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Large Scale Alcohol Fermentation with Cassava Slices at Low Temperature

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Cassava 전분의 저온 증자에 의한 공업적 규모의 알코올 발효

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The conventional alcohol fermentation method requires a large amount of energy for cooking the starchy raw materials prior to saccharification. The aim of this study was to compare the possibility of large scale alcohol fermentation from cassava slices were compared in low and high temperature cooking systems. The same amount of saccharifying and liquefying enzymes were used for cooking at low and high temperature. At low temperature cooking, conversion of glucose consumed in fermented mash to alcohol was 0.468 g alcohol per g glucose of which was higher yield than that obtained at high temperature.

Alcohol produced from sugars and other types of biomass such as naked barley, corn, sweet potato, cassava, etc. is attracting attention as a promising energy source. The production of industrial or nonbeverage alcohol by fermentation is out of a long established industry which has undergone a few recent technological changes.

An update technology of converting sugars to industrial alcohol has been necessary to enable the industry to reduce the overall production cost substantially. Major economic factors in the conventional alcohol production are the cost of steam for cooking and distillation.

Distillation and cooking take 35-40% (1) and 30-40% (2) of the total cost of conversion of sugar into alcohol, respectively. If raw starchy materials could be cooked at low temperature, a large portion of savings in steam as well as other energy can be achieved. Most practical studies on alcohol, reduction of fermentor size, (3,4) and lowering steam consumption for distillation of the alcohol produced. (5)

Application of low temperature cooking could be adapted to alcohol fermentation for savings the energy consumption (6,7,8,9). Using cassava starch as a raw material, however, alcohol fermentation was tried in the present investigation with a special attention on cooking conditions at low temperature with respect to energy saving. Cooking experiments at high temperature are also described for the comparision purposes.

Materials and Methods

Materials

Cassava imported from Vietnam was ground to 177 μm size in a hammer mill. The components of cassava used for alcohol fermentation were shown in Table 1. The microbial strain used in this study was *Saccharomyces cerevisiae* IFO-1-84 (stocked at Laboratory of Il San Trading Co., Ltd.). The yeast was precultured aerobically at 32 °C in shake flask containing koji juice medium on a reciprocal shaker at 120 rpm. Liquefying enzyme, Termamyl 120L (EC. 3.2.1.1) and saccharifying enzyme (refined and crude forms) were purchased

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Table 1. Composition of cassava slices used for alcohol fermentation.

Components	Content, %(w/w)
Starch	76.06
Moisture	12.50
Total Nitrogen	0.70
Crude Fiber	5.0 Max
Sand/silica	3.0 Max
*F/NF Ratio	18.15

* F/NF ratio was expressed as fermentable sugars per non-fermentables sugars.

from Pacific Chemical Ind. Co. and Baehan Ind. Co.

Enzyme assays

Alpha-amylase activity: Alpha-amylase activity was assayed with a minor modification of the NOVO amylase assay method (10). Test tubes (25 × 20 cm) containing a mixture of 20 ml of 2.0% starch solution and 2.0 ml of 0.1M-acetate buffer (pH 5.5) were preheated at 75 °C for 5 min and was added 1 ml of diluted Termamyl 120L. 1.0ml of the reaction mixture was a withdrawn at regular intervals and put into the test tubes containing 2 ml of iodine solution and then, the reaction time was measured at 660 nm in a spectrophotometer until the transparent ratio is reached 66%. With this reaction time, alpha-amylase activity was calculated as follows:

Alpha-amylase activity = $300/T \times n$ (unit/g)

T : reaction time of end point, min

n : dilution ratio

300 : factor of substrate

Beta-amylase activity: The saccharification power (SP)was analyzed with a minor modification of Technical Research Institute, Office of National Tax Administration's regulation method (11). A mixture of 5 ml of soluble starch solution (Hayashi Co., Ltd. Japan) and 30 ml of 0.1N-acetate buffer pH 5.0) were mixed well and preheated at 55 °C for 10 min and then added to 10 ml of enzyme solution. The reaction mixture was saccharified for 60 min and was withdrawn for determination of glucose by the Bertrand method (12). Acidic saccharification power (A-SP) was measured also by Technical Research Institute Office of National Tax Administration's regulation method (11).

Beta-amylase activity was calculated as follows;

amylase activity (A-SP or SP) = saccharification ratio $\times 1/10 \times D$

D: Dilution rate

Assay of cell mass

For biomass assay, cells were grown aerobically in shake flasks containing mash without suspended solid such as fiber, sand in a reciprocal shaker at 32 °C for 12 hours. The cells were centrifuged at 4000 rpm and washed twice with distilled water and then dried at 105 °C to a constant weight.

Total cells were measured in a Hemacytometer (13).

A relationship between dried weight and total cells were estabilished with a factor of 0.26 mg per 10^7 cells. This factor was used for calculation of cell yield in fermented mash with suspended solid including insoluble substances.

Analytical methods

Alcohol contents, total reducing sugar and fusel oil were determined by the methods described in our previous paper. (7)

Starter

All of the experiments were described in our previous paper (7). We used *Saccharomyces cerevisiae* IFO-1-84. This yeast precultured for 24 hours at 32 °C under aerobic condition was transferred. Table 2 shows the analytical data for the starter. Total acidity of starter before inoculation was 1.80 and after cultivation for 24 hours was

Table 2. Analytical data for the starter

	Before incoculation	After inoculation
pH	4.13	4.05
*Total acidity	1.80	3.50
Total sugar g/l	124.6	70.2
Yeast viable count cells/ml	—	1.30×10^8

* Total acidity was expressed as ml of 0.1 N-NaOH soln. required for neutralizing 10 ml of the filtered.

Table 3. Operating conditions of LTC and HTC.

Type	liquefying enzyme (g/kg starch)	Saccharifying enzyme RE[1], RE[2] g/kg starch		Cooking temp (°C)	Cooking time (h)
LTC	0.157	0.257	5.835	90	120
HTC	0.157	0.257	5.835	124	60

1) Refined enzyme 2) Crude enzyme

3.50. Total sugar before inoculation was 124.6 g/l and after cultivation for 24 hours was 70.2 g/l. Yeast viable count was 1.3×10^8 cells/ml after 24h of growth in the late log phase. This starter was used around 9.0% of total fermenting mash in the experiments.

Alcohol fermentation

Table 3 shows the conditions of fermenting mash for low and high temperature cooking. 338 kg of cassava slices as raw material was put into fermentor maintained at 60°C with live steam and was continuous liquefied by Termamyl 120L for 60 min. The operating conditions were maintained at 90°C for 120 min for low temperature cooking and at 120°C for 60 min for high temperature cooking. After rapidly cooling to 58-60°C, refined and crude enzymes were put into the fermentor for saccharification. The yeast starter was then inoculated with an inoculum size of 9.0% of total mash volume for alcohol fermentation at 32°C for 96 hours.

Results and Discussion

The batch experiments for alcohol fermentation of cassava slices were carried out using a 2172 liter fermentor with working volume of 1500 liters. The specificity of this fermentor was described in our previous paper (7). Table 4 shows that liquefaction activity of Termamyl 120L for alcohol fermentation used was 92300 units/g. We used Termamyl 120L at the concentration of 0.156 g/kg starch for liquefaction.

Crude enzyme and refined enzyme activities were 3200 units/g and 25350 units/g for saccharification and also 2630 units/g, 10140 units/g for acidic saccharification.

When the liquefied mash is used for a simultaneous saccharification and fermentation (SSF) with crude and refined enzyme, it is able to complement each other for the acidic saccharification with respect to decreasing under the optimal pH during alcohol fermentation (14).

Alcohol fermentation

Alcohol and reducing sugar contents were compared between low and high temperature cooking processes during fermentation for 96 hours (Fig. 1). There was no significant differences in the reducing sugar content during fermentation for 96 hours between low and high temperature cooking processes. Reducing sugar was rapidly decreased in 48 hours of fermentation.

Initial pH of low and high temperature cooking mash were 6.10, 5.60, respectively, but reached similar pH around 5 after 96 hours. The pH range of 5.0 was observed for maximum ethanol yield, however, when the initial pH value was between 6.10 and 5.60, alcohol and cell yield were not influenced during the fermentation (14) (Fig. 1).

Table 4. The analytical results of liquefying and saccharifying activity of enzymes used for ethanol fermentation.

Enzymes	Activity (unit/g)	Remarks
Termamyl 120L	92,300	for liquefaction
Crude enzyme Gu 201 SP	3,200	for saccharification
* A-SP	2,630	
Refined enzyme SP	25,350	for saccharification
* A-SP	10,140	

* A-SP was expressed for acidic saccharification activity.

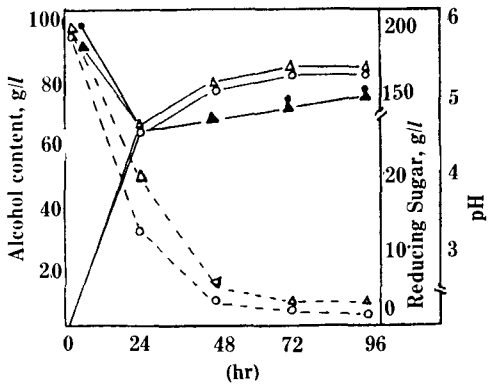

Fig. 1. Changes of alcohol contents, reducing sugar and pH during the fermentation for 96 hours between LTC and HTC

○ - ○ , alcohol content and reducing sugar of LTC
△ - △ , alcohol content and reducing sugar of HTC
● - ● pH of LTC
▲ - ▲ pH of HTC

Fusel oil was compared with between low and high temperature of fermented mash after 60 hours progressed. The fusel oil formed was consisted of high concentrations of iso-amyl alcohol, iso-butanol and n-propanol in order (Table 5). During fermentation, some sugar was converted to products other than ethanol such as glycerol, succinic acid, and fusel oil (7,16). The quantities of fusel oil produced was depended upon a variety of factors such as yeast starter, raw material and temperture. But generally the yield is around 0.2-0.4% based on alcohol contents (19).

Our result, however, fusel oil produced by low temperature cooking were higher as 0.64% than that 0.48% at high temperature cooking, because consumption of sugars and amino sugars by Mailard or Strecker-Abbau reaction was lower than that of high temperature cooking. According to Matsumoto *et al,* (7, 17, 18) changes of alcohol and reducing sugar contents were no significant differences between low and high temperature process during 96 hours fermentation. Therefore, low temperature cooking process has been found same alcohol yield as well as high temperature, so that it could be saved the energy consumption. Alcohol productivity was calculated as 0.887-0.908 gr/*l*.h after 96 hours of fermentation (Table 6).

Alcohol productivity in batch and continuous culture were limited by some factors; alcohol inhibition and a low cell mass concentration etc (5). Some kinetic data were summarized in Table 6. In these experiments, conversion yield of glucose consumed to alcohol was achieved 0.468 g alcohol/g glucose at low temperature cooking, of which was higher than high temperature cooking (0.463g alcohol/g glucose). Therefore, low temperature of energy consumption during cooking process. The cell mass was decreased while the reducing sugar was increased as shown in Table 6. The cell yield was increased because Maillard reaction products were reduced at low temperature process, of which was in agreement with the results by Ghose and Tyagi and Pritzbuer (20).

Table 5. Comparison for impurities of fermented mash after 60 hours progressed

Class	Aldehyde	Methanol	n-Propanol	iso-Propanol	iso-Butanol	iso-Amyl alcohol
LTC	6.6	34.2	51.7	trace	91.6	373
HTC	11.0	50.2	26.6	trace	87.0	291

Diacetyl	Alcohol contents (g/*l*)	Fusel oil contents (%)
trace	80.0	0.64
trace	83.1	0.48

* Fusel oil contents were caculated based on alcohol contents.

Table 6. Kinetic data during alcohol fermentation.

Type	Total sugar (g/l)	Residual total sugar (g/l)	Alcohol concentration (g/l)	Alcohol yield (g/g)	Cell yield	Alcohol productivity (g/l h)
LTC	194.69	14.0	84.64	0.468	0.0186	0.887
HTC	201.03	12.8	87.20	0.463	0.0176	0.908

요 약

알코올 발효의 증자공정중에서 많은 에너지가 소요되므로 이를 줄이기 위한 방안으로서 저온증자법으로 Vietnam 産 cassava 를 원료로 저온증자의 가능성을 고온증자와 비교 검토하였다. 알코올 발효에 당화 및 액화효소는 저온 및 고온증자에서 동일한 량을 사용하였으며 저온증자의 발효 mash 중 소비된 glucose 의 알코올 전환수율은 0.468g alcohol/gr. glucose 로서 고온증자보다 좋은 결과를 보였다. 그러나 발효 불순물인 fusel oil 은 고온증자의 0.48% 보다 저온증자에서 0.64%로 다소 많았으나 증류과정에서 에너지소비 증가는 없었다.

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