Journal of Industrial Technology 7 (1), 1998, 87-93

SHORT COMMUNICATION

ETHANOL PRODUCTION FROM JACKFRUIT SEED

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RINGKASAN: *Hidrolisis kanji dari Artocarpus heterophyllus (nangka) dengan 0.50 M HCl selama 4 jam pada suhu 75°C menghasilkan 54.7% (b/i) glukosa manakala di bawah keadaan yang sama didapati 0.25 dan 0.75 M HCl masing-masing menghasilkan 25.7 dan 34.4 % (b/i) glukosa. Peningkatan kepekatan asid atau penurunan suhu semasa proses hidrolisis tidak menunjukkan tambahan hasil glukosa. Dengan penggunaan Saccharomyces cerevisiae yang di tumbuhkan dalam kultur kelompok maka hasil maksimum etanol yang diperolehi dari 54.7% (b/i) glukosa itu ialah 10.8% (i/i) selepas 12 jam penapaian.*

ABSTRACT: Hydrolysis of starch from *Artocarpus heterophyllus* (jackfruit) using 0.50 M HCl for 4h at 75°C yielded 54.7% (w/v) of glucose whereas under similar conditions of 0.25 M and 0.75 M HCl, it produced 25.7 and 34.4% (w/v) glucose, respectively. Increasing the acid concentrations or decreasing the temperature during the hydrolysis process did not show any increment in the glucose production. With 54.7% (w/v) of glucose obtained, the maximum yield of ethanol was 10.8 % (v/v) after 12 h of fermentation using *Saccharomyces cerevisiae* grown in batch cultures.

KEYWORDS: *Artocarpus heterophyllus,* hydrolysis, fermentation,

INTRODUCTION

Considerable interest in ethanol production from some agricultural wastes such as apples, oranges, peaches, watermelon, tubers have been extensively investigated using selected microbes (Roukas, 1993). However, among the most widely used susbtrates for ethanol production are molasses of sugarcane and sugar beet (Rose, 1976). This is because these substrates are ready for bioconversion to ethanol with limited pretreatments as compared to the starchy or cellulosic materials. Other researchers resolved the use of some edible portion or the skin peeling of the fruits from which juice are extracted. Very little research work on using seeds obtained from fruits as raw materials for ethanol production has been investigated.

Jackfruit, *Artocarpus heterophyllus* probably originated from India (Burhill, 1966). This fruit is grown extensively in the tropical regions of the world. At the ripe age, the fruit weight is reported to range from 3-7 kg whereby the pulp constitutes about 29%, seed 12% and rind 54% (Berry and Kalra, 1988). The large starchy seeds, which are encased in the succulent pulp of the edible portion of the fruit, are often discarded. The seeds were reported to contain 25 - 52% starch (Samaddar, 1985), 9.4% protein (Bibbio *et. al.* 1978) and 24.4% lipid (Berry and Kalra, 1988).

Hydrolytic products made from various sources are virtually identical in terms of chemical, physical and organoleptic properties. Hence, starch hydrolysis products are manufactured from a wide variety of plant materials. The acid conversion is a random action which results in remarkably reproducible saccharide compositions for any given degree of hydrolysis. In this study we report the hydrolysis procedures including the moisture content and the ethanol production from *A. heterophyllus* seeds.

MATERIALS AND METHODS

Jackfruits seeds were chosen for this study because it is a popular fruit predominantly grown in rural areas but can also be seen in urban areas of many countries in the tropics.

Moisture content

Whole seeds were dried in air at room temperature (26°C) for 24 h before being cut into small pieces while whole another set of seeds remained uncut to act as a control. Both cut and uncut samples were then allowed to dry in an oven for 16 h at 105°C until constant weight.

Acid hydrolysis

Some fresh seeds after being cut in smaller pieces, were crushed mechanically to particle size of less than 2 mm. 4 samples (ground seeds) were soaked in a series of quickfit apparatus each containing a set of defined concentrations of HCl at 0.25 M, 0.50 M and 0.75 M, respectively. Each sample was throughly mixed so as to maintain a uniform slurry which was then heated to 75°C and 90°C for two and four hours respectively before being neutralised with 0.5% NaOH and then filtered to separate the salts.

Filtration using Sep-Pak NH Catridge

Hydrolysed sugar samples were extracted using Sep-Pak containing compact NH_2 (Waters, Milford, USA) by injecting 10 ml samples followed by the same volume of hexane and acetonitrile, respectively. Collected samples were then estimated for glucose by HPLC (Jusco DG-98-50, Japan fitted with a detector 830-RI) using Polar C-18 column (3.9 x 300 mm), oven temperature at 25°C and flow rate at 10 ml min^{-1}. Glucose was determined in 5 µl samples peak areas by reference to standard curve prepared with known amounts of pure glucose (analytical reagent grade, Merck, Darmstadt, Germany).

Fermentation procedures

750 ml jackfruit seeds hydrolysate was sterilised in 1 litre LH fermentor (L.H. Engineering Co., Stoke Poges, Bucks, U.K.) by autoclaving at 121°C for 15 min. *Saccharomyces cerevisiae* culture was prepared in nutrient broth containing 6% glucose. Approximately 20 ml culture media was then inoculated aseptically in fermentor at 37°C with continuous stirring at 500 rev. min^{-1}. Air was introduced in the culture using a calibrated peristaltic pump (Watson-Marlow MHRI, Cornwall, U.K.). Samples were taken at regular intervals for microscopic examination and for purity plating, as well as pH measurement. Absorbance was read against uninoculated media in 3 ml cuvettes at 560 nm using spectrophotometer (spectronic 20, Milton Roy Co., USA).

Ethanol determination

The culture samples were centrifuged (Damon IEC centrifuge, Model B-20, USA) at 10,000 x g for 10 min at 4°C. The supernatant obtained was filtered through a millipore filter (0.45 um pore size membrane filter) and 5 µl sampes were injected in replicates into an HPLC as described above using Waters FFJA column (1.9 x 300 mm) with a mobile phase of acetonitrile-water (75 : 25 v/v), flow rate adjusted to 1 ml min^{-1} and

oven temperature 25°C. Ethanol was quantified from peak areas by reference to a standard of pure ethanol (Hamburg Chemicals, Germany).

RESULTS AND DISCUSSION

The average moisture content of cut seeds was 58% whereas for uncut seed was 46.1%. The cut seed has a higher percentage of moisture content because it contains a larger surface area exposed to the air. On the other hand, the uncut seed, with a smaller surface area and with its seed coat intact, obviously prevented dehydration. In either case, it may not be possible to remove all moisture content in the presence of the volatile substances which are incorporated within the cotyledon. Determination of the moisture content is essential since it affects the time period during which the seed can maintain its viability (Chin and Roberts, 1980). If the moisture level is too high, the seed tend to germinate and when this happens, the total quality of starch will be reduced and consequently decrease the fermentable sugars.

The hydrolysis of jackfruit seeds using different acid concentrations, temperature and time reveals some variablity on the amount of glucose obtained. The result indicates that hydrolysis with 0.50 M of HCl for 4 h at 75°C showed a maximum yield of glucose. Increasing the acid concentration or increasing the temperature did not enhance the hydrolysis performance (Table 1). It may be possible that, given an extra time for starch hydrolysis, more glucose is obtainable.

Table 1. Percentage of glucose obtained by hydrolysis at various HCl concentrations, temperature and time.

HCl concentration (M)	Temperature (°C)			
	75.0		90.0	
	2 Hour	4 Hour	2 Hour	4 Hour
	(% w/v glucose)		(% w/v glucose)	
0.25	20.5	25.7	4.7	13.2
0.50	29.7	54.7	15.4	21.9
0.75	24.3	34.4	14.3	17.8

Tsao (1984) reported that concentrated mineral acids including HCl and H_2SO_4 are capable of swelling and dissolving cellulose or starch. Higher temperatures were found to be corrosive and might damage the substrate. Being a catalyst, acid would incorporate water molecules. These results were found to be inconsistant with those reported by

Riera *et al.* (1990) which stated that the yield of furfural via hydrolysis was a function of reaction temperature, particle size and acid concentration.

The biochemical process in the production of yeast fermented alcohol is the catabolism of simple sugars by *S. cerevisiae* to yield ethanol and carbon dioxide. At the initial stage of the fermentation process, the medium contained a high level of sugar and of low acidity. The small addition of oxygen, air (approximately 20 ml min^{-1}) through agitation in the fermentor provides an optimum condition for yeast to grow. The ethanol tolerance of *S. cerevisiae* is an important constraint on the efficiency of the production of ethanol by fermentation. Theoretically, the yield of ethanol through fermentation of 1 kg of glucose is 0.51 kg whereas 1% glucose (w/v) would give 0.68% ethanol (v/v).

Therefore, the percentage of ethanol yield:

$$\frac{\text{Maximum ethanol produced x 100}}{\text{Theoretical ethanol yield (i.e. \% of initial sugar x 0.68)}}$$

It can be assumed that 45 kg of fermentable sugar would yield 18 - 23 kg of ethanol (Rees, 1967). In this study, the initial glucose used was 54.7% w/v and the maximum ehtanol obtained was 10.79 % v/v after 14 h fermentation in batch culture (Figure 1). Kobayashi *et. al.* (1995) reported that yeast growth is affected by the temperature and the accumulation of alcohol. This is in agreement with our findings since the optical density on the yeast growth began to decline after 20 h of fermentation time (Figure 2). However, it may also be due to the depletion of the substate, thus unable to sustain the yeast growth (Rogers *et al.* 1980).

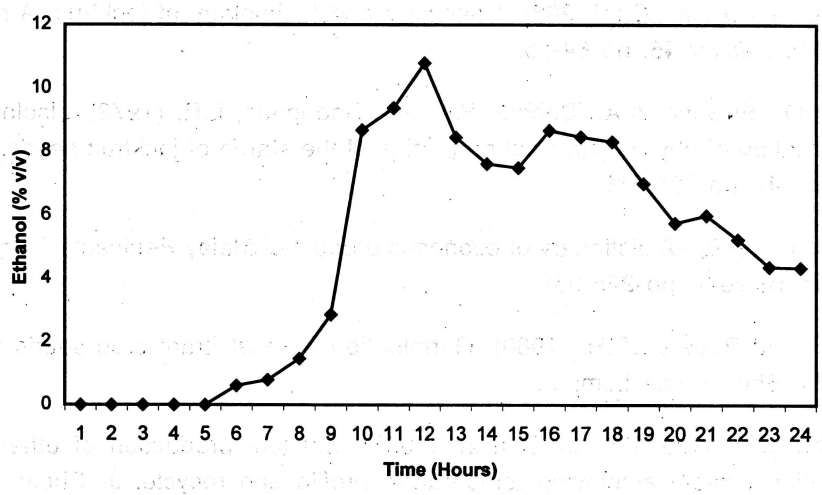

Figure 1: *Production of ethanol in batch culture using Jackfruit seeds hydrolysate*

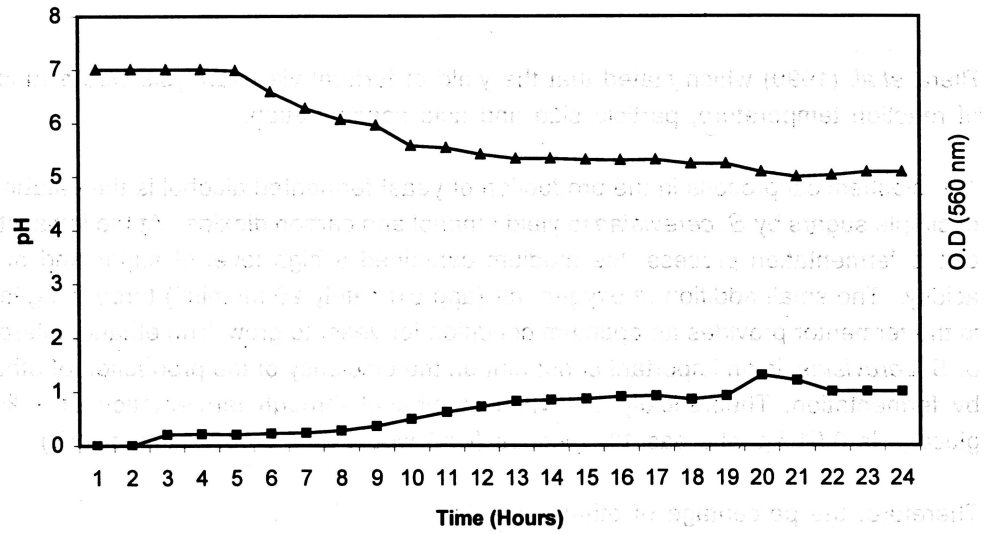

Figure 2: *The optical density of yeast at 560 nm and culture pH during the fermentation of jackfruit seeds hydrolysate. pH (▲), O.C. (■)*

ACKNOWLEDGEMENTS

This investigation was financially supported by IRPA. The authors wish to thank Mr. Onn Ahmad for technical assistance.

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