Current Topics in Biotechnology
Vol. 6, 2011

Original Communication

Cocoyam (*Colocasia esculenta*): An alternative raw material for vinegar production

W. Braide[1,]*, S. Oranusi[1], R. N. Nwaoguikpe[2], C. Akobondu[1], O. I. Oguoma[1], and L. I. Udegbunam[1]

[1]Department of Microbiology, [2]Department of Biochemistry, Federal University of Technology, P.M.B 1526, Owerri, Imo State, Nigeria

ABSTRACT

The potentials inherent in cocoyam (*Colocasia esculenta*) for the production of vinegar were investigated. Corms of cocoyam cultivar were gelatinized and subjected to two stage enzyme hydrolysis using bacterial alpha-amylase (Amylitic TS) and fungal alpha-amylase (AGM) to produce fermentable sugar (wort). The hydrolysed liquor was fermented by viable yeast cells, *Saccharomyces uvarum* to yield 12.9% ethanol. The ethanol was oxidized to vinegar by the metabolic activities of an acetic acid bacterium, *Acetobacter aceti*. Total soluble solids (brix level), pH, and specific gravity of the fermented liquor were determined using standard methods. Percentage ethanol and total acidity of the wort were also determined titrimetrically. Alcoholic fermentation was brought about by *S. uvarum* which lowers the pH (4.50-3.82) that favours the activities of *Acetobacter aceti* that converts the ethanol to vinegar. Total soluble solids and specific gravity of the wort decreased, while the alcoholic content increased as the sugar present in the wort was being exhausted. Total titratable acidity increased rapidly and dropped gradually as the level of ethanol in the medium reduces. Acetic acid production was enhanced by the addition of acetic acid bacteria into the medium. Statistically ($p \leq 0.05\%$) cocoyam vinegar was generally accepted compared to the conventional commercial products (cider and white vinegar). The studies also revealed that colourless vinegar was preferred and distilled cocoyam vinegar tastes better than others. The characteristic taste and aroma was specific to a particular brand of vinegar and was being influenced by the raw materials used, compounds produced and the reactions that took place during the fermentation process. The study concludes that highly perishable cocoyam with no suitable storage or preservative facilities can become an alternative raw material for vinegar production.

KEYWORDS: cocoyam, vinegar production, enzyme hydrolysis, acetification

INTRODUCTION

Vinegar, which is an aqueous solution of at least 4% acetic acid, is the product of the oxidation of alcohol by acetic acid bacteria, *Gluconobacter* and *Acetobacter* species [10]. This conversion according to [12], [19] and [20] was as a result of the ever present bacteria in the air, on the fruits and machine that converts the alcohol into acetic acid, which gives vinegar its characteristic pungent, sharp sour taste and pleasant odour.

Vinegar is produced from a wide range of substances that are capable of producing alcohol by yeast fermentation [3, 5, 10, 14, 13]. Some of

*Corresponding author
wesleybraide2005@yahoo.com

the possible raw materials used for the production of vinegar are palmwine, banana, orange, raspberry, molasses, starchy vegetables such as maize, cassava, etc [8, 12, 18, 20].

Vinegar is a product of double fermentation [12, 19, 20] involving two distinct biochemical processes brought about by the action of microorganisms. The first process known as alcoholic fermentation is brought about by the action of yeast through a process of fermentation that transforms the natural sugar into alcohol [3, 4, 12, 13, 15, 16] with the evolution of carbon dioxide under controlled environmental conditions. The process is an anaerobic fermentation in accordance with Embden-Meyerhoff (EMP) catalysed by enzymes (Amylitic-TS and AGM) produced by bacteria and fungi respectively. The second process results when a wide variety of acetic acid bacteria (eg, *Acetobacter* and *Gluconobacter* spp) oxidized alcohol in the presence of oxygen, thus converting it to vinegar. This process is referred to as the acetous fermentation or acetification.

Vinegar is a multipurpose product used mainly in food industries as condiment and in the preservation of meat and vegetables. The use of vinegar reduces the pH of the food below the level that permits the survival of spore formers and vegetative cells. Vinegar is also useful as skin disinfectants and activates the circulation of blood. It also removes dirts or impurities and also helps in balancing the pH of the skin [12, 14, 20].

Some of the raw materials used in the production of vinegar are becoming expensive as they are also being used in other products. For instance, banana, orange and their juices are fast becoming diet of many and are therefore less frequently used in the production of vinegar.

Cocoyam is cheap and available throughout the year. The high carbohydrate (10%w/v**)** level [11] in cocoyam is yet to be fully harnessed in the industries. [11] reported that the sugars present in a healthy cocoyam are sucrose, maltose, glucose and fructose.

Although cocoyam is one of the stable root crops in Nigeria, however over 20 million tonnes are lost yearly due to inadequate storage facilities [6]. Cocoyam can be successfully cultivated in poor soils with low capital and labour requirement. Since cocoyam is perishable after harvesting, speedy conversion of the surplus harvest will reduce wastage and improve economic gains. The use of cocoyam in the production of valuable economic products should be encouraged because of its high ethanol yield. Studies on the industrial utilization of cocoyam are limited to the production of ethanol [3].

This paper reports on the production of vinegar from cocoyam. Statistical analysis was used to determine the quality and acceptability of the product compared to commercial products.

MATERIALS AND METHODS

Gelatinization of cocoyam cultivar

Corns of cocoyam cultivars were washed, peeled and grated. Two hundred grams of the cocoyam mash was weighed into a beaker containing one hundred milliliters of water and the contents thoroughly homogenized by stirring. The beaker with its content was covered with aluminum foil and cooked in a pressure cooker for 30 mins at a pressure of 10 psi and at a temperature of 108.9°C [2]. The sample became gelatinized by this treatment.

Saccharification of gelatinized sample

The method of saccharification used was the enzyme hydrolysis which involved two stages. The first stage involved the use of bacterial alpha-amylase (a liquefying agent) which break down starch, while the second stage; fungal alpha-amylase (a saccharifying agent) completed the process.

Nine hundred milliliters of water was added to make slurry of the gelatinized sample to give 20% (w/v) solution. In the first stage, 1ml of 0.1N solution of bacterial alpha- amylase (Amylitic-TS) was added to the slurry and pH (controlled by addition of some drops of diluted sulphuric acid) and temperature was adjusted to 6.0 and 95°C - 100°C respectively. A partially liquefied solution was obtained on continuous agitation for 45 mins. In the second stage, the solution was cooled to 60°C - 64°C and the pH was adjusted to 5.4 to favour the activities of fungal alpha amylase. Two milliliters of 0.1N solution of

fungal alpha-amylase (AMG) was added to this slurry. The solution was agitated in a water bath for 45mins to obtain complete liquefaction of the slurry [9]. In order to stop the action of the enzyme and to sterilize the wort, the slurry was further heated for 10 mins at 100°C. The saccharified liquor was cooled to 28°C and the pH adjusted to 4.2 - 4.5.

Alcoholic fermentation of broth sample

Four hundred milliliters of the yeast, (*Saccharomyces uvarum*) was pitched into 4 litres of hydrolysed liquor in a flask and aerated before closing with cotton wool. Fermentation was allowed to take place for 7 days at 30°C. The brix level, pH, specific gravity and percentage alcohol by volume produced were determined daily and recorded [2].

Chemical analysis of sample during alcoholic fermentation

Hydrogen ion concentration (pH) of the sample was determined with a uniscope pH meter by the method described in [2]. Specific gravity was determined according to the method 945.06 [2]. Brix level of the sample was determined by hand refractometer method [2]. Simple distillation method described in [2] was used in the determination of percentage alcohol in the sample. Distillation was done by boiling the liquor at a temperature range of 780°C - 900°C. The evaporated ethanol condensed and was collected into a measuring cylinder and an alcoholmeter dipped into the cylinder. The mark at the top of distillate in the cylinder was recorded as the percentage alcohol produced.

Acetic acid fermentation of hydrolyzed liquor

The fermented liquor (alcohol) was filtered and 1.5 litres of the filtrate was poured into two separate 1000 ml conical flask and labeled "A" and "B". Sample A was allowed to undergo natural fermentation (left - alone method) while sample B was seeded with (25% v/v) cider vinegar of 4% acidity. The mouth of the flask was wrapped with transparent plastic net to prevent contamination from insects and other suspended particles. Aceteous fermentation lasted for 18 days. Samples were analyzed for total titratable acidity at two days intervals.

Clarification and distillation of fermented liquor

The resulting liquor following acetification was centrifuged at 380 rpm for 10mins to obtain clear liquor. The liquor was distilled at 100°C to obtain cocoyam vinegar, which was bottled and pasteurized in a Uniscope water bath at 60°C for 15mins.

Preparation of sample for chemical analysis

Carbon dioxide present in the liquor was removed by shaking gently in a flask and then vigorously at a temperature maintained at 20°C - 25°C. Suspended materials were removed by filtration using a clean millipore filter paper.

Determination of titratable acidity of vinegar produced

This was determined titrimetrically and the result expressed as % acetic acid produced [2]. Formula for the calculation is given below

$$\% \text{ Titratable acidity of acetic acid (vol)} = \frac{\text{ml of NaOH} \times N \times 6.0053}{\text{Vol of sample (ml)}}$$

N = normality of NaOH (0.833N)

Determination of organoleptic properties of cocoyam vinegar

A sensory evaluation of the final products was carried out to ascertain their acceptability compared to commercial products (cider and white vinegar). Parameters determined were colour, taste and aroma. A 5 point scale was used ranging from "like very much" to "(5 points) dislike very much" (1 point).

Statistical analysis

The scores of the panel were analysed statistically. The mean values of each product were determined against the different parameters. Analysis of variance was used to determine any significant difference between the products. The least significance (LSD) was used to compare the means of any significance difference [17].

RESULT

The flowchart of the production of cocoyam vinegar is shown in Figure 1. Figures 2 and 3

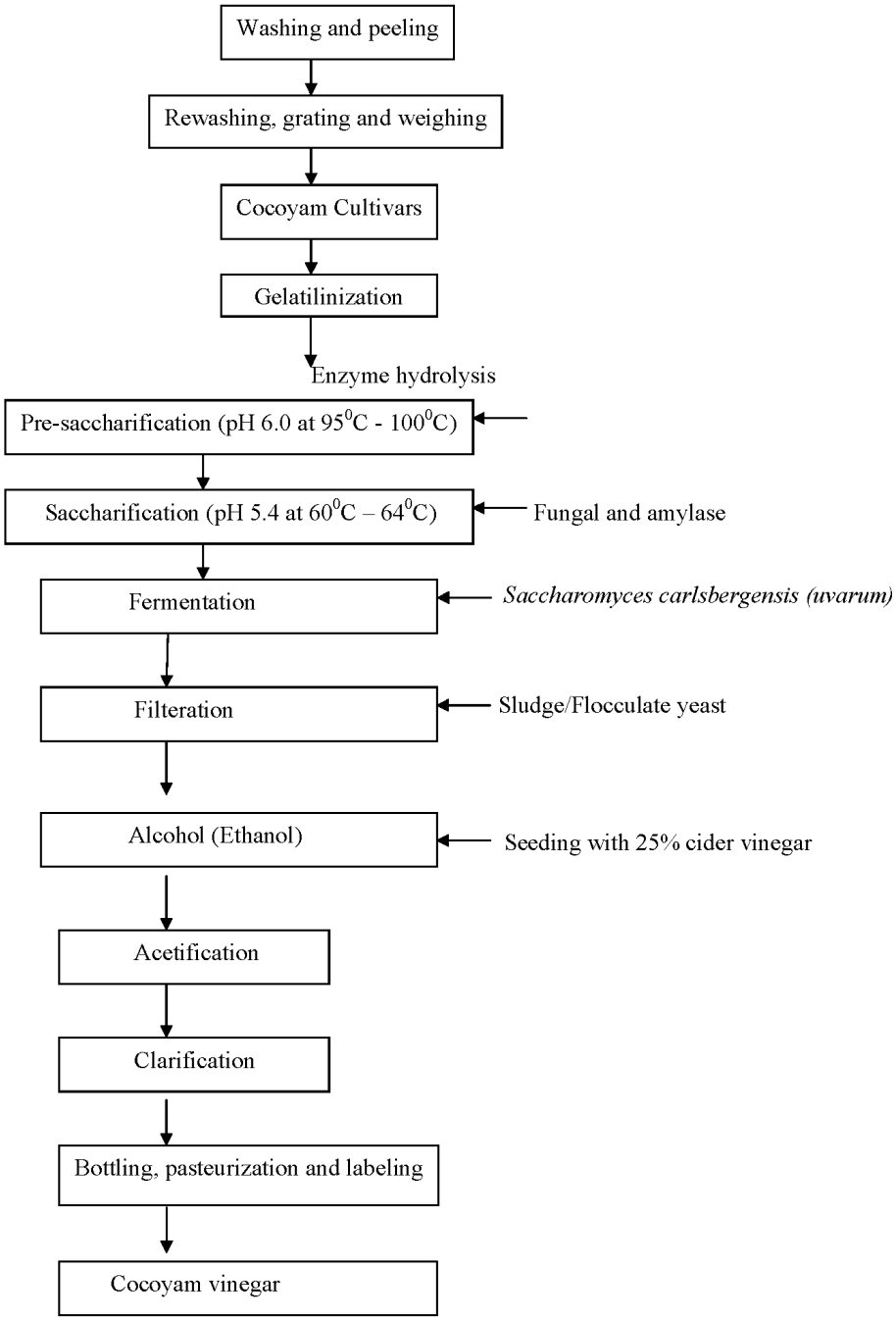

Figure 1. Flow chart of production of cocoyam vinegar.

show the pH and brix level of fermenting broth after 7 day. Table 1 shows the values of specific gravity and percentage alcohol produced from cocoyam after fermentation for 7 days. The values of the titratable acidity (TA) obtained for two samples (A and B) are shown in Table 2. The values were taken for 18 days at 2 days intervals. Table 3 shows the data obtained for the sensory evaluation of four types of vinegar. The acceptability of the cocoyam vinegar among other varieties was determined statistically at 95% confidence limit ($p \leq 0.05$) using the analysis of

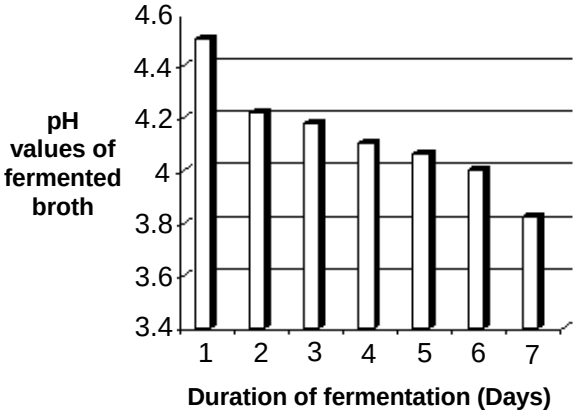

Figure 2. pH of fermenting broth for a period of 7days.

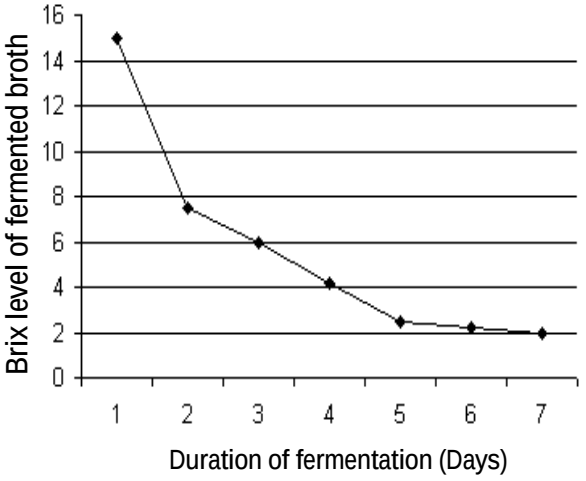

Figure 3. Brix level of fermenting broth for a period of 7 days.

Table 1. Specific gravity and percentage ethanol produced. Adapted from [3].

Duration of fermentation (Days)	Specific gravity	% ethanol produced
1	1.0000	0.00
2	0.9991	0.60
3	0.9963	2.50
4	0.9955	3.00
5	0.9901	7.00
6	0.9850	11.10
7	0.9830	12.90

Table 2. Titratable acidity of acetic acid (vinegar) produced.

Duration of fermentation	Sample A	Sample B
0	1.201	2.001
2	1.301	2.301
4	1.601	2.401
6	1.701	2.801
8	2.301	2.901
10	2.601	3.201
12	2.801	3.601
14	3.302	3.701
16	3.402	4.001
18	3.702	4.202

A, sample exposed to natural fermentation; B, samples inoculated with 25% cider vinegar.

variance (ANOVA) and least significance difference (LSD).

DISCUSSION

Production of vinegar involves two fermentation processes, namely, alcoholic and acetic acid fermentation (Figure 1). Alcoholic fermentation was brought about by *Saccharomyces uvarum*, while the aceteous fermentation was brought about by *Acetobacter* species. The high sugar level present in cocoyam had been exploited in the production of ethanol [3]. The pH of the broth decreased from 4.50 - 3.83 during the fermentation period. The reduced pH favours *S. uvarum* which converts the sugars present in the medium to ethanol [3, 12]. As the pH decreases, the fermenting broth became more acidic, thus changing the metabolic activities of the yeast for increased ethanol production.

The total soluble solids (brix level) of the broth decreased with an increase in the period of fermentation until a constant value was obtained (Figure 3). Fermentation stopped when the sugar present in the broth was exhausted. This was indicated by a large difference between the initial and final brix level. The explanation could be that the yeast exhausted the sugar and fermentation eventually came to a halt. The specific gravity of the wort deceased with an increase in the alcoholic content of the broth (Table 1).

Table 3. Sensory evaluation of four vinegar products.

Panelist	Aroma (p)				Colour (C)				Taste (T)			
	W	**X**	**Y**	**Z**	**W**	**X**	**Y**	**Z**	**W**	**X**	**Y**	**Z**
1	5	4	4	4	5	5	4	5	2	4	5	3
2	3	3	3	1	3	4	4	5	1	5	4	2
3	4	4	4	4	2	5	5	4	3	5	4	3
4	3	5	4	3	2	4	5	4	2	4	5	3
5	4	4	4	4	3	5	4	4	3	4	3	2
6	5	4	4	3	4	3	5	2	3	4	4	3
7	4	3	5	2	5	4	5	3	1	5	4	3
8	5	3	5	3	4	4	5	4	3	5	2	1
9	5	4	4	1	4	5	5	4	1	4	4	3
10	2	2	4	3	2	5	5	3	4	4	5	4
11	4	4	5	3	2	4	5	4	4	5	3	1
12	4	4	4	2	3	4	5	4	3	3	3	2
TOTAL	**48**	**44**	**50**	**33**	**39**	**52**	**57**	**46**	**30**	**52**	**46**	**30**
MEAN	4.0	3.67	4.17	2.75	3.25	4.33	4.75	3.83	2.5	4.33	3.83	2.5

W, undistilled cocoyam vinegar; X, distilled cocoyam vinegar; Y, cider vinegar; Z, white vinegar.

The relationship existing between the alcoholic content, sugar level (Total soluble solids) and the specific gravity of the broth indicates that the activities of the yeast cells were not noticeable at the initial stage of the alcoholic fermentation. This was assumed to be the lag phase when the yeast cells were adjusting to the new medium. Within the next 5 days, the depletion of the sugar was very rapid and the rate of carbon dioxide evolution was vigorous with subsequent increase in alcohol production. This phase was believed to be the exponential phase, which was the period of rapid cell multiplication indicated by active fermentation [1]. The sugar level decreased appreciably while the alcohol level increased from 0 - 12.9% at the end of fermentation (Table 1). The decrease in the specific gravity from 1.0000 - 0.9830 could be attributed to the decrease in the total soluble solids as the sugar present in the broth was fermented to alcohol (Table 1).

Acetic acid is the end product of acetification process. The aceteous fermentation lasted for 18 days. Total titratable acidity increased throughout the duration of fermentation (Table 2). The increase was rapid initially and then became gradual. The explanation could be that alcohol was abundant initially, but as the acetification process lasted, there was a decrease in the level of alcohol until the alcohol was no longer available for the organisms to utilize. The titratable acidity of sample "A" increased from 1.2% - 3.7%, while that of sample "B" increased from 2.0% - 4.2%. Acetification was enhanced or facilitated in sample "B" by the activities of the acetic acid bacteria already present in the vinegar [7, 8, 12, 18].

Results of the sensory evaluations of four products designated W, X, Y and Z are shown in Table 3. Statistically ($P \leq 0.05$), there is a significant difference in taste between product X and Products W and Z. The explanation is that distilled cocoyam tastes better as indicated from the mean of the others. Distilled cocoyam vinegar (X), cider vinegar (Y), and white vinegar (Z) differed significantly from the undistilled cocoyam vinegar (W) in terms of colour. This result suggests that consumers prefer colourless vinegar to the milk coloured vinegar. The aroma of the undistilled cocoyam vinegar, distilled cocoyam vinegar and cider vinegar was significant from that of the white vinegar.

This study had shown that cocoyam can be used in the production of vinegar. The level of acceptability further suggests that cocoyam vinegar competes favourably with other commercial vinegar.

REFERENCES

1. Amerine, M. A. 1988, Encyclopidia Americana, International edition, Macmillan publishing Co. Inc., New York, USA, 36-44.
2. Association of Official Analytical Chemists, 2000, Official Methods of Analysis, 8th edition, USA, 899-911.
3. Braide, W. and Nwaoguikpe, R. N. 2011, International Journal of Plant Physiology and Biochemistry, 3(3): 64-66.
4. Dubey, R. C. 2005, A Textbook of Biotechnology, 3rd edition. S. Chand and Company, New Delhi, India, 264-266, 339-354.
5. Heath, H. B. 1981, Source Book of flavour. AVI Publishing Co. Inc., Westport, Connecticut, USA, 229-231.
6. International Institute of Tropical Agriculture (IITA). 2009, Factors limiting Cocoyam cultivation and processing in developing countries. A paper presented at a workshop by Prof. Olumide Adebayo. IITA, Ibadan, Nigeria.
7. Joslyn, M. A. 1970, Kirk- Other Encylopedia of Chemical Technology. 2nd edition, Wiley press Ltd., New York, 254-269.
8. Kuboye, A. O and Akinrele, I. A. 1971, Derivation of Table Vinegar from Fermented Palmwine. FIIRO Res. Rep. Technology, No 42. Lagos, Nigeria.
9. Miller, B. M. and Litsky, W. 1976, Industrial Microbiology. 2nd edition, McGraw-Hill, Inc., USA, 120-135, 180-186.
10. Nester, E. W., Robert, C. E., Pearsall, N. W., Anderson, D. G., and Nester, M. T. 1998, Microbiology: A Human Perspective, Second edition, WBC/McGraw-Hill Companies, Boston, USA, 770-771.
11. Nwufo, M. I. and Fajola, A. O. 1998, Acta Microbiologica Hungarica, 4, 371.
12. Okafor, N. 2007, Modern Industrial Microbiology and Biotechnology. First edition, Science Publishers, Enfield, New Hampshire, USA, 280-288
13. Okorondu, S. I., Nedosa, I. V., Wesley, B., and Akujobi, C. O. 2009, Current Topics in Biotechnology, 5, 65-70.
14. Perry, J. J. and Staley, J. T. 1997, Microbiology: Dynamics and Diversity. First edition, Saunders College Publishing, New York, USA, 446-447.
15. Saraswati, R. 1988, Paper delivered at a workshop on upgrading of Cassava/ Cassava Waste by appropriate Biotechnologies, 41-49.
16. Saucedo, G., Lonsane, B. K., Navarro, J. M., Roussos, S., and Raimbault, M. 1992, Appl. Biochem. Biotech., 36, 47-61.
17. Spiegel, M. R. 2005, Theory and Problems of Statistics. Schaum's Outline Series. McGraw Hill Book Company, New York, USA, 167-180.
18. Spurgin, M. M. 1964, Journal of First Agricultural Science, 23, 213-233.
19. Talaro, K. and Talaro, A. 1993, Foundations in Microbiology. First edition, Wm. C. Brown Communications, Inc, Los Angeles, USA, 214, 271, 740, 744-745.
20. Willey, J. M., Sherwood, L. M., and Woolverton, C. J. 2008, Prescott, Harley, and Kliein's Microbiology. Seventh edition, McGraw- Hills Companies, Inc, New York, USA, 1043.