African Journal of Biotechnology Vol. 10(79), pp. 18311-18318, 12 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.242
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Study on the alcohol fermentation technology of *Dioscorea zingiberensis* and *D. zingiberensis* residue

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Accepted 4 November, 2011

Using *Dioscorea zingiberensis* and *D. zingiberensis* residue as raw materials, fermentation technology was studied separately under optimal fermentation conditions. This study shows that under the same condition, the final alcoholic concentration of the fermented liquors was 10.5 v/v using *D. zingiberensis* residue as raw material and 6.0 v/v using *D. zingiberensis*; moreover, the utility ratio of the starch in the raw materials was 90.90 and 51.94%, respectively. Scanning electron microscope (SEM) showed that the fiber structure of vinasse fermented from *D. zingiberensis* was more comparatively intact than that of its residue, which showed that *D. zingiberensis* residue was more appropriate for alcohol fermentation. However, the use of *D. zingiberensis* residue can not only achieve the clean production of diosgenine, but can also replace some grains in the production of alcohol, so that there can be a reduction in food crisis.

Key words: *D. zingiberensis, D. zingiberensis* residue, fermentation, alcohol.

INTRODUCTION

Dioscorea zingiberensis C. H. Wright, which belongs to *Dioscorea* of Dioscoreaceae family, contains about 2% dioscin, and 40 to 50% starch and fiber. The hydrolysis product of dioscin-diosgenine has great medical value (Aderiye et al., 1996; Moalic, 2001). High consistency fiberizer was used by Li et al. (2008) to isolate the starch and cellulose from *D. zingiberensis* at first, and then dioscin was hydrolyzed into diosgenine from the turbid liquid. This method was successfully used to isolate the starch from *D. zingiberensis*, though the long process route resulted in high cost, which made it difficult for industrialization to be realized. The method of using *D. zingiberensis* as raw material and fermenting it to produce alcohol was used by Chen et al. (2007) to obtain alcohol from the fermentation of starch at first, after which *D. zingiberensis* residue was hydrolyzed to extract diosgenine. However, the dioscin has the effect of anti-bacteria, which leads to a low alcohol concentration in the fermentation liquor and in the process of fermentation; although, dioscin is subjected to the effect of the microbe of which the steroid ring is broken, thereby resulting in a low purity of the final product - diosgenine. The method of using *D. zingiberensis* as raw material and extracting diosgenine from it by ultrasonic was studied in this paper, of which *D. zingiberensis* residue was fermented to change the starch in the residue into alcohol. On the one hand, this method reduced the amount of acid used in the process of hydrolyzing dioscin into diosgenine (dioscin was only 10% of *D. zingiberensis*; therefore, it reduced the pollution); while on the other hand, it increased the conversion rate of the alcohol in *D. zingiberensis*, in which case, the alcohol concentration in the fermentation liquor reached 10.5 v/v. This paper provides some useful experience in the use of starch in botanical medicines and in exploiting new energy.

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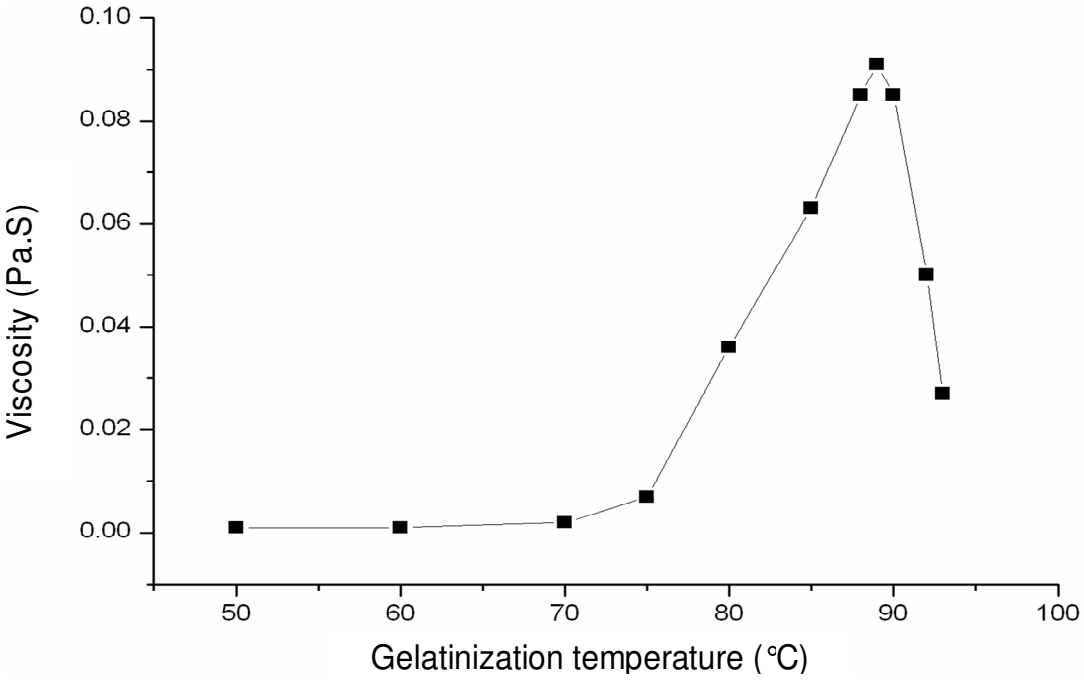

Figure 1. The relationship between viscosity and temperature.

MATERIALS AND METHODS

D. zingiberensis was supplied by Ankang *D. zingiberensis* Research Institution and identified by Prof. Hu Zhenghai from Northwest University. *D. zingiberensis* residue was supplied by the author's lab; thermostable α-amylase (activity 20000 μ/ml) and glucoamylase (activity 20000 μ/ml) are both from Jienuo Enzyme Co., Ltd; high active yeast is from Angel Yeast Co., Ltd; glucose, sucrose, sodium hydroxide and hydrochloric acid are all chemical agents purchased from Xi'an Chemical Agents Ltd. 7230G visible spectrophotometers are from Shanghai Precision and Scientific Instrument Co., Ltd; NDJ-8S digital viscometer is from Shanghai Precision and Scientific Instrument Co., Ltd.; Rotary evaporator (R206D) is from Shanghai SENCO Technology Company Ltd.; SHP-070 biochemistry incubators are from EHSY Lab Company; GMSX-280 portable pressure steam sterilizer is from Jiangmen PengJiang Yue Feng Drinking Water Equipment Co., Ltd.; and PHS-3C precise pH meter and scanning electron microscope (SEM) are from Beijing Elaborate Technology Development Ltd.

Experimental methods

GB/T 12098-1989 was used for the gelatinization temperature of starch in *D. zingiberensis* residue; 3,5-dinitrosalicylic acid (DNS) was used for reducing sugar;

$$\text{The dextrose equivalent (DE) values} = \frac{\text{The reducing sugar} \times 100\%}{\text{The total sugar}}$$

The determination of alcohol was done by GB/T10345 - 2007;

$$\text{The actual liquor yield of the starch} = \frac{\text{The mass of 95 v/v alcohol actually obtained} \times 100\%}{\text{The mass of starch}}$$

$$\text{The utilization factor of starch} = \frac{\text{The actual yield of alcohol} \times 100\%}{\text{Theoretical yield of alcohol}}$$

$$= \frac{\text{The actual liquor yield} \times 100\%}{\text{Theoretical yield of alcohol}}$$

(Pramanik et al., 2003; Vega et al., 1987).

Fermentation technology comparison between *D. zingiberensis* residue and *D. zingiberensis*

Using the following formulas:

$$\text{The actual liquor yield of the starch} = \frac{\text{The mass of 95 v/v alcohol actually obtained} \times 100\%}{\text{The mass of starch}}$$

$$\text{The utilization factor of starch} = \frac{\text{The actual yield of alcohol} \times 100\%}{\text{Theoretical yield of alcohol}}$$

$$= \frac{\text{The actual liquor yield} \times 100\%}{\text{Theoretical yield of alcohol}}$$

RESULTS AND DISCUSSION

Determination of the gelatinization temperature of *D. zingiberensis* residue

30 g of *D. zingiberensis* residue was taken with a granularity of 40 to 60 mesh (11.97% moisture and 48.11% starch) into a 1000 ml beaker, after which 850 ml deionized water was added to it. It was stirred while heating, and at different temperatures, NDJ-8S digital viscometer was used to measure the viscosity of *D. zingiberensis* residue. The results are shown in Figure 1.

Figure 1 shows that when the temperature is within 50 to

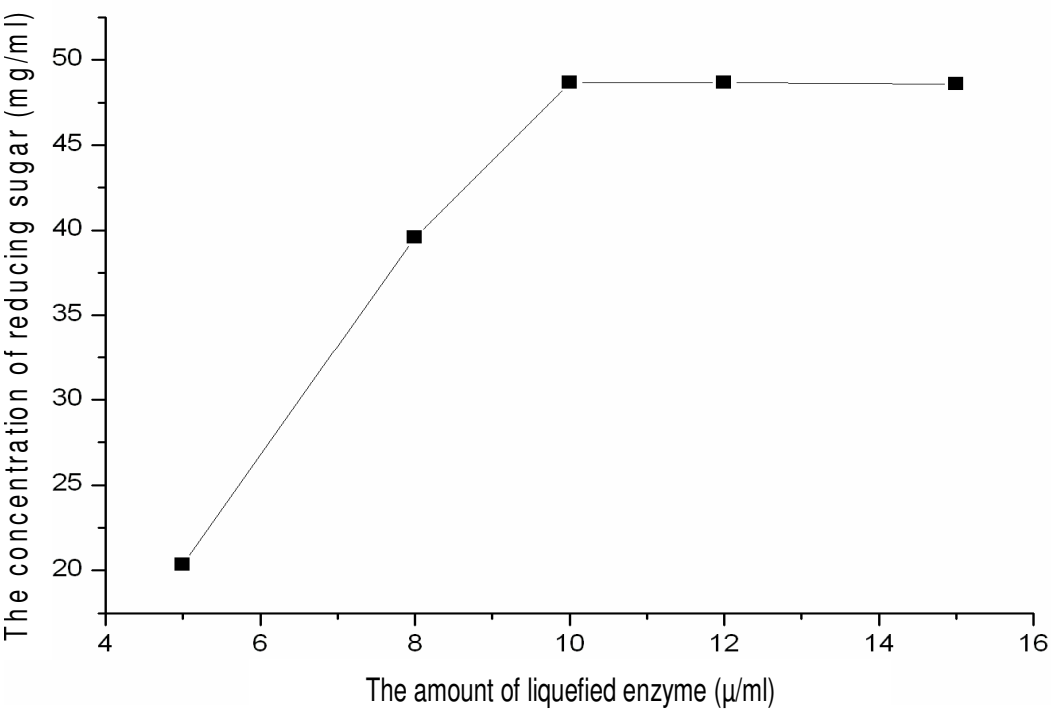

Figure 2. The relationship between the amount of reducing sugar and liquefying enzyme.

75℃, the viscosity of the pasting liquid basically changes slightly, while when it is within 75 to 90℃, the viscosity increases drastically, but when the temperature reaches 90 to 95℃, the viscosity drops drastically; thus, the gelatinization temperature is at 90℃.

Determination of the best ratio of *D. zingiberensis* residue to water and the optimal liquefying time

Four shares of 30 g *D. zingiberensis* residue with a granularity of 40 to 60 mesh were taken into a 250 ml beaker, after which water was added according to the ratio of *D. zingiberensis* residue: water = 1:3, 1:4, 1:5 and 1:6, respectively. It was stirred while heating, and when the temperature got to 90℃, it was put in thermostable α-amylase and liquefied for 50 min. Samples were taken every 10 min, and the concentration of reducing sugar was measured in the liquefied sample, in order to decide the best ratio of *D. zingiberensis* residue to water and the optimal liquefying time. However, the results are shown in Table 1.

Table 1 shows that at the same liquefying time, with the increase of the ratio of solid-liquid, the amount of the reducing sugar reduces in the liquefied sample. Particularly, when the ratio reaches 1:3, the amount of the reducing sugar reaches its highest, but when the ratio is below 1:3, it becomes hard to stir, due to the expansion of the fibers in the residue after the absorption of water, so the best ratio is 1:3. When the liquefying time changes from 10 to 30 min at the same ratio, the amount of reducing sugar increases too with the increase of liquefying time, but after 30 min, the amount has a slight decrease; thus, the optimal liquefying time is 30 min.

Determination of the optimal amount of liquefying enzyme

Five shares of 30 g *D. zingiberensis* residue with a granularity of 40 to 60 mesh were taken into a 250 ml beaker, after which 90 ml of water was added to it. It was stirred while heating, and when the temperature got to 90℃, 5, 8, 10, 12 and 15 μ/ml of thermostable α-amylase were added to it, respectively, and under this temperature, it was liquefied for 30 min. Samples of it were taken and the concentration of reducing sugar was measured in the liquefied liquid, in order to decide the best amount of liquefying enzyme. The results are shown in Figure 2.

Figure 2 shows that when the amount of liquefying enzyme is within 5 to 10 μ/ml, the amount of reducing sugar increases, with its increase, but when the amount of the liquefied enzyme exceeds 10 μ/ml, the amount of the reducing sugar does not show much change, so the optimal amount of liquefying enzyme is 10 μ/ml.

Determination of the optimal conditions for saccharification

Five shares of 30 g *D. zingiberensis* residue with a

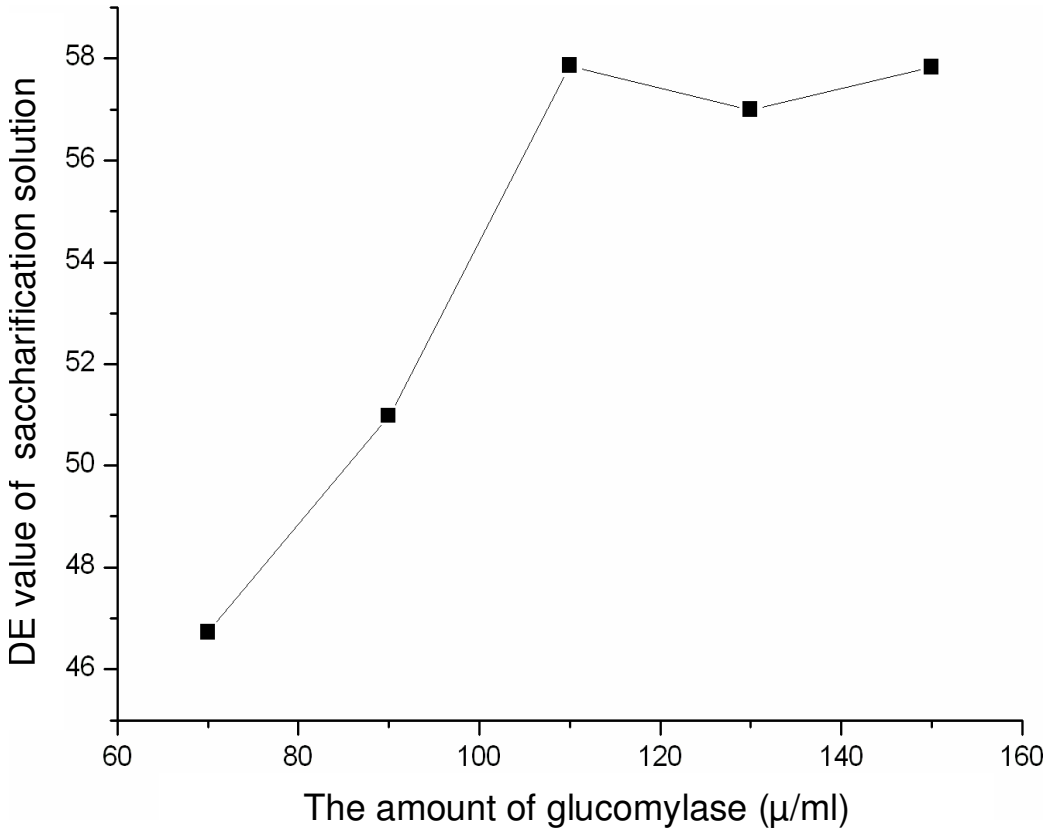

Figure 3. DE value of saccharification solution and the amount of glucomylase. DE, Dextrose equivalent.

granularity of 40 to 60 mesh were taken into a 250 ml beaker, after which 90 ml water was added to it. It was stirred while heating, and when the temperature got to 90°C, 10 μ/ml of thermostable α-amylase was added to it and liquefied for 30 min. It was allowed to cool down to room temperature, before the pH was adjusted to 6.2 to 6.5 and heated up to 60°C. Then, 70, 90, 110, 130 and 150 μ/ml of glucoamylase was added to it, respectively and at this temperature, it was saccharified for 80 min. Samples were taken and the concentration of reducing sugar was measured in the liquefied liquid in order to decide the best amount of saccharifying enzyme.

30 g of *D. zingiberensis* residue with a granularity of 40 to 60 mesh was taken into a 250 ml beaker, after which 90 ml water was added to it. It was stirred while heating, and when the temperature got to 90°C, 10 μ/ml of thermostable α-amylase was added to it and liquefied for 30 min. It was allowed to cool down to room temperature, before the pH was adjusted to 6.2 to 6.5 and heated up to 60°C. Then 110 of μ/ml glucoamylase was added to it and saccharified for 120 min. Starting from 40 min, samples were taken every 10 min, in order to measure the concentration of reducing sugar in the liquefied sample, and the best saccharization time was decided. The results are shown in Figures 3 and 4.

Figure 3 shows that when the amount of glucomylase was between 70 to 110 μ/ml, the DE value of the saccharification solution increased with the increased amount of glucomylase, but when the amount of glucomylase was 110 μ/ml, the DE value reached its highest. When the amount of glucomylase was within 110 to 150 μ/ml, the DE value did not change much, but when the amount of glucomylase was 130 μ/ml, the DE value of the saccharification solution droped a little and this might be caused by some measurement errors.

Figure 4 shows that when the saccharification time was within 40 to 80 min, the DE value of the saccharification solution increased with the increased saccharification time, but when the duration lasted 80 min, the DE value of the saccharification solution reached its highest. When the duration was within 80 to 120 min, the DE value did not change much. Therefore, the optimal amount of glucomylase was 110 μ/ml and the saccharification time was 80 min.

Determination of the optimal conditions for the alcohol fermentation of *D. zingiberensis* residue

Nine shares of 200 g *D. zingiberensis* residue with a granularity of 40 to 60 mesh were taken into a 1000 ml beaker, after which 600 ml water was added to it. It was

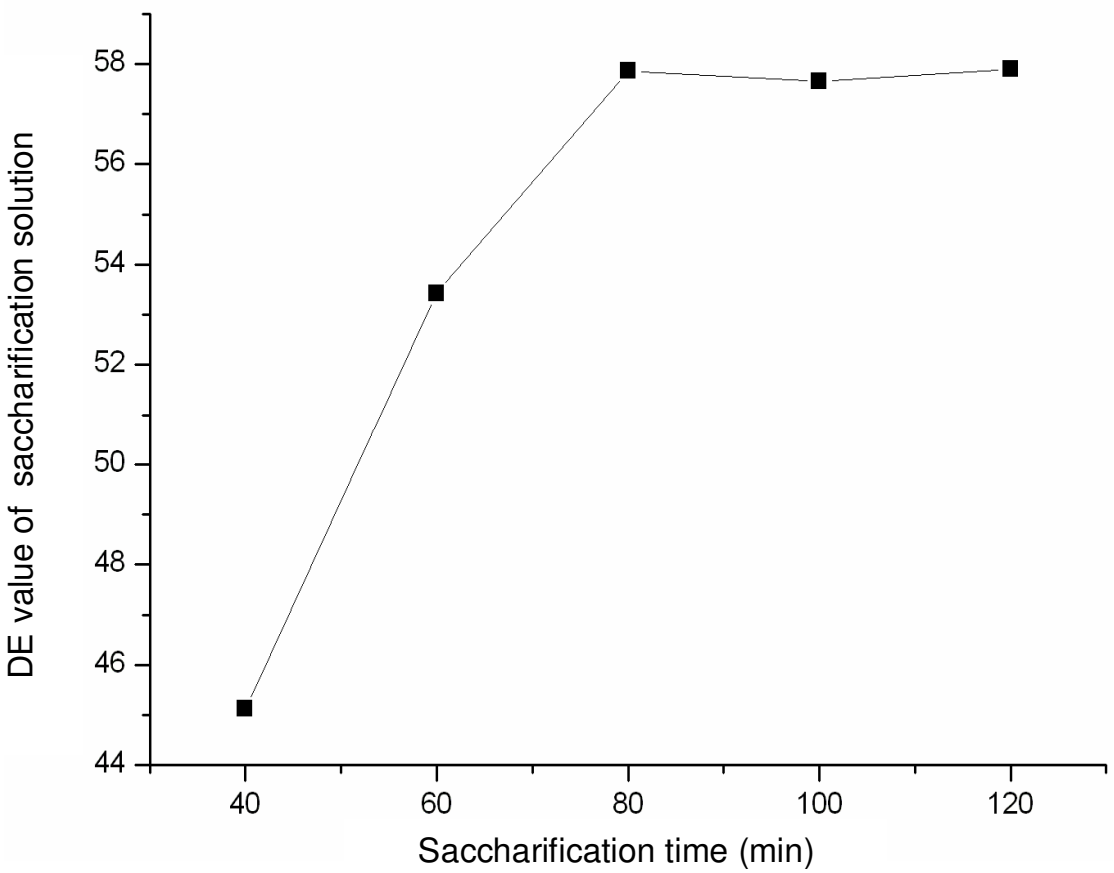

Figure 4. DE value of saccharification solution and saccharification time. DE, Dextrose equivalent.

stirred while heating, and when the temperature got to 90°C, 10 µ/ml of thermostable α-amylase was added to it and liquefied for 30 min. It was then allowed to cool down to room temperature, before the pH was adjusted to 6.2 to 6.5, and the solution was heated up to 60°C. 110 µ/ml of glucoamylase was further added to the solution and it was saccharified for 80 min at 60°C. The pH was adjusted according to Table 1, and then the solution was transferred to sterilized triangular flasks separately. The flasks were plugged in the tampon, sterilized and allowed to cool down to room temperature, before adding activated yeast as shown in Table 2. Subsequently, it was fermented under different temperatures for different time duration. Fermentation liquor was taken and the alcoholic concentration was measured (Rousseau et al., 1992). The results are shown in Table 3.

Table 3 shows that the factors that affected the alcoholic concentration of the fermented liquors of *D. zingiberensis* residue were in the order of fermentation temperature, fermentation time, the original pH of the fermentation broth and the yeast dosage. Moreover, the optimal combination of these factors was when the primal pH of the fermentation broth was 4.5, the yeast dosage was 5%, the fermentation temperature was 34°C and fermentation time was 60 h. From the above study, conclusions are shown in Table 4. However, the experiment was carried out according to the method shown in Table 4, and it was observed that the final alcoholic concentration of the fermented liquors reached 10.5 v/v.

Fermentation behavioral comparison between *D. zingiberensis* residue and *D. zingiberensis*

Three shares of 200 g *D. zingiberensis* residue and *D. Zingiberensi* with a granularity of 40 to 60 mesh were taken into a 1000 ml beaker respectively, after which 600 ml water was added to it. It was stirred while heating, and when the temperature got to 90°C, 10 µ/ml of thermostable α-amylase was added to it, and at this temperature, it was liquefied for 30 min. It was then allowed to cool down to room temperature, before the pH was adjusted to 6.2 to 6.5, and the solution was heated up to 60°C. 110 µ/ml of glucoamylase was further added to it and was saccharified for 80 min at 60°C. It was allowed to cool down to room temperature before the pH was adjusted to 4.5; and then it was transferred to sterilized triangular flasks separately. The flasks were plugged in the tampon, sterilized and allowed to cool down to room temperature, after which 5% activated yeast was then added to them.

Table 1. The ratio of solid-liquid and the amount of reducing sugar in liquefied sample.

Liquefying time (min)	The amount of reducing sugar in liquefied sample (mg/ml)			
	1 : 3	1: 4	1: 5	1: 6
10	40.90	33.78	33.23	31.15
20	46.87	37.78	36.89	35.49
30	48.66	43.13	39.96	39.52
40	48.64	42.62	39.47	39.31
50	48.44	43.10	39.86	39.49

Table 2. The factors and levels of the orthogonal experiment.

No.	Fermentation time (h)	The original pH of the fermentation broth	Fermentation temperature (℃)	Yeast dosage (%)
1	60	4.5	32	5
2	72	4.2	34	6
3	84	4.0	36	7

Table 3. The orthogonal experiment results.

No	Fermentation time (h)	The original pH of the fermentation broth	Fermentation temperature (℃)	Yeast dosage (%)	Alcoholic concentration (v/v)
1	60	4.5	32	5	10.5
2	60	4.2	34	6	9.0
3	60	4.0	36	7	6.5
4	72	4.5	34	7	9.5
5	72	4.2	36	5	5.0
6	72	4.0	32	6	8.5
7	84	4.5	36	6	5.0
8	84	4.2	32	7	5.0
9	84	4.0	34	5	7.5
K_1	26.0	25.0	24.0	23.0	
K_2	23.0	19.0	26.0	22.5	
K_3	17.5	22.5	16.5	21.0	
R	8.5	6.0	9.5	2.0	

Table 4. The optimal fermentation conditions of *D. zingiberensis* residue.

Parameter	Optimal fermentation conditions
Technical process	Process parameter
Gelatinization	90℃
Liquefaction	The amount of liquefying enzyme is 10 μ/ml and liquefying time is 30 min.
Saccharification	The amount of saccharifying enzyme is 110 μ/ml and saccharifying time is 80 min.
Alcohol fermentation	The primal pH of the fermentation broth is 4.5,the yeast dosage is 5%,the fermentation temperature is 34℃ and fermentation time is 60 h.

Table 5. Fermentation behavioral comparison between *D. zingiberensis* residue and *D. zingiberensis*

Parameter	Material	
	***D. zingiberensis* residue**	***D. zingiberensis***
Alcoholic concentration (v/v)	10.5	6.0
Actual liquor yield of the starch (%)	55.07	31.47
Utilization factor of the starch (%)	90.90	51.95

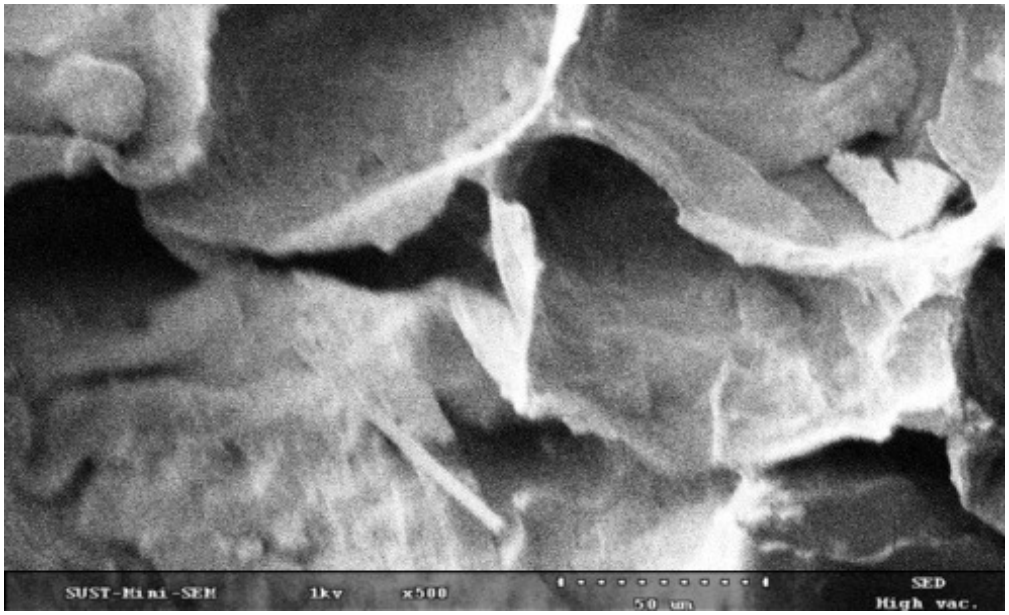

Figure 5. SEM of *D. zingiberensis* after fermentation ×500. SEM, Scanning electron microscope.

They were put in a 34°C incubator and fermented for 60 h. The fermentation liquor's alcoholic concentration was measured and the different liquor yield of starch and the utilization factor of starch in *D. zingiberensis* residue and *D. zingiberensis* were calculated. The results are shown in Table 5.

Table 5 shows that when the same technology was applied, *D. zingiberensis* residue was used for alcohol fermentation, and it was observed that the alcoholic concentration, the liquor yield of the starch and the utilization factor of starch are all higher than those of *D. zingiberensis* used as raw materials. The reasons for this were possibly due to the fact that there were dioscin in *D. zingiberensis* and the dioscin had an inhibiting effect on yeast activity; therefore, it decreased the alcoholic concentration in the fermented liquors (Olayemi et al., 2007; Wang et al., 2001). So, when *D. zingiberensis* residue was used as the raw material for alcohol fermentation, the alcoholic concentration reached 10.5 v/v, the liquor yield of the starch reached 55.07%, and the utilization factor of the starch reached 90.90%. However, this proves that *D. zingiberensis* residue is an ideal material for alcohol fermentation.

The vinasse, after the alcoholic fermentation of both *D. zingiberensis* and *D. zingiberensis* residue, was taken with a granularity of 200 mesh for each, and dried in a vacuum drying oven at 50°C for two days, after which their morphology was compared after a metal spraying. However, Figure 5 shows that the structure of vinasse of *D. zingiberensis* is comparatively intact and most of the fibers are not damaged, while Figure 6 shows that the structure of vinasse of *D. zingiberensis* residue is loose, and most of the fibers are damaged. So, the *D. zingiberensis* residue is more suitable to be alcohol fermented.

Conclusions

Diosgenine, a raw material widely used in medical field, is produced mainly through the process of direct acid hydrolysis of dioscin in *D. zingiberensis* at present. However, the defect of this process is that the starch in *D. zingiberensis* is hydrolyzed into saccharides during the

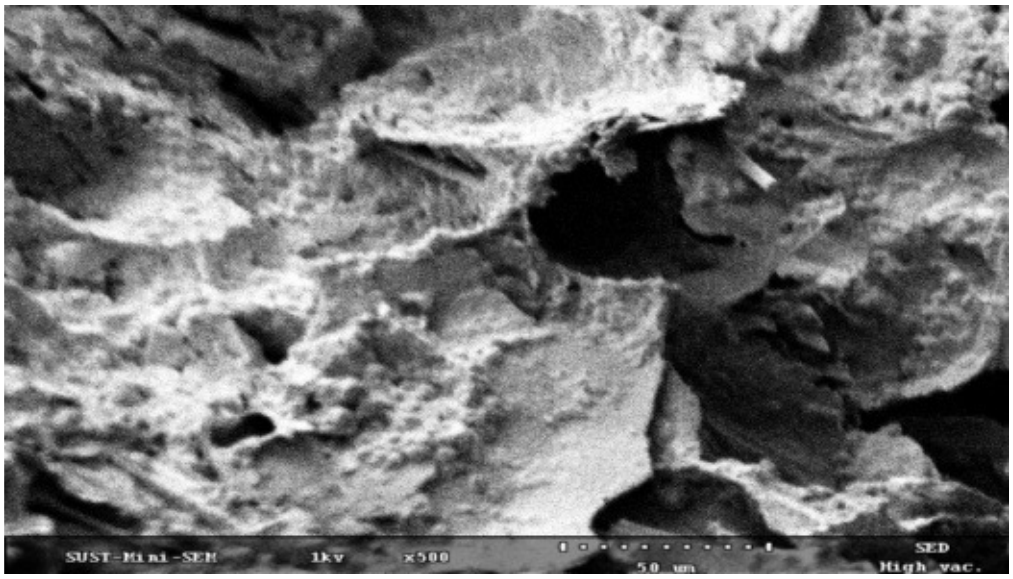

Figure 6. SEM of *D. zingiberensis* residue after fermentation ×500. SEM, Scanning electron microscope.

process of acid hydrolysis, though is not only hard to reclaim saccharides in hydrolysis liquid, but also makes the treatment of wastewater difficult. The indirect technology, which involves the application of ultrasound in the first step of getting dioscin from *D. zingiberensis* and then a hydrolysis of dioscin in getting diosgenine, is considered the only approach that will realize the clean production of diosgenine, but it is expensive. This study, through the alcohol fermentation of *D. zingiberensis* residue, provides a technology for the reduction of cost.

China is a big producer of diosgenine. Ankang of Shaanxi Province and Shiyan of Hubei Province with their unique geographical features have become a natural base for the cultivation of *D. zingiberensis* and production of diosgenine. Presently, the cultivated area is 26667 to 33333 hm^2, and the annual yield is 200 thousand tons. Supposing the content of starch is 48.11% and the liquor yield of the starch is 55.07%, we can produce 53 thousand tons of alcohol, which is equivalent to the output of alcohol produced through the fermentation of 170 thousand tons of corn. However, since *D. zingiberensis* is cultivated in a concentrated area with high annual yield, the high liquor yield of the *D. zingiberensis* residue makes the indirect production technology a possibility.

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