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Alcohol yield from various combinations of cassava and sweet potato flours

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The use of cassava and sweet potato separately as raw materials for ethanol production in the recent past has been demonstrated. The search for the optimum processing conditions to hydrolyse and ferment sugars from the starches in cassava and sweet potato is well studied. The effects of substrate, temperature, enzyme types and concentrations, the reaction times of saccharification and fermentation were investigated for effect on ethanol yield. The objective of this work was to combine cassava and sweet potato as raw material to optimise the yield of ethanol for the combination. Selected cassava and sweet potato varieties were cultivated and harvested after 10 and 3 months, respectively. Liquefaction, saccharification and fermentation were carried out with *Liquozyme SC DS,* a combination of *Spirizyme Fuel* and *Viscozyme L* and *Bio-Ferm XR* (Lallemand) yeast, respectively. The combinations of cassava and sweet potato flours yield more ethanol than processing cassava and sweet potato flours separately. The best combination ratio of cassava and sweet potato, 1:1, resulted in the optimal ethanol yield of 16.2% v/v.

Key words: Cassava, sweet potato, optimization, ethanol yield, combination.

INTRODUCTION

Corn, sugar cane and wheat are major crops used globally to produce ethanol (Zabed et al., 2016; Li et al., 2016; Gupta and Verma, 2015, McMurry, 2015; Vollhardt and Schore, 2014; Boundy et al., 2011). Several studies demonstrate the use of cassava and sweet potato as raw materials for ethanol production (Costa et al., 2018;

Martinez et al., 2018; Pereira et al., 2017; Schweinberger et al., 2016; Archibong et al., 2016; Swain et al., 2013; Oyeleke et al., 2012; Ademiluyi and Mepba, 2013; Ocloo and Ayenor, 2010). The search for the optimal processing conditions to hydrolyse and ferment sugars from the starches in cassava and sweet potato was the major

Figure 1. Image of *Apomuden* (sweet potato variety).

Figure 2. Image of *Sika bankye* (cassava variety).

focus of all these studies. The effects of substrate, temperature, enzyme types and concentrations, the reaction times of saccharification and fermentation were all investigated on ethanol yield.

Cassava and sweet potato are good crops for ethanol production because they contain high concentrations of starch, a potential substrate to produce great amounts of ethanol (Ozoegwua et al., 2017; Lareo et al., 2013). Cassava's ability to grow under poor conditions and store its plant tissue underground until needed, makes it a classic "food security crop" (Parmar et al., 2017; Amarachi et al., 2015). The potential of cassava is significant because it offers the cheapest source of food calories and the highest yield per unit area (Duvernay et al., 2013; Lee et al., 2012). It also has multiple roles as a famine reserve, food and cash crop, industrial raw material and livestock feed (Amarachi et al., 2015). According to the Ministry of Food and Agriculture, Ghana (MoFA Statistics Ghana, 2016), 17,213,000 tonnes of cassava was produced in 2015. From this production, 60 to 70% was used to meet subsistence needs leaving a surplus production of 30 to 40%. Much of the excess cassava is either wasted or remains un-harvested, and can be captured for industrial use without any effect on food security (Grow Africa, 2015). This suggests a large opportunity for other uses and industrial growth (Grow Africa, 2015). Ethanol imports into developing countries over the past decade have been quite high. The over seventy (70) million litres of ethanol imported into Ghana for the various industries in 2016 (Ghana Business News, 2017) could have been produced in Ghana using the excess cassava as raw material. This may only be made possible if the cost of ethanol production is competitive compared to the cost of importing ethanol into Ghana. Sweet potato is an attractive raw material for fuel ethanol, since up to 4800 L ethanol per hectare can be obtained (Lareo et al., 2013). Sweet potato has been considered a promising substrate for alcohol fermentation since it has a higher starch yield per unit land than grains (Duvernay et al., 2013; Lee et al., 2012; Srichuwong et al., 2009; Ziska et al., 2009). It has been reported that some industrial sweet potato breeding lines developed could produce ethanol yields of 4500 to 6500 L/ha compared to 2800 to 3800 L/ha for corn (Duvernay et al., 2013; Ziska et al., 2009).

Investigations by Dziedzoave et al. (2010) show that there is significant β-amylase activity in sweet potato which could aid the degradation of starch during mashing to produce simple sugars. The presence of the β-amylase in sweet potato could have potential benefits on ethanol processing. The combination of cassava and sweet potato together with the support of external commercial saccharifying enzymes could have synergic effects on ethanol yield. This study was to establish the effect of combination of cassava and sweet potato on ethanol yield.

MATERIALS

Cassava flour processed from 10 months old *Sika bankye* (cassava variety) and sweet potato flour processed from three months old *Apomuden* (sweet potato variety) were used for ethanol production (Figures 1 and 2). The starch degrading enzymes: *Liquozyme SC DS, Viscozyme L* and *Spirizyme Fuel* were supplied by Novozymes, Denmark. The yeast type used for fermentation was Bio-Ferm XR (unique yeast strain of *Saccharomyces cerevisiae)* produced by Lallemand, Georgia, USA.

METHODS

Processing of *Sika bankye* flour

Ten months old freshly harvested *Sika bankye* was weighed, washed, sliced thinly with peels on, dried immediately at 62°C in a forced air oven dryer for 6 h, cooled and milled with a hammer mill, sieved with a 350 μ mesh sieve (motorized sifter) and starch content determined using Litner's method (see below). The cassava flour sample was subsequently used for ethanol production.

Processing of *Apomuden* flour

Three months old freshly harvested *Apomuden* was weighed, washed, sliced thinly with peels on, dried immediately at 62°C in a forced air oven dryer, cooled and milled, sieved with a 350 μ mesh sieve size and starch content determined. The flour sample was subsequently used for ethanol production.

Production of ethanol from cassava and sweet potato flour samples

Ten months old *Sika bankye* flour and three months old *Apomuden* flour were mixed in the ratios of 7:3, 1:1 and 3:7, respectively. Fifty grams each of the mixtures was mixed with 250 ml of distilled water in an Erlenmeyer flask. The pH of the sample was measured. *Liquozyme SC DS* enzyme (3 ml) was then added to the mixture, stirred gently and heated on water bath (Grant OLS 200) at speed of 100 strokes/min at 85°C for 2 h. The mixture was then cooled to a temperature of 50°C and the pH measured. *Viscozyme L* and *Spirizyme fuel* enzymes (1.5 ml) each was then added and maintained on the water bath for 4 h. The extract of the sample was determined at the end of the 4 h. The sample was then cooled to 34°C and 0.1 g Bio-Ferm XR yeast added to the mixture and fermented for 60 h in an Erlenmeyer flask at 30°C. The brix of the fermented sample was assayed after the 60 h. The alcohol yield by weight after 60 h was determined using the Cutaia et al. (2009) formula: $A_{w/w} = 0.38726 \times (OE - AE) + 0.00307 \times (OE - AE)^2$, where $A_{w/w}$ is Alcohol content by weight, OE is original extract and AE is the apparent extract. The alcohol by volume conversion was done using the Probrewer conversion table. The experiment described was repeated three times for each of the flour mixes (*Sika bankye* flour: *Apomuden* flour; 7:3, 1:1, 3:7) to determine the alcohol yield. This experiment was also carried out using 100% cassava and sweet potato flour samples separately.

Starch content determination (Litner's method)

Five grams of cassava and sweet potato flour samples were triturated with 10 ml of water, and 20 ml hydrochloric acid (sp.gr.1.15) added in small portions. The mixture was washed into a 100 ml flask with hydrochloric acid (12% w/w HCl) and 5 ml of 5% phosphotungstic acid added to precipitate proteins and the volume made up to 100 ml with 12% hydrochloric acid. The mixture was shaken, filtered and the optical rotation of the filtrate was measured in a 200 mm tube. The mean specific rotation of starch was taken as +200.

$$\text{Starch (\%)} = \frac{2000 \times \text{optical rotation}}{\text{Specific rotation}}$$

The experiment was repeated three times for each flour sample.

Moisture content determination

The moisture content of the samples was determined using AOAC (1990) method.

pH determination

The pH of the samples was measured with a Mettler Toledo (Seven Compact pH meter, Mettler Toledo group, Switzerland) pH meter. Ten grams of each flour sample was homogenized in 50 ml of distilled water. The pH of the resulting suspensions was determined using the standardized pH meter. The measurement was repeated three times.

Pasting characteristics determination

The pasting characteristics of the flour sample were determined using Visco-Amylograph (Viscograph-E), Brabender GmbH & Co, KG, Illinois, USA. The moisture content of the flour sample was determined using Sartorius MA 45 (Sartorius AG, Goettingen, Germany) moisture analyzer and the value fed into the software of the Viscograph-E. The software determined the quantities of flour sample and distilled water to mix for the test. The sample was then weighed and poured in distilled water, mixed well to form a consistent slurry with no lumps. The sample was transferred into the reaction chamber of the Viscograph-E machine and the head of the lever carefully lowered into the sample. The machine was run to analyse the sample. The data generated at the end of the analysis was copied and saved from which the gelatinisation temperature was identified.

RESULTS AND DISCUSSION

Physico-chemical properties

The physico-chemical properties of *Sika bankye* and *Apomuden* studied are presented in Table 1. *Sika bankye* and *Apomuden* were selected for the study because they are improved cassava and sweet potato varieties in Ghana that have higher starch and lower fresh root moisture content compared to the other cassava variety (*Ampong*) and sweet potato variety (*Tuskiki*) used and analysed in earlier study (Komlaga, 2018). Ethanol yield from a starchy raw material is largely dependent on the starch content and dry matter of the raw material (Li et al., 2016; Ademiluyi and Mepba, 2013; Teerawanichpan et al., 2008). The gelatinisation temperatures of *Sika bankye* and *Apomuden* ranged between 69 and 72°C, respectively. Gelatinisation of starch is a thermal point at which the intermolecular bonds of starch molecules are broken down in the presence of water and heat. The process irreversibly dissolves the starch granule in water. Gelatinisation improves the availability of starch for amylase hydrolysis. The gelatinisation temperatures observed fall far below the optimum temperature (85°C) of the *Liquozyme SC DS* enzyme used for dextrinization in this study. This was an assurance that all the starch present in the cassava samples gelatinised so as to be

Table 1. Physico-chemical properties of *Sika banye* and *Apomuden*.

Sample	Parameter			
	Starch content (% on dry weight basis)	Moisture content (% fresh weight basis)	Gelatinisation temperature (°C)	pH
Sika Bankye	65.8	58.5	69.0	5.9
Apomuden	78.0	68.0	72.0	5.5

Table 2. Mean alcohol yields from *Sika bankye* and *Apomuden* composite flour samples.

Sample	Limit attenuation (%)	Alcohol yield (%/$_{v/v}$)
Sika bankye:*Apomuden* flour mix (7:3)	83.9 ± 0.3	15.5a* ± 0.2
Sika bankye:*Apomuden* flour mix (1:1)	84.7 ± 0.2	16.2b ± 0.1
Sika bankye:*Apomuden* flour mix (3:7)	84.5 ± 0.2	15.9b ± 0.3
Sika bankye flour	82.5 ± 0.3	14.9c ± 0.1
Apomuden flour	83.5 ± 0.3	15.2d ± 0.1

*Means in the same column with different letters (a-d) are significantly different ($p<0.05$).

broken down into short chain carbohydrates for subsequent hydrolysis by saccharifying enzymes. The pH values for *Sika bankye* and *Apomuden* are 5.9 and 5.5, respectively. The pH values observed were ideal for the dextrinization, saccharification and fermentation of the samples. This is because the enzymes and yeast used in the study have their optimum pH values between 5 and 6. There was no need therefore to adjust the pH of the medium which could have economic implication on the production of the ethanol.

Ethanol yields from *Sika bankye* and *Apomuden*

Results of limit attenuation and corresponding mean alcohol yields from different combinations and individually processed *Sika bankye* and *Apomuden* flours are shown in Table 2. The attenuation of the samples ranged from 82.5 to 84.7% which are all higher than the recommended figure of 80.0% (Kunze, 2004). Degree of fermentation is an important indicator of brewery production and profitability, since it determines the yield of alcohol in the brewing process as well as malt fermentation efficiency. Limit of attenuation of wort is a relatively simple and efficient method for the determination of fermentable extract. Limit attenuation is an indication of how many fermentable sugars in a wort that has been converted into alcohol and carbon dioxide using yeast procedure. The greater the attenuation, the more sugar that has been converted into alcohol (Krstanovic et al., 2019). The alcohol values observed in the study ranged between 14.9 and 16.2% v/v. Cutzu and Bardi (2017), Ocloo and Ayernor (2010) and Begea et al. (2010) reported maximum ethanol yields of 10.22, 8.3

and 15.18% v/v, respectively from cassava and agro waste bio-fermentation. The flour mixture from 10 months old *Sika bankye* and 3 months old *Apomuden* in the ratio of 1:1, respectively resulted in the highest ethanol yield of 16.2% v/v. Analysis of variance (ANOVA) on the results at 95% confidence level using Minitab version 17.1 software showed significant differences among four out of the five samples analysed. This means that the different cassava and sweet potato samples evaluated yielded different ethanol values when they were treated with the same processing conditions. The results also indicated that ethanol yields were higher when *Sika bankye* and *Apomuden* flour combinations were processed together compared to processing *Sika bankye* and *Apomuden* separately. The relative higher ethanol values obtained for processing the various flour mixtures of *Sika bankye* and *Apomuden* could be due to the chemical composition and possible biochemical synergies between the two flours during processing. The starch content of the sweet potato variety studied is significantly higher than the cassava variety studied. The higher ethanol yields from the individually processed *Apomuden* and mixtures of *Sika bankye* and *Apomuden* is due to the relatively higher starch content of the *Apomuden*. It was reported that there is significant β-amylase activity in sweet potato which could aid the degradation of starch during mashing to produce simple sugars (Dziedzoave et al., 2010). This fact could also be a contributing factor to the higher ethanol values recorded for processing *Apomuden* individually and the mixtures of *Sika bankye* and *Apomuden* compared to processing *Sika bankye* separately. The relatively higher ethanol yields produced from the combination of the two flours could be exploited to process the 30 to 40% production surplus of cassava

to cut down the importation of ethanol which indirectly saves foreign exchange for developing countries (Grow Africa, 2015).

Conclusion

The results from the study indicate that combinations of cassava and sweet potato varieties for ethanol production yield more ethanol than processing cassava and sweet potato individually. The best combination ratio of cassava and sweet potato for optimum ethanol yield from the results of this study is 1:1 with a yield of 16.2% v/v ethanol.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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