blended with specific flavor components to form a commercialized product. To evaluate the possibility of using the HS-pretreated rice husk for the solid-state fermentation as bulking agent, the HS-pretreated rice husk was completely characterized.

The chroma of the HS-pretreated rice husk was characterized, shown in Fig. 2a. As seen, the chroma of the rice husk was significantly reduced after pretreatment, which indicated that some colored substances were consumed during the HS pretreatment (Anda et al., 2008). The moisture content the HS-pretreated rice husk was characterized, shown in Fig. 2b. As seen, the moisture content of rice husks was reduced (the drying step was strictly similar with that for the control). As an auxiliary material for fermentation, the lower moisture content allowed rice husks to absorb more liquid produced by solid-state fermentation, so that the moisture content of the fermentation tank was more uniform, which was beneficial to the growth of brewing microorganisms (Nagel et al., 2001). Moreover, the bulk density of rice husks was also significantly reduced (Fig. 2c). Rice husk with lower bulk density allowed more oxygen to enter the fermentation pond and increased the fermentation rate of aerobic microorganisms (Asha Poorna and Prema, 2007). The decrease of moisture content and bulk density in rice husks indicated a reduction in the hardness of the rice husk (Seth et al., 2015). We further tested the hardness of the rice husks and the results validated our inference that the hardness of the rice husks was significantly reduced (Fig. 2d). HS contains multiple organic acids, such as, acetic acid, formic acid, lactic acid, oxalic acid, and citric acid (Xia et al., 2014). In general, the pH of HS solution is ∼3.5 (Xia et al., 2014). Based on the above results, it can be suggested that the organic acid in the HS softens the plant fibers of the rice husk. In addition, the major microbes in HS include *Lactobacillales* (Zhang et al., 2015), and *Saccharomycetales* (Wu et al., 2013), which originate from the *Jiuqu* powder. When the rice husks were pretreated with HS, the microorganisms significantly changed the physicochemical properties of the rice husks by producing hydrolytic enzymes such as cellulases (Uzuner and Cekmecelioglu, 2019). The elasticity of the pretreated rice husks was tested, as shown in Fig. 2e, the elasticity of the rice husks was significantly enhanced. The lack of elasticity of the skeleton structure of rice husk was the main factor limiting its efficiency as an auxiliary material for improving microbial fermentation (Jearanaisilawong et al., 2015; Nie et al., 2015). Therefore, the pretreated rice husks were applied to solid-state fermentation experiments to explore its effects on microbial fermentation efficiency and Baijiu yield.

The solid-state fermentation experiment using pretreated rice husks were performed and the Baijiu samples were collected. Three groups of Baijiu samples were tested, namely: the first group was obtained from the rice husk that had not been pretreated with HS (blank control group); the second group was obtained from the rice husk pretreated

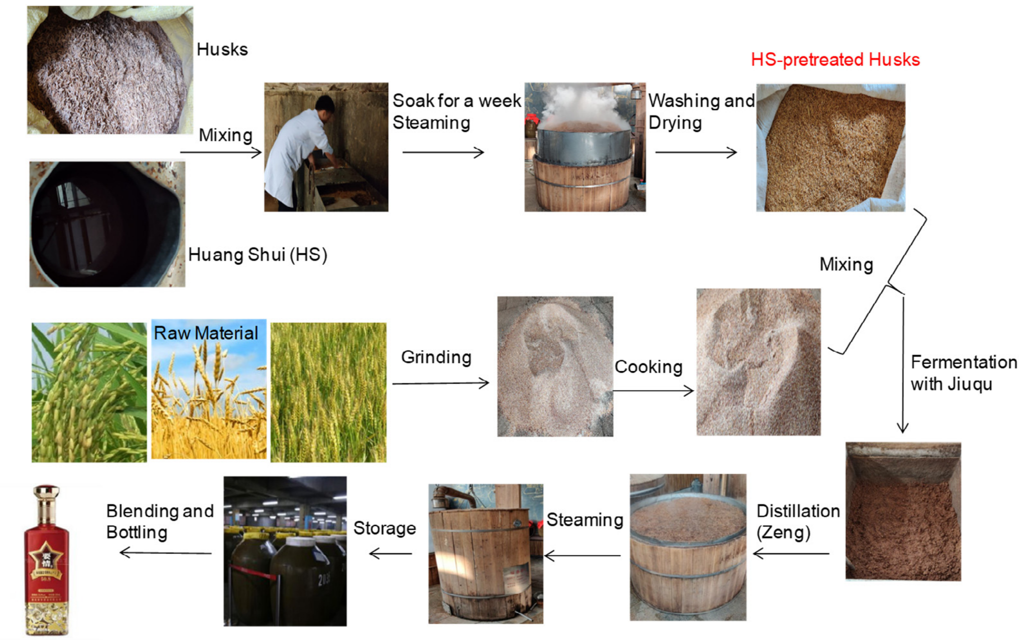

Fig. 1. Scheme of the proposed Baijiu production method: Solid-state fermentation with HS-pretreated rice husk.

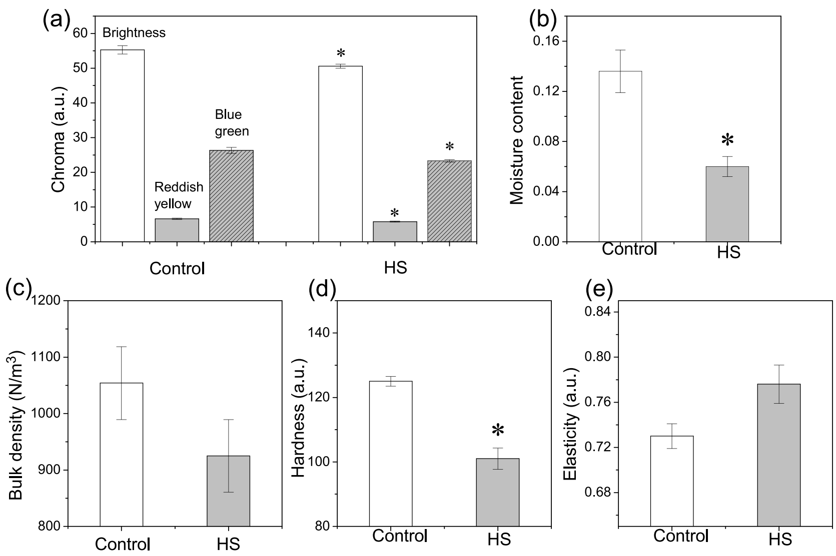

Fig. 2. Characterization of the chroma (a), moisture content (b), bulk density (c), hardness (d) and elasticity (e) of the HS-pretreated rice husk. *P < 0.05, compared with the control, n = 3.

with HS (experimental group); the third group was obtained by treating the rice husks with the yeast containing active microorganism (comparing the treatment effect of HS and yeast). In order to avoid the difference in solid-state fermentation at different heights (Arora et al., 2018), we selected raw materials at three different heights for the production of Baijiu, the upper layer, the middle layer, and the lower layer. The results of the total acid test are shown in Fig. 3a. After the rice husk was pretreated with HS, the total acid content of the Baijiu sample was significantly lower than that of the control group. According to the Chinese national standards (GB/T 10781.1-2006) (Qin et al., 2012), the total acid content of fine quality Baijiu should be higher than 0.4 g/L. However, if the total acid content was too high (highly than 1.4 g/L), it would enhance the pungent taste of liquor (Zheng et al., 2014). The total acid content of the experimental group was about 0.6 g/L, which was in line with the standard of high-quality liquor, and avoided the pungent taste caused by excessive total acid. Therefore, the total acid content was suitable. After treating the rice husks with active microorganisms, the total acid content of the liquor samples was very close to that of the experimental group. The results

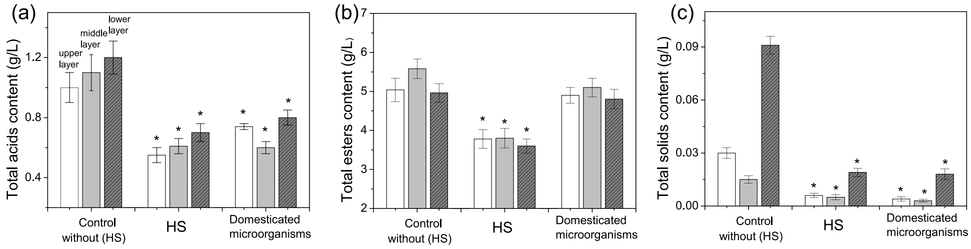

Fig. 3. The total acid content (a), total ester content (b) and total solids content (c) of three groups of Baijiu samples: Baijiu obtained from the rice husk that had not been pretreated with HS (control group, without HS), Baijiu obtained from the rice husk pretreated with HS (HS), Baijiu obtained by treating the rice husks with the yeast containing active microorganism (Domesticated microorganisms). Samples at three different heights were presented in different color (Upper layer in white, middle layer in gray, lower layer in black). *$P < 0.05$, compared with the control, $n = 3$.

showed that the efficiency of pretreatment of rice husks in HS was close to the efficiency of pretreatment of rice husk by active microorganisms in the yeast. The balanced distribution of active microorganisms in the yeast was a key factor affecting the effect of solid-state fermentation (Wang et al., 2019b). HS can achieve similar functions, confirming the potential of HS to be effectively utilized.

The esters in Baijiu mainly include ethyl lactate, ethyl acetate, and ethyl hexanoate, and the content thereof accounts for more than 90% of the total esters (Cui et al., 2018). According to the Chinese national standards above mentioned, the total ester content of high-quality liquor should be higher than 2 g/L, but the high total ester content will destroy the balance of fragrance in the liquor (Li et al., 2017). Usually the excessive content of esters were removed by re-distillation (de Almeida Lima et al., 2012). The results of the total ester test are shown in Fig. 3b. After the rice husk was pretreated with HS, the total ester content of the liquor sample was significantly lower than that of the control group. The total ester content of the experimental group was about 3.8 g/L, which not only met the high-quality liquor standard, but also prevented the total ester content from being too high. After the rice husk was treated with the active microorganism, the total acid content of the Baijiu sample was close to the control group. The results showed that the effect of active microorganisms on rice husk pretreatment was not obvious. Conversely, rice husks treated with microorganisms in HS could significantly reduce the total ester content of the Baijiu sample. This might be due to the strong acid resistance of the microorganisms in the HS (Xu et al., 2019), and the lower pH of the HS was beneficial to the pretreatment of the rice husk.

Baijiu solid referred to the residue after evaporation of ethanol, water and other volatile components at the measured temperature (100 °C–105 °C) (Zhu et al., 2016). The inorganic constituents in brewing water were the main source of solids. If there was a large amount of inorganic salts and insoluble in the water, not only would the finished Baijiu solids exceed the standard, but also affected the taste of the liquor, and even caused sedimentation or turbidity. According to the Chinese national standards, the solid content of fine quality liquor should be less than 0.40 g/L. The total solid content test results are shown in Fig. 3c. The solid contents of all the tested samples were in line with national standards. In addition, the solid contents of the HS-pretreated sample and the active microorganism pretreated sample were significantly lower than the control group. On the premise of meeting national standards, the total solid content was further reduced. In this case, it can be suggest that the Baijiu obtained with HS-pretreated rice husk has a better quality. It can also be seen that raw materials of different heights used for the production of Baijiu have significantly different total solid contents. For example, for the control group, the content of the solids produced by the lower layer of raw materials was significantly higher than that of the upper layer. This phenomenon is reasonable when we take consideration of the gravity.

It can be seen from the above results that after pretreatment of rice husks with HS, the total acid, total esters and total solid content of the obtained Baijiu were within a reasonable range, and met the national standards. In order to comprehensively analyze the effect of HS pretreatment of rice husk on Baijiu production, we conducted principal component analysis with the electronic tongue on the Baijiu samples of the above three groups (Li et al., 2019). Similarly, in order to avoid the difference in solid-state fermentation at different heights, we selected raw materials at three different heights, the upper, middle and lower layers. The results are shown in Fig. 4. As seen, data points in each group were distributed separately. After the rice husks were pretreated with HS, or the rice husks were treated with active microorganisms, both the samples at the upper (Fig. 4a), middle (Fig. 4b) and lower (Fig. 4c) layers were significantly different from the control group, confirming that the pretreatment with HS has a obvious effect on enhancing the physicochemical properties of the rice husks.

The yield of base liquor is the main indicator affecting the production cost of Baijiu (Lever et al., 2010). Therefore, we further compared the yield of base liquor, η, as shown in Fig. 5. After pretreatment of rice husks with HS, η was significantly higher than that of the control group. After the rice husks were treated with active microorganisms, η was close to that of the experimental group. There were two main factors related to the yield of base liquor: 1, the starch and sugar content of the raw materials; 2, the degree of fermentation of the raw materials (Li et al., 2017). The above three groups all used the same raw materials, so it can be concluded that the skeletal structure of the rice husk was enhanced after the HS pretreatment, and as a filler for solid-state fermentation, the contact interface between microorganisms and food in the solid-state fermentation process was improved, thereby the degree of fermentation of raw materials was improved.

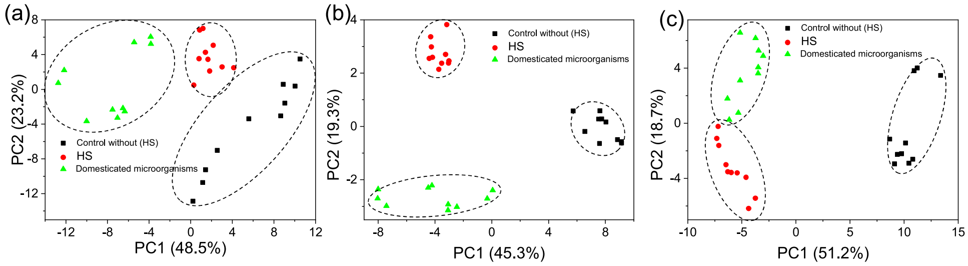

Fig. 4. Principal component analysis on the Baijiu samples in the control group, HS-pretreated group and domesticated microorganism pretreated group respectively. At different heights: the upper (a), middle (b) and lower (c) layers.

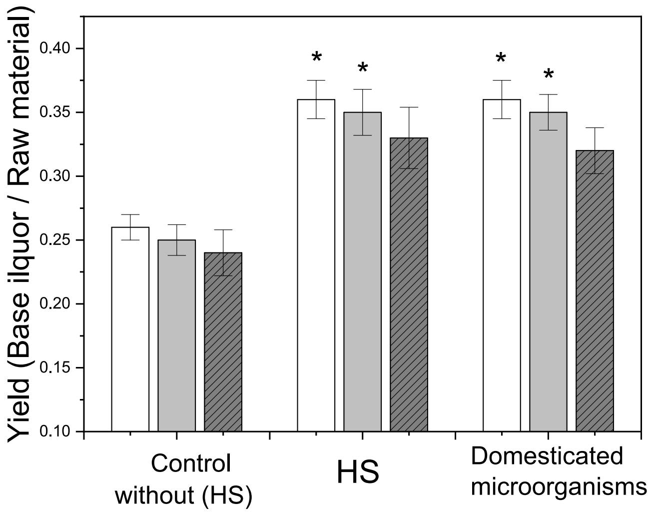

Fig. 5. The yield of base liquor (η) in the control group, HS-pretreated group and domesticated microorganism pretreated group respectively, $^{*}P < 0.05$, compared with the control, $n = 3$.

4. Conclusions

In this paper, the rice husks pretreated with HS were used for solid-state fermentation to produce Baijiu. The conclusions are as follows: first, the rice husks were pretreated with HS, and the microorganisms in the HS utilized the nutrients in the rice husks for metabolism, which significantly changed the physicochemical properties of rice husks by enhancing the elasticity of rice husks and improving air permeability. Second, the total acid, total ester and total solid content of the obtained liquor produced by pretreated rick husks were within a reasonable range, and met the national standards. Under the premise of the same Baijiu production, the HS pretreatment of rice husks can significantly reduce the amount of grain used in Baijiu production, and conform to the development concept of green chemistry.

CRediT authorship contribution statement

Binqiang Fan: Investigation. **Liping Xiang:** Investigation. **Yougui Yu:** Supervision, Writing - review& editing. **Xuepeng Chen:** Investigation. **Qiang Wu:** Writing - review& editing. **Kun Zhao:** Investigation. **Zhilong Yang:** Investigation. **Xiang Xiong:** Investigation. **Xiaobei Huang:** Project administration. **Qing Zheng:** Project administration, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We gratefully acknowledge the financial support from Hunan Provincial Natural Science Foundation of China (Grant No. 2020JJ5516, 2016JJ4080), Science and Technology Bureau of Shaoyang, China (Grant No. 2018CG18, 2017NS17) and Science and Technology Department of Hunan Province, China.

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