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Full Length Research Paper

Application of acid-hydrolyzed cassava (*Manihot esculenta*) and cowpea (*Vigna unguiculata*) for the production of yeast (*Saccharomyces cerevisiae*)

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Much progress has been made in the cultivation and production of cassava (*Manihot esculenta*) and cowpea (*Vigna unguiculata*) in Nigeria. In the present study, investigation was carried out on the possibility of using cassava flour as a source of glucose as well as cowpea as source of nitrogen in the production of yeast. Acid hydrolysis (using dilute H_2SO_4) of cassava and cowpea was undertaken to release the sugars and amino acids. The pH of the growth medium using hydrolyzed cassava as carbon source and cowpea as nitrogen source was varied from pH 2.5 - 6.5. The results obtained show that pH 6.5 gave optimum yeast biomass. The hydrolyzed cassava was also varied in the growth medium. The result obtained shows that increased concentrations of acid-hydrolyzed cassava increased yeast biomass, indicating that hydrolyzed cassava is a good carbon source of glucose for yeast production. It was also observed that yeast biomass using acid hydrolyzed cowpea extract as nitrogen source was high. This is due to the fact that cowpea contains 66.35% of carbohydrate in addition to about 25% protein and hence a good source of carbon and nitrogen in the culture medium. The residual glucose concentration of the yeast culture for each medium was also determined. The result obtained indicates that with increased yeast biomass, there was significant decrease in the residual glucose. Also, there was a significant decrease in pH of the culture media following yeast culture; the culture media tends to be acidic after yeast culture. Therefore, yeast can be produced using acid hydrolyzed cassava flour as carbon source with cowpea as nitrogen source.

Key words: Cassava, cowpea, yeast, acid hydrolysis, glucose, nitrogen source.

INTRODUCTION

Yeasts (*Saccharomyces cerevisae*) can grow in the presence or absence of air. Anaerobic growth which is growth in the absence of oxygen is quite slow and inefficient. For instance, in bread dough, yeast grows very slowly. In this case, the sugar that can sustain either fermentation or growth is used mainly to produce alcohol and carbon dioxide. Only a small portion of the sugar is used for cell maintenance and growth. In contrast, under aerobic conditions, in the presence of a sufficient quantity of dissolved oxygen, yeast grow by using most of the available sugar for growth and producing only negligible quantities of alcohol (Bekatorov et al., 2006). Studies show that organic nitrogen sources such as yeast extract support rapid growth and high cell yields of microorganisms because it contains amino acids and peptides, water soluble vitamins and carbohydrates (Peppler, 1982; Watson, 1976).

The basic carbon and energy source for yeast culture are sugars (Dubai and Muhammad, 2005). Starch cannot be used because yeast does not contain the appropriate enzymes to hydrolyze this substrate to fermentable sugars. Beet and cane molasses are commonly used as raw materials because the sugars present in molasses, a mixture of sucrose, fructose and glucose, are readily fermentable (Ohara et al., 1992). In addition to sugar, yeast also requires certain minerals, vitamins and salts for growth. Some of these can be added to the blend of beet and cane molasses prior to flash sterilization while others are fed separately to the fermentation (Stanbury et al., 1995). Required nitrogen is supplied in the form of ammonia and phosphate is supplied in the form of phosphoric acid (Zheng, 2005).

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Cassava (*Manihot esculenta*) ranks very high among crops that convert the greatest amount of solar energy in soluble carbohydrates per unit area of land. It is grown for its large starch filled roots, which contain nearly the maximum theoretical concentration of starch on a dry weight basis among food crops. Among the starchy stables, cassava gives a carbohydrate production which is about 40% higher than rice and 25% more than maize (Tonukari, 2004; Akinfala et al., 2002; Nwokoro et al., 2002). Fresh cassava contains very little protein and fat (Okezie and Kosikowski, 1982). The approximate compositions of the cassava tuber are: moisture (75 – 80%), starch (20 – 30%), protein (2 – 3%), ash (1.15%), fibre (1.0%) and fat (0.1%) (Ihekonronye and Ngoddy, 1985; Alais and Linden, 1999). The future demand for fresh cassava mainly depends on improved storage methods even as the market for cassava as a substitute for cereal flours in bakery products and as energy source in animal feed ratios are likely to expand (Tonukari, 2004).

Cowpea (*Vigna unguiculata*) belongs to the legume family (fabaceae – legumunosae). It is an annual legume which is commonly referred to as blackeye bean, blackeye pea (Quinn and Myers, 2002). Cowpea is one of the world's important legume food crops (Bressani, 1985; Awonaike et al., 2001). At least 12.5 million hectares of cowpea are cultivated with an annual production of over 3 million metric ton worldwide. Development of new cultivators with early maturity, acceptable grain quality and resistance to important diseases and pest has significantly increased (Miller, 1998). The international institute of tropical Agriculture (IITA) has been working on the improvement of cowpea for more than 30 yrs and over 60 countries receive cowpea cultivars improved by IITA for testing and adoption where needed (Davis et.al., 1991). Cultivars which are developed by the IITA in Nigeria are now widely grown in over 60 countries and the production has increased up to 341 from 1961 to 1995 in Nigeria.

The industrial and commercial use of yeast started at the end of the 19th century after it was identified and isolated by Pasteur (Broach and John, 1991). Yeasts are included in starter culture for the production of specific types of fermented foods like cheese, bread, fermented meat, vinegar and vegetable products (Gilland, 2002). *Saccharomyces cerevisiae* and other yeasts have industrial and medical applications beneficial to human life. *S. cerevisiae* also known as baker's yeast is the most common yeast, which is used worldwide for the production of bread and baked products (Corriher, 2001). The need to design feasible and financially viable processes and the ultilization of low-cost raw materials for edible yeast biomass production is extremely important (Albers et al., 1996). The purpose of this project work is to develop a rapid method for producing glucose and simple sugars from cassava as well as amino acids from cowpea through acid hydrolysis and combining this for yeast production. This will enhance the use of locally abundant agricultural products (cassava and cowpea) for the industrial production of yeast.

MATERIALS AND METHODS

Cassava and cowpea

Cassava tubers were purchased from Abraka market in Delta State, Nigeria. The tubers were peeled and thoroughly washed (to reduce the cyanide content), sliced into small pieces, dried under the sun, ground to flour and passed through a sieve of 0.25 mm before being stored in a dry plastic container, ready for use. Cowpea seeds also known as beans were also purchased from Abraka market in Delta State. They were ground to flour and passed through a sieve of 0.25 mm before it was stored in a dry plastic container, ready for use.

Acid hydrolysis of cassava

To get the best hydrolysis of cassava flour, the weights of cassava flour was varied at constant acid volume. 5 to 25 g of cassava flour was weighed into six (6) conical flasks. To each of the conical flask, was added 0.5 ml of 0.5% (v/v) H_2SO_4 acid. The conical flask was then made up to 100 ml with distilled water and shaken thoroughly to have an even mix. It was autoclaved at about 115°C for 20 min, cooled and filtered into different sterilized test tubes as hydrolyzed glucose (sample glucose) and stored in a cool dry place.

Acid hydrolysis of cowpea

This was done to get the best hydrolysis of cowpea flour by varying the weights of cowpea flour used at constant acid volume. 5 - 30 g of cowpea flour was weighed into six (6) conical flasks. To each of the conical flask was added 0.5 ml of conc. H_2SO_4 acid. The conical flask was then made up to 100 ml with distilled water and shaken thoroughly to have even mix. The conical flask was then autoclaved for 30 min, cooled and filtered into different sterilized test tubes using filter paper. It was then stored in a cool dry place.

Glucose estimation

The sample glucose estimation was carried out to determine the optimum hydrolysis of cassava flour. This was done using glucose estimation kit (Randox Laboratories Ltd, Diamond Road, Crumlin, Co. Antrim United Kingdom, BT29 4QY). Five test tubes were labeled test tubes 1 - 5 with tube 1 serving as the control. To each of the test tubes, 100 µl of hydrolyzed glucose filtrate was added, 2.0 ml of distilled water and 2.0 ml glucose buffer was also added to the test tubes. This was incubated for 5 min at room temperature and optical density (O.D) taken using the spectrophotometer at 500 nm to estimate the amount of glucose as well as the optimum hydrolysis of cassava flour (Barham and Trinder, 1972).

Yeast tablets

Yeast (*S. cerevisiae*) tablets were purchased from a chemist in Abraka, Delta State, Nigeria.

Preparation of yeast culture (YPD media) for inoculation

To prepare an uncontaminated yeast culture which was used for

Table 1. Proximate analysis of cowpea and cassava flours.

Parameter	Cowpea	Cassava
Lipid	2.23 ± 0.153	0.92 ± 0
Crude protein	25.38 ± 0.290	1.93 ± 0.03
Ash	3.66 ± 0.025	4.83 ± 0.106
Fibre	2.35 ± 0.132	1.99 ± 0.014
NFE (carbohydrate)	66.35 ± 0.300	90.34 ± 0.163
Moisture	8.4 ± 0.158	6.40 ± 0.141
Energy (KCal)	387.01 ± 1.140	377.34 ± 0.481

Values are mean ± standard deviation of triplicate experiments; values are in % (except for energy); NFE, nitrogen free extract.

inoculation of different reaction media for yeast production, 2 g peptone, 2 g D-glucose and 1 g yeast extract were weighed into a 250 ml autoclaved conical flask. The content was mixed with a little amount of distilled water and the solution was made up to 100 ml with distilled water. The content was autoclaved for 30 min; it was brought out, cocked with cotton wool to make it air tight and then autoclaved again for another 30 min. After which, it was brought out and allowed to cool at room temperature. After cooling, 100 µl of 20% (w/v) antibiotics (ampiclox) was added to the solution to prevent bacteria growth before adding two tablets of yeast. Its content was shaken very well to allow the tablet dissolve and the culture was incubated for 6 - 12 h before using the culture for inoculation of other substrate for yeast culture.

Yeast culture with varying acid-hydrolyzed cowpea extract

To determine the yeast biomass using acid hydrolyzed cassava as carbon source and acid-hydrolyzed cowpea extract as nitrogen source, 20 ml of the acid-hydrolyzed cassava was measured into six (6) conical flasks having the glucose control flask; the acid-hydrolyzed cowpea extract control flask containing 10 ml of acid-hydrolyzed cowpea extract and four other flasks containing 2.5, 5.0, 7.5 and 10.0 ml of acid-hydrolyzed cowpea extract, respectively. To the acid-hydrolyzed cowpea extract control flask, there was no addition of 20 ml acid hydrolyzed cassava and no cowpea extract was added to the glucose control flask. The pH in the conical flasks was adjusted to 6.5. This was done using the pH meter and NaOH solution (1.0 M) as alkaline medium. The total volume in each conical flask was made up to 100 ml with distilled H_2O. The flasks were then autoclaved for 30 min, allowed to cool, corked with cotton wool and re-autoclaved for another 30 min. After autoclaving the second time, it was allowed to cool and 100 µl of 20% (w/v) antibiotics (ampiclox) was added to each flask to prevent bacteria growth. 2 ml of the incubated yeast culture was then used to inoculate each of the conical flasks. The flask was shaken very well and allowed to grow for 36 h. After the growth period, the optical density (OD) was taken at 600 nm to determine the yeast biomass.

Yeast culture with varying acid-hydrolyzed cassava

To determine the yeast biomass using varying amount of hydrolyzed cassava as carbon source with acid-hydrolyzed cowpea extract as nitrogen source, a varied amount of the acid-hydrolyzed cassava was added from 2.5 to 20 ml into different conical flasks (flasks 2 – 9). To flask one, there was no addition, serving as control. 5% of acid-hydrolyzed cowpea extract was then added to the different conical flasks. After these additions, the subsequent procedures following the yeast determination is as previously described. In estimating the glucose content of the yeast culture using varied acid-hydrolyzed cassava, nine test tubes were used and labeled test tubes 1 – 9. All other procedures were followed as previously described. After the yeast biomass has been determined, the pH readings were also determined as previously described.

Yeast culture with varying pH

To determine the yeast biomass with varying pH using acid hydrolyzed cassava as carbon source and acid hydrolyzed cowpea as nitrogen source, 20 ml of the acid-hydrolyzed cassava was measured into six (6) conical flasks followed by the addition of 5 ml of acid-hydrolyzed cowpea extract to the conical flasks. The pH of contents of the conical flasks labeled 2-6 was adjusted to 2.5, 3.5, 4.5, 5.5, and 6.5, respectively, with the exception of conical flask 1, which served as the control. This was done using the pH meter and NaOH (1.0 M) solution as alkaline medium. The total volume in each conical flask was made up to 100 ml with distilled H_2O after adjusting the pH. It was then autoclaved for 30 min, allowed to cool, corked with cotton wool and re-autoclaved for another 30 min. After autoclaving the second time, it was allowed to cool and 100 µl of 20% (w/v) antibiotics (ampiclox) was added to each flask to prevent bacteria growth. 2 ml of the incubated yeast culture was used to inoculate each of the conical flasks. The flask was shaken very well and allowed to grow for 36 h. After the growth period, the optical density (OD) was taken at 600 nm to determine the yeast biomass.

RESULTS

Proximate analysis

Prior to the commencement of yeast culture using locally available raw materials, proximate analysis of experimental samples was carried out to determine the biochemical components of cassava and cowpea. The result of the analysis carried out on the lipid, crude protein, ash, fibre, nitrogen free extract (NFE), moisture and energy level of cassava and cowpea is as shown in Table 1.

Acid hydrolysis of cowpea flour

Acid hydrolysis of the cowpea flour was also carried out

Table 2. Glucose estimation after acid-hydrolysis of cassava.

Acid-hydrolyzed cassava	5%	10%	15%	20%	25%
O.D (500 nm)	1.757	1.723	1.707	1.785	1.268
Glucose concentration (mmol/L)	9.800	9.611	9.521	9.956	7.072
Amount of glucose (g)/100 ml	1.764	1.730	1.714	1.792	1.273

Table 3. Yeast biomass estimation after 36 h culture* with varying amount of acid-hydrolyzed cassava.

Acid-hydrolyzed cassava (%)	Acid-hydrolyzed cowpea (%)	Yeast biomass (O.D 600 nm)**	Residual glucose (mmoles/L)**	pH after 36 h of yeast culture
0	10	1.023 ± 0.006	1.473 ± 0.002	5.26
2.5	10	1.132 ± 0.006	1.445 ± 0.002	5.3
5.0	10	1.149 ± 0.002	1.210 ± 0.002	5.28
7.5	10	1.167 ± 0.004	1.244 ± 0.003	5.25
10.0	10	1.185 ± 0.005	1.149 ± 0.001	5.22
12.5	10	1.206 ± 0.003	1.132 ± 0.002	4.92
15.0	10	1.213 ± 0.004	1.132 ± 0.002	4.79
17.5	10	1.225 ± 0.001	1.032 ± 0.001	4.67
20.0	10	1.275 ± 0.006	1.071 ± 0.001	4.62

*The medium contained varying amount (%) of 20% acid-hydrolyzed cassava (20 ml of acid-hydrolyzed cassava contains 0.36 g glucose) and 5% poultry manure extract. The pH in the conical flasks was adjusted to 6.5. The total volume in each conical flask was made up to 100 ml with distilled H_2O and autoclaved for 30 min. 100 µl of 20% antibiotics (ampiclox) was added and inoculated with 2 ml of yeast culture ($OD_{600\ nm}$ = 1).
**Values are mean ± standard deviation of triplicate experiments.

to determine the best hydrolysis of cowpea flour and from the results obtained, 10% cowpea flour gave the optimum hydrolysis using 0.5% concentrated H_2SO_4 with 30 min autoclaving. It was easier to filter compared to higher cowpea flour amounts. Thus, subsequent analysis for yeast production was carried out using 10% cowpea flour for acid hydrolysis, which was cooled and stored as cowpea extract serving as nitrogen source for yeast culture.

Acid hydrolysis of cassava flour

Acid hydrolysis of the cassava flour was carried out to determine the best hydrolysis of cassava flour varying the weights of cassava flour from 5 to 25 g at constant acid volume. At the end of the hydrolysis, the hydrolyzed glucose (sample glucose) was filtered into different sterilized test tubes and stored in a cool dry place. Glucose estimation of the varied hydrolyzed cassava was carried out as well as standard glucose estimation in comparison. This was to test for the optimal hydrolysis of cassava. The glucose estimation was carried out using glucose kit and the result shows that 20% of the cassava flour gave the optimum hydrolysis as shown in Table 2. This gave the highest amount of glucose on hydrolysis. Thus, subsequent analysis for yeast production was carried out using 20% cassava flour for acid hydrolysis and the glucose produced was used for yeast production.

Yeast biomass estimation with varying acid-hydrolyzed cassava

The yeast biomass estimation with varied acid-hydrolyzed cassava (glucose) was aimed at determining the yeast biomass level using various percentage of acid-hydrolyzed cassava (glucose). The amount of residual glucose and the pH level (whether acidic or alkaline) of the yeast culture was also determined. The result is shown in Table 3.

From Table 3, it was observed that, as the percentage of acid-hydrolyzed cassava increased from 0 – 20%, there was also a concurrent increase in the yeast biomass. The yeast biomass observed in the control is due to the extra carbon content contained in the cowpea. After the yeast culture had been incubated at the specified period, the residual glucose concentration as well as the pH of the media after growth was measured. From Table 3, it was observed that yeast biomass is inversely related to the residual glucose after yeast culture. Thus, indicating that, the more the yeast biomass, the lesser the residual glucose after yeast culture while the lesser the yeast biomass, the more the residual glucose after yeast culture.

Also measured was the pH of the yeast culture, which was seen to be acidic (reduced pH). The reduced pH observed indicates that, after the optimal growth of yeast, the media in which the growth occurred becomes more acidic because of the production of organic acids like

Table 4. Yeast biomass estimation after 36 h culture* with varying acid-hydrolyzed cowpea.

Acid-hydrolyzed cassava (%)	Acid-hydrolyzed cowpea (%)	Yeast biomass (O.D 600 nm)*	Residual glucose (mmoles/L)*	pH after 36 h of yeast culture
20	0	0.507 ± 0.002	5.438 ± 0.002	4.39
20	5	0.535 ± 0.003	7.212 ± 0.008	4.44
20	10	0.693 ± 0.002	3.984 ± 0.002	4.46
20	15	0.726 ± 0.006	3.720 ± 0.002	4.48
20	20	0.746 ± 0.009	3.564 ± 0.004	4.49
0	20	0.684 ± 0.014	0.039 ± 0.000	4.51
YPD		0.992 ± 0.002	7.351 ± 0.045	4.79

*The medium contained 20 ml of the 20% acid-hydrolyzed cassava (20 ml of acid-hydrolyzed cassava contains 0.36 g glucose) and 5% poultry manure extract with varying pH. The total volume in each conical flask was made up to 100 ml with distilled H_2O and autoclaved for 30 min. 100 µl of 20% antibiotics (ampiclox) was added and inoculated with 2 ml of yeast culture ($OD_{600\ nm}$ = 1).
** Values are mean ± standard deviation of triplicate experiments; **YPD,** Yeast peptone dextrose culture medium containing 2% glucose.

lactic and malic acids (Roble et al., 2003).

Yeast biomass estimation with varying acid-hydrolyzed cowpea

The yeast biomass estimation analysis was aimed at determining the amount of yeast as well as determining the amount of residual glucose and the pH level (whether acidic or alkaline) of the yeast culture. This was done using the acid-hydrolyzed cassava (glucose) as carbon source and cowpea extract as nitrogen source. The result obtained is shown in Table 4.

Yeast grows very well in the presence of carbon and nitrogen. From Table 4, it is observed that, under the normal yeast culture (YPD), the yeast grew optimally compared to when other carbon (acid-hydrolyzed cassava filtrate – glucose) and nitrogen sources (acid-hydrolyzed cowpea) were used. Using constant percentage of acid-hydrolyzed cassava filtrate as carbon source together with varied percentage of acid-hydrolyzed cowpea as nitrogen source, increased yeast biomass was observed as the percentage of the cowpea extract increases (Table 4). This indicates that, Yeast grows very well in the presence of high amount of nitrogen and carbon, with cowpea containing extra amount of carbon from the carbohydrate it contains. The plot of the yeast biomass using cowpea extract as nitrogen source.

Also, in Table 4, there is glucose filtrate (acid-hydrolyzed cassava) control and cowpea extract control. It was observed from the table that, in the presence of high amount of glucose filtrate (acid-hydrolyzed cassava) and no amount of cowpea extract (cowpea extract control), there was high amount of yeast biomass. The high yeast biomass observed with cowpea accounts for the extra carbon that is contained in cowpea as carbohydrate together with the nitrogen content.

The residual glucose as well as the pH of the media after yeast culture was also measured. From Table 4, it was observed that, with increase in yeast biomass, there was significant decrease in the residual glucose concentration, which indicates that, much of the glucose have been used during the yeast growth process.

Yeast biomass estimation with varying pH

The yeast biomass estimation with varying pH was aimed at determining the pH at which optimal growth is observed. This experiment is necessary because, after acid hydrolysis, the medium was very acidic (about pH 1.3); thus, varying the pH helps to ascertain the minimum amount of NaOH needed to adjust the pH. Hence, neutralizing with least amount (few drops) of base will save cost using this method. The amount of residual glucose and the pH level (whether acidic or alkaline) after the yeast biomass was also determined. The results obtained are shown in Table 5.

Yeast grows best (optimally) at pH 6.5. From Table 5, the pH of the yeast media was adjusted to varying pH range, from pH 2.5 to 6.5 using 1 M sodium hydroxide (NaOH) solution as the alkaline medium while the acid-hydrolyzed cassava (glucose filtrate) was used as the acidic medium as well as carbon source and the cowpea extract was used as the nitrogen source. The pH of the medium before adjusting with 1 M NaOH (serving as the control for the analysis) was 1.40.

Using constant percentage of acid-hydrolyzed cassava and cowpea, increased yeast biomass was observed as the pH variation increases from pH 2.5 to 6.5 and pH 6.5 gave the best (optimum) yeast biomass (Table 5).

After the growth of the yeast at the specified incubation period, the residual glucose concentration as well as the pH of the media after growth was measured. From Table 5, it was observed that, as the pH increases, the residual glucose concentration of the yeast culture decreases.

This indicates that there was increased yeast biomass which resulted in decreased residual glucose concentration due to the high utilization of the carbon

Table 5. Yeast biomass estimation after 36 h culture* with varying pH using acid-hydrolyzed cassava and cowpea.

Acid-Hydrolyzed cassava (%)	Acid-hydrolyzed cowpea (%)	pH	Yeast biomass (O.D 600 nm)**	Residual glucose (mmoles/L)**	pH after 36 h of yeast culture
20	10	1.40***	0.220 ± 0.003	11.557 ± 0.011	2.35
20	10	2.5	0.306 ± 0.002	12.433 ± 0.005	3.24
20	10	3.5	0.478 ± 0.005	12.322 ± 0.021	4.13
20	10	4.5	0.552 ± 0.002	11.368 ± 0.024	4.35
20	10	5.5	0.652 ± 0.003	10.882 ± 0.001	5.39
20	10	6.5	1.046 ± 0.003	7.435 ± 0.004	5.86

*The medium contained 20 ml of the 20% acid-hydrolyzed cassava (20 ml of acid-hydrolyzed cassava contains 0.36 g glucose) and 5% poultry manure extract with varying pH. The total volume in each conical flask was made up to 100 ml with distilled H_2O and autoclaved for 30 min. 100 µl of 20% antibiotics (ampiclox) was added and inoculated with 2 ml of yeast culture ($OD_{600\ nm}$ = 1).
** Values are mean ± standard deviation of triplicate experiments;
*** = pH after acid hydrolysis and before adjusting with NaOH (1 M).

content in the acid-hydrolyzed cassava (Stewart and Russel, 2002). Also observed was the pH of the yeast culture, which was seen to be acidic (reduced pH). However, it was observed that, at other adjusted values of pH ranging from 2.5 to 4.5 with the controls inclusive, the pH values of the yeast culture was higher than the adjusted values, which indicates that, yeast do not grow very well in very acidic medium (Gaudreau et al., 1997). Fair yeast growth was observed for pH 5.5, as the pH value after the yeast culture was seen to be reduced (acidic) but not as good as pH 6.5 (Table 5).

DISCUSSION

Cassava is basically made up of starch. Cassava is rich in carbohydrate and can be used both industrially and as an important food source (El-Sharkawy, 2004). Various industries have exploited the use of cassava in the production of many items such as textiles, cosmetics, glue and adhesive, pharmaceutical and cement (Tonukari, 2004; Altschul and Von, 1973; Nduele et al., 1993). The acid catalyzed hydrolysis of starch is a complex heterogenous reaction. It involves physical factors as well as the hydrolytic chemical reaction. The hydrolysis is therefore controlled by both the reaction conditions (which are acid concentration and temperature) and the physical state of starch (Oboh and Akindahunsi, 2003). -Amylose and amylopectin glycosidic bonds are broken to produce glucose and oligosaccharide residues (Burelli, 2003). The major hydrolytic product of cassava starch (glucose) can serves as a suitable carbon source for the production of yeast. The production of yeast is an important step to the commercial use of the product in baking, brewing and other applications. Yeast has been known to humans for thousands of years as they have been used in fermentation processes including the production of alcoholic beverages and bread leavening (Chao et al., 2001; Hough, 1998).

From the results of the present study, it was shown that, acid hydrolyzed cassava flour (glucose) can serve potentially as a good and locally available carbon source for the production of yeast. Moreover, there is relative high availability of cassava (Ihekonronye and Ngoddy, 1985; Alais and Linden, 1999). The processes involved in acid hydrolysis of cassava flour is fast and economically feasible, as little amount of the acid can hydrolyzed large amount of the cassava flour within 2 h. The results obtained showed that the hydrolysis of cassava flour gave a high yield of glucose which serves as good and locally available carbon source for yeast production. This is in agreement with the findings of Oboh and Akindahunsi (2003), who observed that, the hydrolytic product of cassava starch – glucose, serves as a suitable substrate in providing carbon source for the growth of yeast.

Cowpea is rich in protein which can be used industrially as well as an important food source (Quinn and Myers, 2002). The results obtained from proximate analysis of cowpea and cassava showed that cowpea is a good source of nitrogen since the protein content is 25.38%. Also, cassava is a good source of carbohydrate (90.36%). This indicates that cowpea and cassava can be good sources of nitrogen and carbon, respectively, for yeast culture. This present research shows that yeast culture can be enhanced with the use of cowpea which serves as an alternative to peptone. Peptone is commonly used as nitrogen source for the growth of yeast. Cowpea also contains carbohydrate in addition to protein.

The results of the present study showed that yeast grows best closer to neutral pH with a sharp increase at pH 5.5 - 6.5. This result is in agreement with the findings of Glen and Dilworth (2002). Yeast grows poorly when the pH of the medium is lower than 4. Yeast biomass was also determined at varied values of the acid-hydrolyzed cassava. The results showed that yeast biomass increases with increasing amount of acid-hydrolyzed cassava as carbon source, thus, confirming

Dubai and Muhammad's (2005) observation that the basic carbon and energy source for yeast culture are sugars. Increasing acid-hydrolyzed cowpea also increased yeast biomass after 36 h of culture.

Conclusion

The methods described in this work can be used in the development of a rapid method for producing glucose and simple sugars from cassava through acid hydrolysis and combining this with cowpea for yeast production. It is of significance that locally abundant agricultural products such as cassava and cowpea can be used for the production of an industrial raw material, yeast. This research has taken the advantage of cassava and cowpea flour as alternative carbon and nitrogen sources because of its availability. Yeast is currently imported into Nigeria for various uses. Local industries should take advantage of the results presented here to start yeast processing plants using locally and readily available cassava and cowpea.

REFERENCES

Akinfala EO, Aderibigbe AO, Matanmi O (2002). Evaluation of the nutritive value of whole cassava plant as replacement for maize in the starter diets for broiler chicken; Livest. Res. Rural Dev. pp. 14-16. Retrieved March 17, 2009, from http://www.cipav.org.co/lrrd/lrrd14/6/akin146.htm.

Alais C, Linden G (1999). Food Biochemistry. Aspen publishers Inc. Maryland.

Albers E, Larsson C, Lidén G, Niklasson C, Gustafsson L (1996). Influence of the nitrogen source on Saccharomyces cerevisiae anaerobic growth and product formation. Appl. Environ. Microbiol. 62(9): 3187–3195.

Altschul S, Von R (1973). Drugs and Food from Little known plants Harvard Univ. Press, Cambridge.

AOAC (1995). Association of Official Analytical Chemists. Official Methods of Analysis. Washington DC.

Awonaike MJ, Ferrer J, Ejiofor AO (2001). Nitrogen contribution in cowpea. Biores. Technol. 57: 275-288.

Barham D, Trinder P (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97(151): 142-145.

Bekatorov A, Psarianos C, Athanasios A (2006). Production of food grade yeasts. Food Technol. 44(3): 407-415.

Bressani R (1985). Nutritive Value of Cowpea. John Wiley and Sons, Chinchester.

Broach JR, John R (1991). Pringle and biology of the yeast Saccharomyces cerevisiae: Genome dynamics protein synthesis and energetics, Cold Spring Harbour Laboratory Press, New York.

Burelli MM (2003). Starch: the need for improved quality or quantity – an overview. J. Exp. Bot. 54(382): 451-456.

Chao HJ, Joo Ms (2001). Utilization of Brewer's Yeast Extract Part 1: Effects of Different Enzymatic Treatment on Solid and Protein Recovery and Flavour Characteristics. Biores. Technol. 76: 253-258.

Corriher S (2001). Yeast's crucial roles in bread making. Fine cooking. 43: 80-81.

Davis DW, Oelke EA, Oplinger ES, Doll JD, Hanson CV Putnam DH (1991). Cowpea alternative field crops manual. University of Wisconsin- Madison, United States pp. 1-10.

Dubai YU, Muhammad S (2005). Cassava starch as an alternative to agar-agar in microbiological media. Afr. J. Biotechnol. 4(6): 573-574.

El-Sharkawy MA (2004). Cassava biology and physiology. Plant. Mol. Biol. 56: 481-501.

Gaudreau H, Champagne CP, Goulet J, Conway J (1997). Lactic acid fermentation of media containing high concentration of yeast extracts. J. Food Sci. 62: 1072-1075.

Gilland B (2002). World population and food supply In:`can food production keep pace with population growth in the next half century'. Food policy.

Glen ST, Dilworth EA (2002) Growth and Survival of Yeast in dairy product. Food Res. Int. 34: 791-796.

Hough JS (1998). The biotechnology of malting and brewing pp. 93-96.

Ihekonronye AI, Ngoddy PO (1985). Integrated food science and technology for the tropic. Macmillian Education Ltd. Oxford 2nd ed. pp. 15-35.

Miller P (1998). Growth rate of cowpea. J. Appl. Food Sci. 29: 253-255.

Nduele M, Ludwig A, Van Ooteghem M (1993). The use of cassava starch in the formulation of gelatin capsules. J. de Pharm. Belg. 48(5): 325-334.

Nwokoro SO, Orheruata AM, Ordiah PI (2002). Replacement of maize with cassava sieviates in cockerel starter diets: effects on performance and carcass characteristics. Trop. Anim. Health Prod. 34(2): 103-107.

Oboh G, Akindahunsi AA (2003). Biochemical changes in cassava products (flour and garri) subjected to *Saccharomyces cerevisiae* solid media fermentation. Food Chem. 52(4): 599-602.

Ohara H, Hiyama K, Yoshida T (1992). Non-competitive product inhibition in lactic acid fermentation from glucose. Appl. Microbiol. Biotech. 36: 773-776.

Okezie BO, Kosikowski FV (1982). Cassava as a food. Crit. Rev. Food Sci. Nutr. 17(3): 259-275.

Peppler HJ (1982). Yeast extract. In fermented foods ed. Rose, A.H. London: Academic press pp. 93-312.

Quinn J, Meyers R (2002). Cowpea: A versatile legume for hot, dry conditions In: "Alternative crop guide". Jefferson Institute pp 4. Singh, B. B. 1987.

Roble ND, Ogbonna JC Tanaka H (2003). L-lactic acid production from raw cassava starch in a circulating loop bioreactor with cells immobilized in loofa (Luffa cylindrical). Biotechnol. Lett. 25(13): 1093 -1098.

Stanbury PF, Whitaker A, Hall SJ (1995). Media for industrial fermentations. In principles of fermentation Technol. Oxford; Pergamon Press pp. 93-121.

Stewart G, Russel I (2002). Biochemistry and genetics of carbohydrates utilization by industrial yeast strains. Pure Appl. Chem. 59: 1493-1500.

Tonukari NJ (2004). Cassava and the future of starch. Electron. J. Biotechnol. 7(1). www.ejbiotechnology.info//content/vol7/issue1/issues/2/.

Watson TG (1976). Amino acid pool composition of *Saccharomyces cerevisiae* as a function of growth rate and amino acid nitrogen source. J. Gen. Microbiol. 96: 263-268.

Zheng S, Yang M, Yang Z. (2005). Biomas production of yeast isolate from salsd oil manufacturing waste water. Bio. Res. Technol. 96: 1183-1187.