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RESEARCH ARTICLE

A COMPARATIVE ANALYSIS OF THE ALCOHOL PRODUCTION BY ENZYMATIC TECHNOLOGY FROM CASSAVA

Kalaiarasu, S*. and Uma, C.

Department of Microbiology, Annamalai University, Annamalai Nagar
Tamilnadu - 608 002. India

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ABSTRACT

The continuous depletion of the fossil fuel reserves and consequent escalation in their price has stimulated the researchers to find alternate sources to meet the global energy demand. Alcohol was prepared from different substrates of cassava by comparing two step enzyme-enzyme process and single step enzyme process followed by yeast fermentation. The study reveals 96 per cent fermentation efficiency, when the slurry was hydrolyses by two step enzyme-enzyme method and fermented by a strain of *Saccharomyces cerevisiae.*

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INTRODUCTION

The growing demand of fossil fuels has prompted search for alternate sources of energy Alcohol has been identified as a renewable source of energy. Ethanol can serve as a chemical feed stock as well as liquid fuel is obtained from renewable sources such as corn, whey sugarcane molasses, cassava, sweet potato etc. Cassava serves as a nucleus for many industries with the application of biotechnology, especially fermentation industries (Balagopalan *et al.,* 1988). (1) One of the important uses of this abundantly available raw material is conversion to ethyl alcohol by fermentation. (2) Yang *et al.* (1977) reported that bioconversion of starch tuber crop for alcohol production consists of major steps such as preparation or pretreatments, enzymatic hydrolysis (liquefaction and saccharification), fermentation and distillation.(3) *Suseela et al*. (1980) compared the efficacy of acid, enzyme and acid-enzyme and enzyme-enzyme hydrolysis on the extent of saccharification of cassava starch factory waste. The present study focused on alcohol production from different substrates of cassava employing enzyme - enzyme conversion (two step enzyme process and single step enzyme process) followed by yeast fermentation.

Corresponding Author Address
karasu2010@rediffmail.com

MATERIALS AND METHODS

Cassava tuber varieties H-1687 (Sree visakham) H-165 were collected from central tuber crop Research Institute field. Various substrates of cassava like fresh tubers, peel, whole tuber, rotten tuber, flour, starch, thippi were used for this study.Samples were prepared from each substrate and they were subjected to absolute starch and total sugar estimation by titration method described by (4) Moorthy and Padmaja (2002). Estimation of reducing sugars was done according to modified (5) Calorimetric method of Nelson Somogyi. (Nelson, 1944).

Alcohol Production

Alcohol production requires 3 main stages. Liquefaction, Saccharification and fermentation. Enzymatic hydrolysis of substrate was done by 2 methods. 1 Two step enzyme hydrolysis 2 Single step enzyme hydrolysis. *Two step enzyme hydrolysis:*1kg of the substrate was slurred with 2l distilled water and the pH was adjusted to 6.5. It was autoclaved for 1 hour at 15 lb pressure. The flasks were cooled, to this 0.8 ml Liquezyme x was added and incubated at 90°C for 1 hr in water bath. The lignified slurry was cooled and the pH was adjusted to 4.0. To this 2 ml of Dextrozyme GA was added. The saccharification was carried out at 60°C for 48 hours. *Single step enzyme hydrolysis:*Single step enzyme hydrolysis was done by

simultaneously adding amylase and glucomylase to the substrate. One kg of the substrate was slurried with 2l distilled water and the pH was adjusted to 5.0. It was autoclaved for 1 hr at 15 lb pressure. The flasks were cooled, to this 0.8 ml Liquezyme x and 2 ml of Dextrozyme GA was added. The mixed enzyme reaction was carried out at 70°C for 48 hours.

Fermentation

The hydrolysate obtained after saccharification was filtered using muslin cloth to remove unhydrolysed materials. The clear supernatant was enriched with 10 gm urea. The yeast starter culture *Saccharomyces cerevisiae* was added to the broth and the flasks were kept for fermentation. The fermentation was allowed to continue for 3 days at ambient temperature 30°C.The fermented hydrolysate was distilled to recover the total ethanol yield by using laboratory model distillation unit, after filtering the hydrolysate through muslin cloth. The alcohol formed was distilled and redistilled 3 or more times with $CaCl_2$ to get 96 per cent alcohol. The yield of 96 per cent alcohol was quantified and purity was ascertained with alcohol meter

RESULTS AND DISCUSSION

The initial starch content in the various sources of cassava is presented in the Table 1 to 7. The starch and sugar content of tubers were estimated by titrimetric method. The reducing sugar was estimated by calorimetric method. It was found that the starch content of the tubers was slightly less than the normal range (22-25%). This could be due to pre-harvesting of tubers. The various substrates of cassava were converted to glucose using either a two step enzyme hydrolytic process (or) single step process. In the former case, starch and other sources were first liquefied to form limit dextrins using alphaamylase (liquezyme GA). This is then completely hydrolyzed to free glucose using another enzyme Dextrozyme GA. This attempt was made to find out whether the yield of glucose is affected by combining the two enzyme reactions.

Table 1. Starch content of various sources of cassava H-1687 and pH-165

S. No.	Substrate	Starch (g/100 g) H1687	Starch (g/100 g) H165
1.	Peeled tuber (fresh)	18.90	18.50
2.	Peel (fresh)	11.27	11.07
3.	Whole tuber (fresh)	20.00	19.65
4.	Rotten tuber (fresh)	16.18	15.96
5.	Flour (dry)	75.00	70.31
6.	Starch (dry)	88.00	87.00
7.	Thippi (dry	58.44	52.9

The results of the two steps and single step process are presented in the table 2. It was observed that the glucose formation is drastically affected by combining the two steps. The results were similar for the two cassava verities. The feasibility of combining the two processes

Table 2. Amount of reducing groups formed during liquefaction (Two step enzyme process)

S. No.	Substrate	Amount of reducing groups (g/100 g) H1687	Amount of reducing groups (g/100 g) H165
1.	Peeled tuber (fresh)	4.71	4.58
2.	Peel (fresh)	2.83	2.51
3.	Whole tuber (fresh)	6.58	6.01
4.	Rotten tuber (fresh)	3.50	3.79
5.	Flour (dry)	11.10	11.15
6.	Starch (dry)	13.05	10.75
7.	Thippi (dry)	7.90	7.06

Table. 3. Amount of Glucose formed during saccharification (Twostep enzyme process)

	Cassava H1687		Cassava H165	
Substrate	**Amount of reducing sugar (g/100 g)**	**Percentage conversion to glucose on absolute starch basis)**	**Amount of reducing sugar (g/100 g)**	**Percentage conversion to glucose on absolute starch basis**
Peeled tuber (fresh)	15.19	80.39	15.16	81.50
Peel (fresh)	8.70	77.60	7.99	71.80
Whole tuber (fresh)	17.75	88.71	16.72	85.12
Rotten tuber (fresh)	10.98	67.70	11.11	69.00
Flour (dry)	66.10	78.00	65.30	81.00
Starch (dry)	88.30	94.40	86.95	92.00
Thippi (dry)	37.70	64.40	37.25	70.40

Table.4. Amount of reducing groups formed from cassava in one step enzyme process

Table. 5. Yield of 96 per cent alcohol by two step enzyme process (ml)

Substrate	*Cassava H1687*	*Cassava H165*
Peeled tuber	145.83	135.00
Peel	95.83	84.58
Whole tuber	205.83	175.00
Rotten tuber	90.00	70.00
Flour	430.20	420.56
Starch	651.94	544.43
Thippi	252.10	223.60

was investigated, with the objective of economizing the whole process. However, the present study showed that the hydrolysis of starch to glucose was never completed, unless otherwise saccharification is done after liquefaction.

The saccharified slurry was fermented by yeast for 3 days at room temperature and then distilled and redistilled to obtain 96 percent alcohol. The yield of 96 per cent alcohol was quantified and purity ascertained with the alcohol meter. Highest yield of alcohol obtained from H1687 and H165. In the present study, the yield of alcohol is drastically reduced in one step, liquefaction – saccharification process. The low yield results mainly from the poor percentage conversion to glucose after the saccharification.

	Cassava H1687		Cassava H165	
Substrate	Amount of reducing sugar (g/100 g)	Percentage conversion to glucose on absolute starch basis)	Amount of reducing sugar (g/100 g)	Percentage conversion to glucose on absolute starch basis
Peeled tuber (fresh)	12.47	65.90	11.97	64.30
Peel (fresh)	4.58	40.86	4.35	38.80
Whole tuber (fresh)	14.28	71.30	13.96	71.00
Rotten tuber (fresh)	7.50	46.10	7.77	48.60
Flour (dry)	41.10	35.80	40.65	39.50
Starch (dry)	46.05	52.00	41.85	47.00
Thippi (dry)	426.40	44.90	25.00	46.00

Table. 6. Yield of 96 per cent alcohol by one step enzyme process

Substrate	*Cassava H1687*	*Cassava H165*
Peeled tuber	105.00	83.33
Peel	55.55	59.89
Whole tuber	140.00	145.00
Rotten tuber	81.25	68.74
Flour	395.83	291.67
Starch	444.16	427.50
Thippi	194.79	154.00

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