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Effect of harvest age of cassava roots and sweet potato tubers on alcohol yield

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Several studies have been conducted in the past using cassava and sweet potato as feedstock to optimise the yield of alcohol. Harvest age of cassava and sweet potato may have some effects on the fermentable carbohydrates quantity. This study aims to establish the best harvest age of cassava and sweet potato for alcohol production. Two varieties of cassava (*Sika bankye* and *Ampong*) cultivated and harvested at 8, 10 and 12 months and two sweet potato varieties (*Apomuden* and *Tuskiki*) harvested at 3, 4 and 5 months were used for the study. Starch hydrolysis was performed with two sets of enzymes followed by fermentation with Bio-Ferm XR (Lallemand) yeast. The nutrients in *Sika bankye* were generally higher than in *Ampong*, except for ash. *Sika bankye* had the highest alcohol yield (14.8% v/v) between the two cassava varieties, with the best harvest age of cassava for ethanol production being 10 months. *Apomuden* had relatively higher nutrients than *Tuskiki* at all levels of growth except for fat. *Apomuden* had the highest alcohol yield (15.7% v/v) between the two sweet potato varieties with 3 months being the economical harvest age of sweet potato for ethanol production.

Key words: Cassava, sweet potato, harvest age, saccharification, fermentation, alcohol yield.

INTRODUCTION

Cassava and sweet potato contain high concentrations of starch which could be converted into ethanol (Ozoegwua et al., 2017; Lareo et al., 2013). Several investigations in the recent past confirmed the potential of cassava and sweet potato as feedstock 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). The major crops that are usually used globally for ethanol production are corn, sugar cane and wheat (Zabed et al., 2016; Li et al., 2016; Gupta and Verma, 2015; McMurry, 2015; Vollhardt and Schore, 2014; Boundy et al., 2011).

Fresh cassava roots contain about 30% starch (Amarachi et al., 2015) and 1l of ethanol could be produced from 5-6 kg of fresh roots (containing 30% starch) and 3 kg of cassava chips (14% moisture content).

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It is also reported that cassava and sweet potato have higher starch yield per unit area than grains (Duvernay et al., 2013; Lee et al., 2012; Srichuwong et al., 2009; Ziska et al., 2009). Cassava can be grown under relatively poor agronomic conditions; therefore, cassava is a "food security crop" (Parmar et al., 2017; Amarachi et al., 2015). Sweet potato is an excellent feedstock for ethanol production (Lareo et al., 2013). Industrial sweet potato could produce 4500–6500 l/ha of ethanol compared to 2800–3800 l/ha for corn (Duvernay et al., 2013; Ziska et al., 2009).

Importation and use of ethanol in developing countries such as Ghana in recent times has been on the rise. In 2016 for instance, a total of over seventy million litres of ethanol was imported into Ghana for various industrial uses (Ghana Business News, 2017), this quantity could have been produced using cassava as raw material. The Ministry of Food and Agriculture, Ghana (MoFA Statistics Ghana, 2016), reported 17,213,000 tonnes of fresh cassava production by Ghana in 2015. A surplus of 30 to 40% production figure was reported from the above production figure in 2016, which could be utilised in industries as raw materials for other products, without adverse effects on food security (Grow Africa, 2015).

The amount of fermentable carbohydrates available in cassava root and sweet potato tuber depend on the variety and growth conditions of the crops (Teerawanichpan et al., 2008). The harvest age of the crops could therefore have some effects on the fermentable carbohydrates, which could have direct relation with the amount of alcohol produced. The study was to establish the best harvest age of cassava and sweet potato for alcohol production.

MATERIALS

Flour processed from two varieties of cassava (*Sika bankye* and *Ampong*) cultivated at Caltech Ventures Ltd farms, Ho, in the Volta region, Ghana and sweet potato flour processed from two varieties of sweet potato (*Apomuden* and *Tuskiki*) cultivated at Mantsi, in Greater Accra region, were used for the study. The cassava roots were harvested at 8, 10 and 12 months and the sweet potato was harvested at 3, 4 and 5 months and processed into flour for the study. *Liquozyme SC DS, Viscozyme L and Spirizyme Fuel* enzymes were provided by Novozymes, Denmark. Bio-Ferm XR yeast (unique yeast strain of *Saccharomyces cerevisiae)* produced by Lallemand, Georgia, USA was used for fermentation.

METHODS

Processing of cassava (Sika bankye and Ampong) and sweet potato (Apomuden and Tuskiki) flours

The 8, 10 and 12 month old lots of *Sika bankye* and *Ampong* (freshly harvested) and the 3, 4 and 5 month old lots of freshly harvested *Apomuden* and *Tuskiki* were weighed, washed, sliced thinly (average of 2 mm thick) with peels on, steam blanched for 10 m, dried at 62°C in an oven for 6 h, after which it was cooled and milled through a sieve with 350µ mesh size. The flour samples were subsequently used for ethanol production. Amount of starch in the flour was determined using Litner's method, proximate analysis carried out, visco-amylograph analysis and other physicochemical properties determined.

Ethanol production from cassava and sweet potato flour

The alcohol content of 50 g each of the cassava (8, 10 and 12 months) and sweet potato (3, 4 and 5 months) flour samples processed was determined using the method described by Komlaga et al. (2021), followed by alcohol yield by weight calculation using the Cutaia et al. (2009) formula: $A_{w/w} = 0.38726^* (OE - AE) + 0.00307^* (OE - AE)^2$. 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 (Probrewer, 2018).

Determination of Starch content A (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.

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

The experiment was performed in triplicates.

Crude fibre determination

Three grams of cassava and sweet potato flour samples was weighed and transferred into Erlenmeyer flask. Petroleum ether (15 ml) was added, the solution stirred, allowed to settle for 15 min and decanted. The washing procedure was repeated three times. The extracted sample was air dried and transferred into a 1000 ml conical flask. 200 ml H_2SO_4 (0.1275M) was added and the solution brought to boiling for 30 min by heating. The boiling sample was poured into a prepared Buckner funnel after allowing it to stand for 1 min. Insoluble matter was washed with hot water until the washing was free from acid. The insoluble matter was washed back into the 1000 ml conical flask with 200 ml of 0.313M NaOH solution by means of a wash bottle. The solution was boiled for 30 min, allowed to stand for 1 min and filtered through a No. 1 filter paper. The insoluble material was transferred to the filter paper with boiling water. The material was washed with 1% HCl and finally with boiling water until free from acid. The sample was washed twice with 20 ml ethanol and 3 times with 20 ml of petroleum ether. The insoluble material was then transferred to a dry pre-weighed filter paper, dried at 100°C to constant weight. The filter paper and contents was incinerated in the Thermconcept furnace, (Thermconcept GmbH, Bremen, Germany), at 550°C for 8 h to ash. The weight of the ash was subtracted from the increase of weight on the filter paper due to insoluble material and the difference reported as crude fibre.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of insoluble material} - \text{Weight of ash}}{\text{Weight of sample}} \text{ x } 100$$

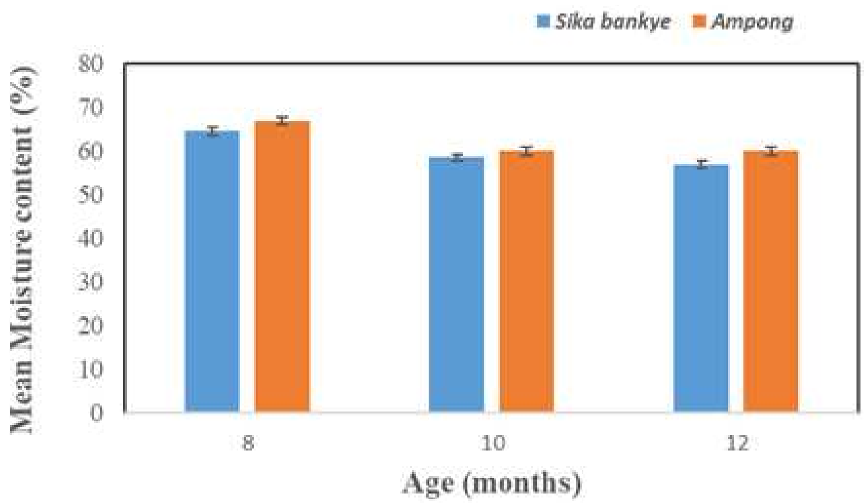

Figure 1. Mean moisture contents of *Sika bankye* and *Ampong* varieties.

Water absorption capacity determination

Water absorption capacity of the cassava and sweet potato flour samples was determined based on a modification of the centrifugation method of American Association for Clinical Chemistry methods (AACC, 1989). 2g of the flour sample was mixed with 20 ml distilled water. Samples were then allowed to stand at 30°C for 30 min, then centrifuged at 3500 rpm for 30 min. The reduction in the volume of the supernatant in a graduated cylinder was noted and recorded as water absorption capacity. Means of triplicate determinations were recorded. Water absorption capacity (%) = $V_1 - V_2$, where V_1 is the initial level (volume) of supernatant and V_2 is the final level (volume) of supernatant.

Swelling power determination

Swelling power was determined using the method described by Afoakwa et al. (2012). 1 g of flour was transferred into a weighed graduated centrifuge tube (50 ml). Deionized water was added to give a total volume of 40 ml. The sample in the centrifuge tube was heated at 85°C in a thermostatically controlled temperature water bath (Grant OLS 200, Keison products, Chelmsford, UK) for 30 min with constant shaking (80 strokes/min). The tube was then taken out, wiped dry on the outside and cooled to room temperature. It was centrifuged (Hermle Z 206 A, Hermle Labortechnik GmbH, Germany) for 15 min at 2200 rpm. The swelling power was determined by evaporating the supernatant in a hot-air oven (Gallenkamp Oven, England, UK) and weighing the sediment paste and supernatant residue. The swelling power was then calculated using the formula:

$$\text{Swelling power} = \left(\frac{\text{Weight of precipitated paste}}{\text{Weight of sample}}\right) - \text{Weight of residue in supernatant}$$

Proximate analysis

Moisture and protein contents were determined according to Helrich (1990), while ash and fat contents were determined according to Horwitz (2000) methods.

pH determination

The pH of the samples was determined by homogenizing 10g of flour samples in 50 ml of distilled water, after which the pH of the resulting mixture was measured with a Mettler Toledo (Seven Compact pH meter, Mettler Toledo group, Switzerland) pH meter. The experiment was performed in triplicates.

Pasting characteristics determination

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

Data analysis

Analysis of variance (ANOVA) was carried out on the ethanol yields from the samples using Minitab version 17.1 (Kutner et al., 2005).

RESULTS AND DISCUSSION

Moisture content

The moisture contents of the fresh cassava and sweet potato varieties studied are represented in Figures 1 and 3, respectively. The moisture content ranged from 57 to

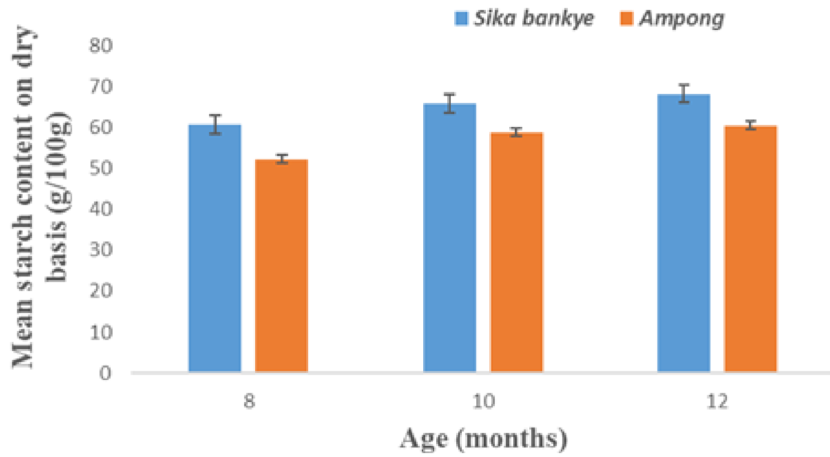

Figure 2. Mean starch contents of *Sika bankye* and *Ampong* varieties.

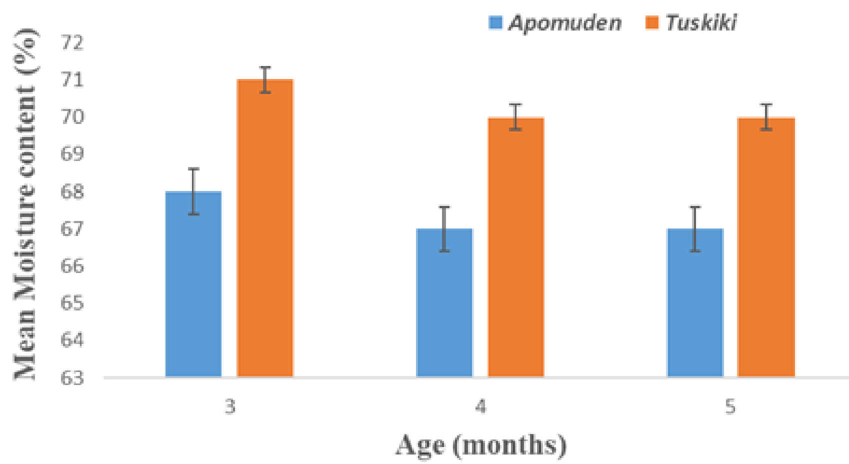

Figure 3. Mean moisture contents of *Apomuden* and *Tuskiki* varieties.

67% for cassava varieties and 67 to 71% for sweet potato varieties for the three growth stages. The moisture content of the cassava roots was comparable with the 68.1% value reported by Amarachi et al. (2015). The moisture content of *Ampong* roots were significantly higher ($p < 0.05$) compared to *Sika bankye* variety for all three growth levels. The amount of moisture is related to dry matter content of root crops. The higher the moisture content, the lower the dry matter content. It therefore implied that, *Sika bankye* variety has relatively higher dry matter content than *Ampong* variety at the same harvest age. It was also observed from the two varieties that, the more matured the cassava roots, the less moisture it has. The cassava roots were harvested in June, August and October with highest root moisture content recorded in June and the least moisture documented in October. There was much rain in June at the time of the 8 month harvest than in August and October in the location of the cultivation. The moisture levels in the soil at the time of harvest could make the roots absorb more water which could lead to higher moisture content in the 8 months matured roots than the 10 and 12 months matured roots. The moisture content of the fresh *Tuskiki* variety was significantly higher compared to *Apomuden* variety for all three harvesting stages. It implied that, *Apomuden* variety has relatively higher dry matter content than *Tuskiki* variety at same maturity levels.

Starch content

Figures 2 and 4 represent the starch contents of the cassava and sweet potato varieties studied. The starch content of the cassava varieties ranged between 52% and 69% and that for sweet potato ranged between 78 and 90% on dry basis. It was observed that the starch

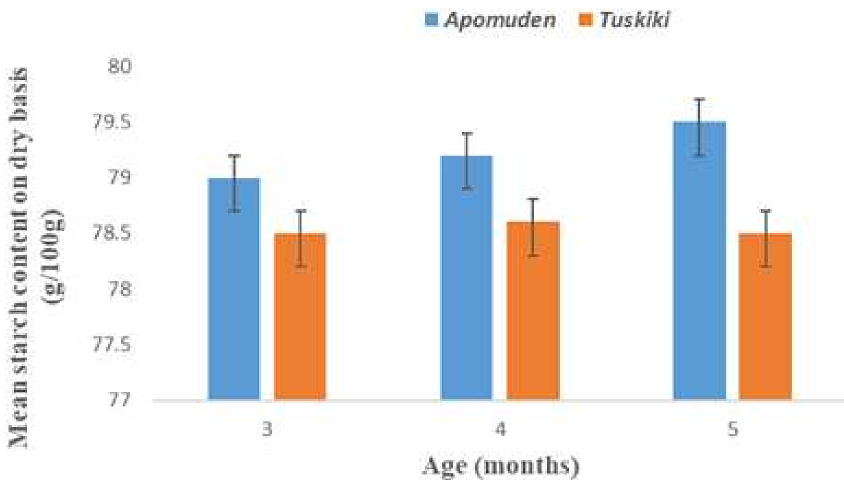

Figure 4. Mean starch contents of *Apomuden* and *Tuskiki* varieties.

Table 1. Mean proximate composition of 8, 10 and 12 months old *Sika bankye* and *Ampong* varieties (N=3).

Nutrient (g/100 g)	***Sika bankye* variety**			***Ampong* variety**		
	8 months	**10 months**	**12 months**	**8 months**	**10 months**	**12 months**
Ash	2.37 ± .01	2.39 ± .01	2.42 ± .08	2.99 ± .02	2.99 ± .02	3.05 ± .06
Protein	1.67 ± .08	1.69 ± .10	1.70 ± .90	1.62 ± .06	1.63 ± .09	1.64 ± .08
Fat	0.70 ± .00	0.73 ± .02	0.73 ± .00	0.54 ± .00	0.55 ± .01	0.55 ± .00
Crude fiber	4.22 ± .08	4.54 ± .03	4.67 ± .04	2.32 ± .06	2.36 ± .05	2.57 ± .05
Total carbohydrates	91.01±.17	90.66± .16	90.47± .17	92.53± .26	92.48 ± .32	92.20± .34

content of *Sika bankye* variety was significantly higher at all levels of growth than in the *Ampong* variety. The starch content of *Apomuden* variety was significantly higher at all levels of maturity than in the *Tuskiki* variety. Ethanol yield from a starchy raw material is largely dependent on the starch content and dry matter of the raw material (Li et al., 2015; Ademiluyi and Mepba, 2013). Teerawanichpan et al. (2008) reported that the amount of hydrolysable carbohydrates available in cassava root and sweet potato tuber depended on the variety and growth conditions of the crops. *Sika bankye* variety is a better variety for ethanol production compared to *Ampong* variety based on the fact that it has higher dry matter and higher starch contents. Likewise, for the sweet potato varieties, *Apomuden* is a better variety for ethanol production compared to *Tuskiki* variety.

Proximate composition

The proximate composition of the cassava and sweet potato varieties studied are presented in Tables 1 and 2, respectively. The nutrients (ash, fat, protein, carbohydrates and crude fibre) are generally higher in *Sika bankye* than in *Ampong* variety except for ash. The nutrients determined (ash, fat, protein, carbohydrates, crude fibre) in the sweet potato varieties were generally higher in *Apomuden* than in *Tuskiki* variety, except for fat content. The fat content of *Tuskiki* variety was relatively higher for all levels of growth than that of *Apomuden* variety (Table 2). Nutrients in Wort during brewing (fermentation) are essential to how well the sugar is fermented into ethanol (Kunze, 2004). The yeast cells need amino acids to build proteins and new cells, they need vitamins and minerals to make enzymes work-well and they need phosphorous to create new DNA.

Nitrogen is a key factor in determining the ethanol yield in brewing (Agu et al., 2009). Nitrogen is approximately 10% of the dry weight of yeast cells. Since the nutrients are relatively higher in *Sika bankye* than in *Ampong* variety and higher in *Apomuden* than in *Tuskiki* variety, especially that of protein, it suggested that *Sika bankye* and *Apomuden* could supply, to a large extent, the needed nutrients to yeast cells during fermentation than *Ampong* and *Tuskiki*. *Sika bankye* and *Apomuden* varieties could therefore be the best varieties in terms of nutrients supply for ethanol production than *Ampong* and *Tuskiki* varieties.

Table 2. Mean proximate composition of 3, 4 and 5 months old *Apomuden* and *Tuskiki* varieties (N=3).

Nutrient (g/100 g)	*Apomuden* variety			*Tuskiki* variety		
	8 months	10 months	12 months	8 months	10 months	12 months
Ash	0.60 ± .00	0.61 ± .01	0.62 ± .01	0.56 ± .01	0.56 ± .01	0.56 ± .01
Protein	1.11 ± .02	1.12 ± .02	1.16 ± .03	1.07 ± .03	1.13 ± .02	1.14 ± .04
Fat	0.45 ± .01	0.47 ± .00	0.46 ± .00	0.70 ± .01	0.81 ± .00	0.80 ± .00
Crude fiber	0.10 ± .01	0.14 ± .00	0.14 ± .02	0.11 ± .01	0.11 ± .01	0.12 ± .00
Total carbohydrates	97.60± .30	97.66± .06	97.61± .03	97.56± .08	97.40± .02	97.39± .06

Table 3. Mean physico-chemical properties of cassava and sweet potato varieties.

Sample	Gelatinisation temperature (°C)	pH	Swelling power	Water absorption capacity
Sika bankye	$68.8 \pm 0.3^{a*}$	5.8 ± 0.2^{c}	9.4 ± 0.3^{d}	0.5 ± 0.1^{f}
Ampong	69.1 ± 0.3^{a}	6.0 ± 0.0^{c}	10.0 ± 0.3^{d}	0.4 ± 0.1^{f}
Apomuden	72.2 ± 0.6^{b}	5.8 ± 0.0^{c}	5.8 ± 0.0^{e}	3.1 ± 0.1^{g}
Tuskiki	72.3 ± 0.6^{b}	5.8 ± 0.2^{c}	5.6 ± 0.1^{e}	2.9 ± 0.2^{g}

*Means in the same column with different letters are significantly different (p<0.05)

Physico-chemical properties

The physico-chemical properties of the cassava and sweet potato varieties studied are presented in Table 3. The gelatinisation temperatures of the cassava varieties ranged between 68 and 70°C and that of the sweet potato varieties ranged between 72 and 73°C. Gelatinisation irreversibly dissolves starch granules in water in presence of heat, by breaking the intermolecular bonds of the starch molecules. The process improved the availability of starch molecules for hydrolysis by amylases. The gelatinisation temperatures observed for the cassava and sweet potato varieties were far below the optimum temperature (85°C) of the Liquozyme SC DS enzyme used for dextrinization in this study. The starch molecules in the cassava and sweet potato varieties would therefore gelatinise before the optimum temperature of the liquozyme SC DC enzyme is attained. This would ensure that all the starch molecules present in solution would be broken down into short chain carbohydrates for subsequent hydrolysis by saccharifying enzymes. The pH values recorded during the study for cassava and sweet potato varieties were between 5.5 and 6.1. The pH values obtained in the study are conducive for the hydrolysis and fermentation of the samples, since all the enzymes and the yeast used have their optimum pH values between 5 and 6. The adjustment of pH of the reaction medium, with economic implications for the ethanol production, is therefore not necessary.

The swelling capacities of the cassava varieties studied ranged from 9.4 to 10.0, with *Sika bankye* generally having relatively low swelling capacity compared to *Ampong* variety. The swelling capacity of the sweet potato varieties were 5.8 and 5.6 for *Apomuden* and *Tuskiki*, respectively. Swelling capacity is a measure of the ability of starch to imbibe water and expand in volume at a particular temperature (Amarachi et al., 2015). Low swelling capacity of flour suggested that the starch granules have strong binding force and low amylose content. Low-amylose starch has an excellent functionality of easy digestibility when compared with high-amylose starch (Amarachi et al., 2015). In addition, low swelling power in cassava flour is a clear indication of restricted starch which shows a high resistance to breaking during cooking. Since the cassava and sweet potato varieties studied have relatively low swelling capacities, they could all be digested easily, hence ideal for ethanol production. The water absorption capacity of the cassava and sweet potato varieties ranged between 0.3 and 3.2. *Sika bankye* variety had relatively higher water absorption capacity than *Ampong*, and *Apomuden* had relatively higher water absorption capacity than *Tuskiki* variety.

Water absorption capacity is the ability to take up and retain water either by adsorption or absorption. It is influenced by the extent of starch disintegration. Low water absorption capacity could be attributed to the protein content in a product because protein has been reported to limit the ability of water uptake in food (Amarachi et al., 2015). There were no significant differences between the water absorption capacities of *Sika bankye* and *Ampong* and between *Apomuden* and *Tuskiki* varieties.

Ethanol yield

Results of limit attenuation and corresponding mean

Table 4. Mean alcohol yields of cassava and sweet potato varieties.

Cassava/sweet potato variety	Limit attenuation (%)	Alcohol yield ($\%/_{v/v}$)	Limit attenuation (%)	Alcohol yield ($\%/_{v/v}$)	Limit attenuation (%)	Alcohol yield ($\%/_{v/v}$)
	8 months old		**10 months old**		**12 months old**	
Sika	81.3 ± 0.5	13.3^{a*} ± 0.2	81.9 ± 0.2	14.8^{b} ± 0.4	81.3 ± 0.3	14.7^{b} ± 0.1
Ampong	80.3 ± 0.5	11.5^{c} ± 0.2	80.5 ± 0.6	12.8^{d} ± 0.2	81.3 ± 0.3	12.7^{d} ± 0.1
	3 months old		**4 months old**		**5 months old**	
Apomuden	85.5 ± 0.3	15.2^{e} ± 0.2	82.7 ± 0.4	15.1^{e} ± 0.1	83.7 ± 0.2	15.1^{e} ± 0.1
Tuskiki	82.4 ± 1.0	14.8^{e} ± 0.3	81.9 ± 0.2	14.9^{e} ± 0.2	81.9 ± 0.2	14.8^{e} ± 0.1

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

alcohol yields from the cassava and sweet potato varieties studied are presented in Table 4. The attenuation of the samples ranged between 80 and 86%, which are comparable to other previous studies (Kunze, 2004). Attenuation refers to the conversion of fermentable sugars in a Wort into alcohol and carbon dioxide by yeast during fermentation. 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 from 11.5 to 15.2% v/v. The results obtained are comparable with that of other studies (Cutzu and Bardi, 2017; Ocloo and Ayernor, 2010; Begea et al., 2010). Flour processed from 10 months old Sika bankye produced the highest alcohol (14.8% v/v) among the two varieties of cassava studied, while 3 months old *Apomuden* flour produced the highest alcohol (15.2% v/v) among the two varieties of sweet potato studied. The ethanol content of *Sika bankye* is higher and significantly different ($p < 0.05$) compared to those of *Ampong* variety of same level of growth (Table 4). This is attributed to the higher dry matter, starch content and other nutrients like protein and fat which are relatively higher in *Sika bankye* than *Ampong* variety (Table 1 and Figure 2). There was no significant difference between the ethanol yield of cassava samples of 10 and 12 months old. There is therefore no economic value, according to the findings, to keep cassava roots on the field after 10 months if they are meant for processing alcohol.

The economic harvest age of cassava meant for ethanol production is therefore 10 months. The starch contents of the sweet potato varieties studied are significantly higher than the cassava varieties studied. This reflected in higher ethanol yields in the sweet potato than cassava varieties. Apart from the higher starch content and other nutrients, the higher ethanol yields from the sweet potato varieties could also be attributed to significant β-amylase activity in sweet potato, which could aid the degradation of starch during mashing to produce simple sugars (Dziedzoave et al., 2010). There was no significant difference between the ethanol yield of sweet potato samples of 3, 4 and 5 months old. There is no economic value, according to the findings, to keep sweet potato on the field after 3 months if they are meant for ethanol production. The economic harvest age of sweet potato meant for ethanol production is therefore 3 months. The ethanol yields from the 10 months old *Sika bankye* and 3 months old *Apomuden* flours could be exploited to process the 30 to 40% surplus cassava (documented in Ghana in 2016) into ethanol 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 nutrients in *Sika bankye* variety at the same harvest age are generally higher than in *Ampong* variety, except for ash. *Sika bankye* variety has more dry matter and higher starch content at the same harvest age which resulted in higher ethanol yield than *Ampong* variety. *Sika bankye* variety had highest ethanol yield (14.8% v/v) between the two cassava varieties at 10 months. The best harvest age of cassava for ethanol production is 10 months. *Apomuden* variety has relatively higher nutrients than *Tuskiki* variety at all levels of maturity except for fat. *Apomuden* variety has more starch and produced much ethanol than *Tuskiki* variety at the same harvest age. *Apomuden* variety had the highest ethanol yield (15.7% v/v) between the two sweet potato varieties at age of 3 months. The best economical harvest age for ethanol production from sweet potato is 3 months.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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