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Study on Ethanol Production from Cassava Leaves and Pulp using *S.cerevisiae*

S Anbuselvi and T Balamurugan

Department of Industrial Biotechnology, Bharath University, Chennai-73, Tamil Nadu, India.

ABSTRACT

Ethanol is generally produced by the fermentation of sugar, cellulose and lignocelluloses rich fruits and vegetables. It is an alternate to fossil fuel and used as a commercial gasoline additive. Ethanol reduces country's dependence on petroleum. Cassava used as substrate for fermentation medium using yeast. This work shows the study of optimization for ethanol production using cassava pulp and leaves. The enzymatic hydrolysis was carried out by barley and α-amylase. The maximum yield of ethanol(5.89%) was observed in cassava leaves treated with enzymatic hydrolysis of barley. The cassava pulp also showed ethanol production(2.56%)in barley used fermentation medium.

Keywords: Fermentation,Barley,Starch,Reducing sugar,Protein

Corresponding author

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INTRODUCTION

Cassava(Manihot esculenta cranz) belongs to *Euphorbiaceae,* a perennial crop of tropical America. It is a major industrial crop in Tamil Nadu. Cassava starch is used for human consumption, production of monosodium glutamate, aminoacids, sweeteners and ethanol etc[1]. Cassava waste contains 29.3% of starch on wet basis and 77.9% on dry basis [2]. Cassava starch shows some characteristics such as high purity, neutral flavor and solubility. It is highly viscous and has low ability retrograde compounds with other starches such as poyato,rice and corn[3]. Cassava leaves rich in proteins, antioxidants,vitamins A and B which are consumed as vegetable and animal feed [4]. Cassva plant act as antimicrobials, photoreceptors, visual attractors and feeding repellents[5].

Cassava pulp from starch industry and is a good raw material for ethanol production. The cellulose and starch were subjected to enzymatic hydrolysis which convert fermentable sugars to ethanol by s.cerevisiae TISTR 5596. The maximum amount of reducing sugar was obtained from cassava starch by cellulase, α-amylase and glucoamylase [6]. Thus the cassava wastes left over these production processes are abundant and can be used to produce ethanol. This is not only reduces the waste material from starch industry but also lowers the cost of ethanol production. Different treatments and pre treatments to improve the split of cassava starch by acid hydrosis[7]enzymatic hydrolysis[8],wet oxidation[9] and ultrasonic pretreatment[10,11] to use cell surface engineering technology, an arming yeast was constructed especially for ethanol production. This armingyeast co-displays α-amylase,glucoamylase andoglucanase,cellobiohydrase and β-glucosidase on the surface of yeast cells were constructed[11].The ethanol produced from cassava plant is not poisonous and doesnot cause pollution. The main objective of this study was to investigate the conversion of different concentration of cassava pulp and leaves were subjected to fermentation using barley and α amylase to produce ethanol using s.cerevisiae.

MATERIALS AND METHODS

Fresh cassava samples were obtained from the farms near Tuticorin and pulp was collected from starch industry. The basal growth media was purchased from Hi media and yeast culture and α amylase were consumed commercially. Initially cassava mash was prepared and distributed into 3 flasks and cooled to 62.8^{o}C . Different concentration of barley malt (10 %) in the form of slurry was added in equal amount to each treatment. This mixture of malt and mash was placed on shaker and conversion of cassava starch to reducing sugar was carried out for specified times. The mash were cooled to 22^{o}C and made up to 100 ml. The pH was adjusted to 4.8 to 5.0. Mash in each treatment was divided into four portions. To this sterilized basal medium actively growing yeast was inoculated aseptically. It was kept in an incubator for overnight at 37^{o}C. To three portions, *S. cerevisae* (yeast) was added to the fourth one acted as control. Fermentation was permitted to continue for 68 to 70 hours. During fermentation period,the biochemical and physical parameters were analyzed at regular intervals[12-16].

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Production of barley malt

Barley was taken and placed in a petriplate containing filter paper .Then water was sprayed so as to soak the grains .then it was left undisturbed for about 24 hrs for germination. Proper ventilation and aeration was ensured. After a certain level of growth was achieved the germinated barley was dried in hot air oven at the temperature of 70º C. This arrested further growth of the grains. The dried barley was ground to fine. Powder and used as a source of malt. This malt provided the required amylase enzyme required for the breakdown of starch

D)Fermentation of cassava tubers

100 grams of cassava leaves and pulp were weighed and boiled. The boiled pulpand leaves were peered offs mashed into a puree. This puree was taken in a flask and about 150 ml of water was added to it. α-amylase obtained from barley malt was added. It was left undisturbed for a few hours. Then the supernatant was decanted into another flask. Different concentration of α-amylase was also subjected to another set of experiments. About 12 -18 ml of the yeast innoculum was pitched into the fermentation broth. The mouth of the flask was tied with balloon avoiding any leakage and left for incubation for 10 days at 28^{o}C[12].

E)Distillation of ethanol produced

The alcohol produced from cassava potato was taken in a distillation flask. Few glass beads were added to it. Then a thermometer was inserted into the flask through the corked opening. The thermometer bulb was just below the condenser. Water was allowed to circulate through condenser.The flask was heated by a Bunsen flame and thus condensed vapour were collected in the collecting vessel. The heating was so adjusted that 1 drop was collected in 1 second. The collected ethanol was analyzed qualitatively and quantitatively by standard methods[17].

Table:1 Biochemical characteristics of raeecassava plant

S.No	Parameters	Pulp	Leaves
1	Starch(mg)	32.6	28.7
2	Total sugar(mg)	18	29
3	Reducing sugar(mg)	25	29
4	Non Reducing sugar(mg)	6.6	5.4
5	Protein(mg)	6.8	7.0
6	Ascorbic acid (mg)	0.2	1.6

Table2: Physical changes in fermentations of Cassava using yeast

S.NO	Parameters	8-10days		10-16days		16-22days	
		Pulp	Leaves	Pulp	Leaves	Pulp	Leaves
14	pH	4	4.8	8.5	7.6	6.8	6.9
5	Temperature	29^{o}C	28ºC	32^{o}C	36ºC	26^{o}C	25ºC
6	Turbidity(NTU)	59	350	110	480	80	200

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Figure 1: Biochemical changes in fermentation of cassava pulp using yeast

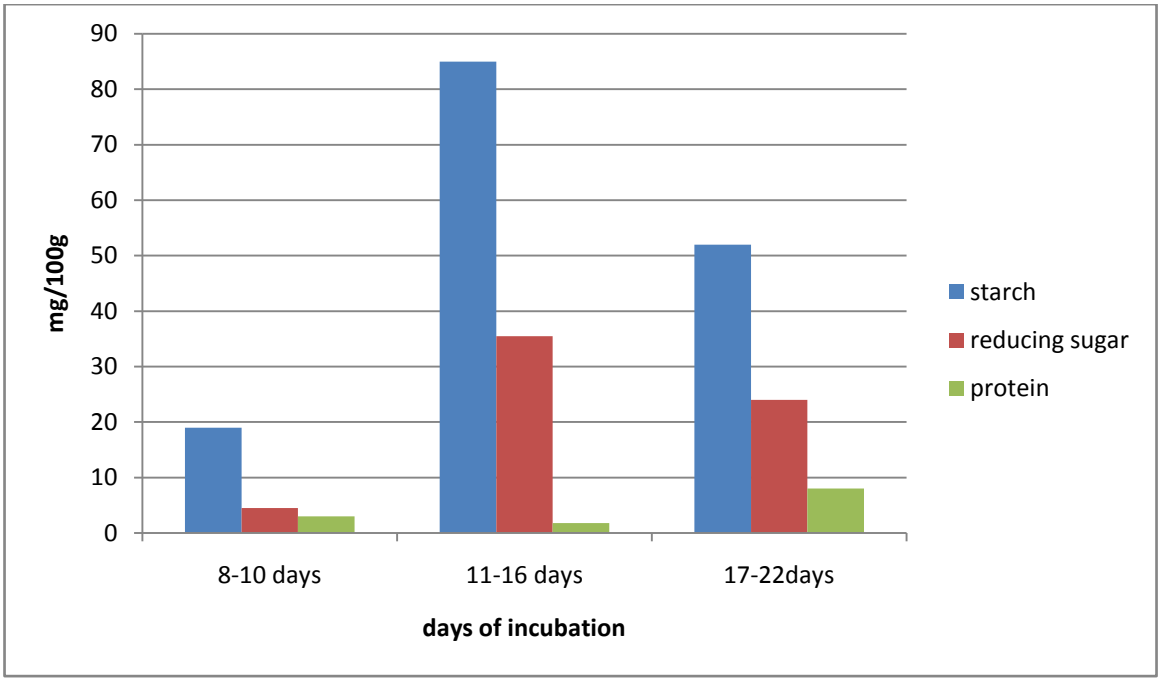
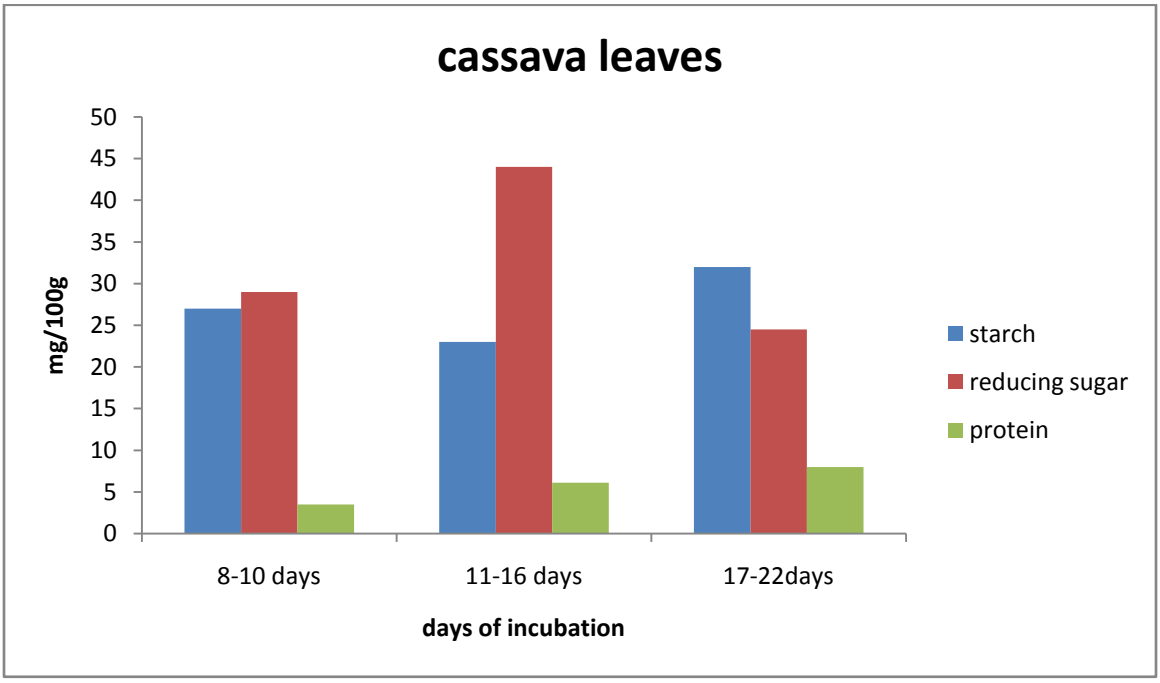


Figure 2: Biochemical changes in fermentation of cassava leaves using yeast



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RESULTS AND DISCUSSION

The pH of pulp waste and leaves were found to be acidic. This was due to presence of ascorbic acid. The maximum temperature was observed in leaves (36^0C) when compared with pulp. The maximum turbidity was found in pulp. The high moisture content 82.7% was observed in waste. The ash content in leaves showed maximum amount 20.5% when compared with tubers(12.8%). The low content of ash was found in cassava sample. Renata et.al, 2012 has been reported that moisture and ash content of cassava were found to be 62.4% in wet basis and 0.7% for wet sample and 1.8% for dry samples(Table1).

The biochemical constituents of cassava pulp and leaves were summarized in table 1. The cassava is a rich source of carbohydrates. The maximum amount of sugar was observed in cassava leaves (29 mg/10g) when compared with tubers. Similar results were observed in nutritive value of cassava leaves 18mg/100g and tubers 3.5mg/100g(Renata et.al,2012). Cassava pulp contains about 50-70% starch on dry basis. The maximum amount of starch was found in pulp 30.6%.Therefore cassava is used as substrate for ethanol production. Renata et.al,2012 reported that starch content of cassava pulp was found to be 29.3%.

The cassava leaves showed maximum amount of reducing sugar (29mg/100g) and non-reducing sugar (6.6mg/100g) and protein(7mg/100g) when compared with tubers. Most cassava leaves can be used as animal feed due to its high content of protein and other nutrients which are necessary for animal growth. Cassava starch always contaminated with fibers more efficiently saccharified after treatment with fungal cellulose. Use of enzymes cellulose or pectinase alone not only used effectively to improve starch extraction reported by sriroth et al, 2000.

Cassava pulp and leaves from cassava plant is a good raw material for ethanol production. The experiment was carried out at with barley used for enzymatic hydrolysis of starch which reacts directly with starch to fermentable sugars andenhance ethanol production using yeast. The biochemical constituents of fermented products were analyzed at regular intervals. The amount of starch was rapidly increased from 19mg to 85mg upto 16 days of fermentation in pulp and decreased from 27 mg to 23 mg in leaves (Figure1,2). It indicates the process of fermentation starts and decreased in leaves due to high amount of anthocyanin(Suresh et.al,2011). Reducing sugar also gradually increased in pulp from 29mg to44mg in leaves and pulp from 4.5mg to 35.5mg upto 16 days of fermentation. The starch and reducing sugar were utilized at the end of fermentation.

The protein content was gradually lowered from 3mg to1.8mg in cassava pulp, after 10 days of fermentation and attained maximum (8mg) at end of fermentation using yeast. Whereas in leaves, the protein content of fermentation medium , was found to be increased from 3.5mg to6.1mg and slowly increased at end of fermentation medium. The change in amino acid and carbohydrates were utilized for production of ethanol(Figure1,2).

The pH of fermentation medium of cassava pulp was rapidly increased at 11th day of

fermentation and attained nearly 6.8 at end of fermentation. But cassava leaves attained neutral pH and slowly reduced to 6.9.The temprature of fermentation medium was gradually increased at 12th day of fermentation and slowly reached room temperature at 22 days of fermentation. At the end of fermentation, pH of medium was found to be nearly around 7 before subjected to ethanol distillation. The turbidity content of fermentation medium was found to be high at 10-16days incubation in which leaf and pulp used as a substrate. The production of ethanol was collected in fermentation medium, turbidity was rapidly reduced at the end stages of ethanol production. The ethanol was qualitatively conformed by appearance of yellow colour. The concentration of ethanol was calculated by titration with potassium dichromate. The maximum yield of ethanol(5.89%) was observed in cassava leaves treated with enzymatic hydrolysis of barley. The cassava pulp also showed ethanol production(2.56%)in barley used fermentation medium.

CONCLUSION

Cassava waste and pulp can be used as raw material for production of monosodium glutamate starch in food Industry. It is also used as substrate for production of single cell protein, ethanol. This work evaluated the physicochemical characteristics of cassava leaves and wastes. Maximum use of pulp for production of monosodium glutamate and in food industry and leaves used for animal feed due to high protein content.Cassava waste used as a substrate of ethanol waste used as a substrate for ethanol production. Thus cassava used as a raw material for so many industries to improve the economy of country through its cultivation at cheaper rate

REFERENCES

[1] Akpan I, Uraih N, Obuekuwe C and Kenebomah MJ. Acta Biotechnological 2004;18 (1):39 – 45.
[2] Renata CM, Miklasevicius VS, Mariana MB, Salau PG and Terra MT. Biomedicine and Biotechnol 2012; 13 (7) : 579 – 586.
[3] LL Zamora, JAG Calderon, ET Vazquoz and EB Reynoso. J Mex Chem Soc 2010, 54 (4) : 198 –203.
[4] Lenis JL, Calle F, Jaramillo G, Perez JC, Ceballos H. Field Crop Res 2006,95:126-134.
[5] Pieatta GP. J Nat Prod 2000,63:1035-1042.
[6] J Sci Res Chula Univ 2006;31 (1):77 – 84.
[7] Agu RC, Amadife AE, Ude CM, Onyia A, Ogu EO, Okafor M, Ezejiofor E. Waste Manage 1997;17(1):91-96
[8] Saoharit N, Kumar RS, Grewell D, Van leeuwan J, Kumar KS. Biotechnol.Bioeng, 2008 Saoharit N,Shresha P,Rasmussen ML,Lamsel BP,Van Leeuwan J,Kumar KS, Bioresour Technol,2010,101,2741-2747.
[9] Martin C,Thomsen AB. J Chem Technol Biotechnol 2007;82:174-181.
[10] Apiwatanapiwat W, Murata Y, Yamada R, Kondo A, Arai T, Rugthawom P, Mori Y. Appl Microbiol Biotecnol 2011:377-384.

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[11] AOAC (Association of Official Analytical Chemists) , 16th Edition Virginia(1995).
[12] Ranganna S. Ma nual of Analysis of fruit and vegetable products, New Delhi(1997).
[13] Hodge JE, and BT Hofreiter BT. Methods in Carbohydrate chemistry, New York(1962).
[14] Miller GL. Anal Chem 1972; 31:426.
[15] Sadasivam S, and Theymoli B. Practical manual in Biochemistry, TNAU(1987).
[16] Badger. Trends in new crops and uses ASHS press, Alexendria.,2002, 17-21
[17] CN Ogbonna and EC Okoli. Process Biochem 2010; 45 (7) : 1196 – 1200.