Bio-Ethanol Production from Non-Food Parts of Cassava (*Manihot esculenta* Crantz)

Ephraim Nuwamanya, Linley Chiwona-Karlton,
Robert S. Kawuki, Yona Baguma

Received: 23 February 2011 / Revised: 27 July 2011 / Accepted: 5 August 2011 / Published online: 11 October 2011

Abstract Global climate issues and a looming energy crisis put agriculture under pressure in Sub-Saharan Africa. Climate adaptation measures must entail sustainable development benefits, and growing crops for food as well as energy may be a solution, removing people from hunger and poverty without compromising the environment. The present study investigated the feasibility of using non-food parts of cassava for energy production and the promising results revealed that at least 28% of peels and stems comprise dry matter, and 10 g feedstock yields >8.5 g sugar, which in turn produced >60% ethanol, with pH $\approx$ 2.85, 74–84% light transmittance and a conductivity of 368 mV, indicating a potential use of cassava feedstock for ethanol production. Thus, harnessing cassava for food as well as ethanol production is deemed feasible. Such a system would, however, require supportive policies to acquire a balance between food security and fuel.

Keywords Cassava feedstock · Food security · Energy production · Bio-ethanol

INTRODUCTION

Bio-fuels (carbohydrate based or oil based; Chandel et al. 2007) are increasingly recognized as important forms of renewable energy (Londo et al. 2010). Bio-ethanol is produced by hydrolysis and fermentation of carbohydrate feedstocks. This ethanol may be used as a fuel as is, or in a mixture with fossil fuels, using various proportions. Bio-diesel is produced from oil plants like *Jatropha curcas* where the oil is blended with diesel to produce fuel (Davies 2006). Bio-fuels (especially second-generation bio-fuels) hold the promise of sustainable and environmentally friendly energy forms (Chandel et al. 2007) and consequently, production

has increased by 8% from the levels in 2005 to 33 thousand million liters in 2009. It has been established that production of bio-ethanol and other domestic forms of energy is economically viable and feasible with available technologies (Londo et al. 2010; Tillman et al. 2009). Crops grown for energy production purposes may be sugar cane (Hall et al. 2009), cassava, corn, and sweet potato (Ziska et al. 2009) as well as other high sugar and high biomass producing crops and nontraditional food or cash crops. In addition, municipal and agricultural wastes may be used for energy production (Rist et al. 2009). However, the choice of feedstock is based on availability, competition between food and non-food products, and cost (Londo et al. 2010), thus making the search for an optimal feedstock of uttermost importance. The high productivity and yield of cassava (Ziska et al. 2009), along with its ability to grow on marginal soils (Dixon et al. 2002), requiring a minimum of labor (Chiwona-Karlton et al. 1998) and management costs (Jansson et al. 2009), have placed it among the candidates for bio-ethanol production.

In countries such as Uganda, cassava is at present predominantly used for food and production of cassava remains low in terms of yield per hectare compared to its potential (FAO 2005–2008). The volume presently produced may not meet the demand of ethanol production as well as reducing food insecurity in situations of food deficit. This calls for exploitation of alternative forms of feedstocks. In terms of cassava, the above ground biomass, including stem and leaf residues, is often not utilized for economical purposes (Ahamefule 2005), apart from being a source of planting material (Pattiya et al. 2007) and the unintended use as fertilizer (Fermont et al. 2008). Root residues, especially peels, which are poisonous due to high levels of cyanogenic glycosides (Guo et al. 2008; Pattiya et al. 2007), may be exploited for energy production taking into account their role in nutrient recycling (Fermont et al. 2008). Sustainability may be

achieved if energy production is linked to food production and if energy production is harmonized with the livelihoods of people. Livelihood diversification would require the understanding of society dynamics in terms of domestic energy consumption as well as investigating possible ways of producing energy from available resources (Amigun et al. 2008). Non-food parts of the cassava may play a very significant role in the production of energy since they produce relatively high amounts of biomass, are easily hydrolysable and have a high content of dry matter (Kosugi et al. 2009). Furthermore, starch extraction industries produce lignitic and cellulosic material that may be used for generating ethanol (Akpan et al. 2004). Using cellulosic and lignocellulosic material as feedstocks in production of bio-ethanol is as efficient as starch-based feedstocks and is important since the green house gas emission from this bio-ethanol is reduced by up to 80% compared to the 40–60% of the first generation bio-fuels (Londo et al. 2009). The aim of the present study was to explore the feasibility of using non-food parts of cassava for bio-fuel production. The inexpensive nature of cassava stems and peel biomass as well as their abundance, create an opportunity for cassava exploitation in the production of bio-ethanol.

MATERIALS AND METHODS

Feedstocks

The cassava variety TMS 30572 was selected for the study. It is characterized by high yield (44 ton ha⁻¹) and above ground biomass as well as a high content of dry matter (~40%). The leaves and roots of this variety hold high levels of cyanogenic glycosides (HCN-equivalents ~800 mg kg⁻¹, dry weight) compared to other common Ugandan cassava varieties. The different plant parts (stem, leaves, root, and root peels) were collected separately, 5 kg of each, in triplicate. The material was collected uniformly from individual plants to avoid tissue differences, immediately stored at 4°C and transferred to the laboratory.

Treatment of Feedstocks

Stem and peel samples were chopped, washed, and dried at room temperature for 3 h before manually crushing them with a hammer, allowing the resulting particles to pass through a sieve (size 5 mm). Samples were put into glass bottles prior to hydrolysis. Leaf and root samples were washed, dried for 3 h and chopped into fine particles (<2 cm diameter).

Compositional Analyses of Feedstocks

The treated samples were analyzed for dry matter, ash, lipid, and protein content as well as for starch. Dry matter

content (DMC) was determined by placing the samples in an air-forced oven at 105°C until a constant weight was attained, after which the percentage of dry matter was calculated. The ash content was determined by burning the air-dried samples at 550°C for 8 h in a furnace. The samples were cooled in a desiccator and the percentage ash was calculated. Total organic content of the feedstocks was calculated as the difference in weight between the ash content of the feedstock and the dry matter content. Total protein content of the samples was analyzed using the Bradford method (Bradford 1976) and the sample preparation procedures included analyzing the colored compounds in the stem, peel, and leaf matter, measuring the absorbance of untreated samples prior to the protein test. Starch content of the feedstocks was determined in triplicates (100 g wet weight) after enzymatic hydrolysis, using an enzyme solution comprising amylase (3000 IU ml⁻¹, BDH Laboratories) and amyloglycosidase (60 IU ml⁻¹, SIGMA, Aldrich). The liberated amount of glucose was quantified as described by Dubois et al. (1956). Lipid content of the samples was determined by means of extraction, using a hexane chloroform mixture (1:1, volume ratio), after which the weight loss of the samples was estimated and the percentage lipid content was calculated (Nuwamanya et al. 2010).

Hydrolyses of Samples

Stem, leaf, root, and peel samples were hydrolyzed using the following hydrolysis methods. Acid and alkaline hydrolyses were carried out on triplicate samples of matter (stem, leaf, peel, and root separately), 200 g each in 200 ml solution, 1 M HCl for the acid hydrolysis, and 1 M NaOH for the alkaline hydrolysis. From each solution, samples for analyses of reducing sugar content were taken on an hourly basis for 8 h and thereafter daily for 5 days. Enzymatic hydrolyses were carried out on triplicate samples of 200 g wet weight, using a combination of different enzymes; amyloglycosidases (60 IU ml⁻¹, SIGMA, Aldrich), amylase (3000 IU ml⁻¹, BDH Laboratories) and cellulases (75 IU ml⁻¹, SIGMA, Aldrich) in a 200-ml solution. The amount of the different sugars liberated by the enzymes was noted along with the environmental conditions under which the liberation occurred. Samples for analyses of the content of reducing sugars were taken from each solution on an hourly basis for 8 h, and thereafter daily for 5 days.

Estimation of Reducing Sugars

The method of Dubois et al. (1956) was used to estimate the total amount of reducing sugars produced during hydrolysis.

Microorganism Preparation and Sugar Fermentation

The glucose-fermenting microorganism *Saccharomyces cerevisiae* was used. Cells were grown on 1% (wv⁻¹) yeast extract, 2% (wv⁻¹) peptone, and 2% (wv⁻¹) glucose and mixed with 20% (ww⁻¹) glycerol and subsequently stored in vials at −20°C.

Sugar Fermentation and Estimation of Fermentation Efficiency

After successive saccharification procedures, the mash was cooled to room temperature (~27°C), keeping the pH at 4.5–5.0. A yeast extract was then added to allow fermentation. Inoculum was produced by inoculating a 1000 ml shake flask (500 ml culture volume constituted from the hydrolysis mixtures above) with 7.5 ml of frozen yeast cells. The contents were subsequently transferred to a 2-l shake flask (1 l culture volume) and consequently used for the fermentation, which was carried on for 6 days with regular mixing of the mash (Sassner et al. 2006). Fermentation efficiency was assessed by measuring the decrease of the amount of reducing sugars in the fermenting solution (beer) and was performed hourly for 8 h and thereafter daily for 6 days.

Ethanol Recovery and Ethanol Evaluation

Ethanol was separated from the beer (500 ml) using a three-phase distillation procedure in which the first distillation was carried out at a temperature range of 20–94°C to recover the first distillate. The distillate was then redistilled at 90°C twice consecutively, to produce ethanol (60–65%) for further evaluation. The amount of ethanol produced from each 500 ml batch of the beer, was used to calculate the ethanol yield. To ascertain the quality of the ethanol produced, various procedures were utilized as described below.

Visual measurements based on the visible properties of ethanol were used to evaluate the color and aspect of ethanol at different processing stages and concentrations according to National Response Team (NRT) quick reference guide (www.nrt.org/production/NRT/NRTWeb.nsf).

Ethanol clarity was analyzed by transferring samples of the produced ethanol to a cuvette and measuring transmittance of light at 650 nm in a spectrophotometer, using double distilled, deionized water and 95% ethanol as standards. The level of acidity of the processed ethanol was assessed by titration with NaOH (aq.) (Titratable acidity), using phenolphthalein as indicator (Fabro et al. 2006). Titratable acidity was expressed as the total volume of 0.1 M NaOH (aq.) used to neutralize the existing acid. The pH-value of the produced ethanol was appraised using a pH-meter. The conductivity of ethanol was measured with a

conductivity meter, with a cell constant equal to 0.1 cm⁻¹. Presence of sulfate and chloride in the produced ethanol was assessed by means of precipitation analysis. A 0.1-M barium chloride solution was added drop wise (up to 0.5 ml) to 0.5 ml ethanol to study possible formation of barium sulfate, a salt insoluble in water solutions. Similarly, presence of chloride ions was studied by drop wise adding up to 0.5 ml of silver nitrate solution to a 0.5-ml ethanol sample and the possible precipitate of solid silver chloride were studied.

To determine lipid content, dried samples were extracted using a hexane chloroform mixture (1:1, volume ratio), after which the weight loss of the samples was estimated and the percentage of lipid content was calculated (Nuwamanya et al. 2010).

The percentage of ethanol in the azeotropic mixture was calculated as below.

$$(a) * (sga) + (v - a) * (sgb) = m;$$

i.e. $a/v = (m - v * sgb) / (sga - sgb) / v$, where $a/v = \%$ ethanol, a is the volume of ethanol, $(v - a) = b =$ volume of water, $sga = 0.789$ is the specific gravity of the ethanol, $sgb = 1$ = the specific gravity of water, m = the mass of mixture, v = the volume of mixture.

Statistical Analysis

The Microsoft Excel software was used for descriptive statistics of the feedstocks used. Composition of untreated feedstocks was defined as the average of at least three independent samples originating from the same lot of material, while in the treated feedstocks, two samples of the test solution were used to estimate the composition of reducing sugars. Further statistics involved the analysis of variance as well as correlation and regression analyses, using the GENSTAT Discovery Edition 3 software. All tests were performed at 5% level of significance. ANOVA was used to test the difference between the means of different parameters for feedstock composition as well as the properties of ethanol produced from different feedstocks. After ANOVA, the least significant differences were used to test the difference in means of quantitative ethanol properties. Correlation analysis highlighted relationships between the different ethanol properties and the composition of feedstocks. Covariance was used to test the effect of other factors on the resulting properties of the ethanol.

RESULTS

Composition

The amount of dry matter varied among different plant parts with dry matter content of peels (30.5%) and stems

(28.77%) comparable to that of roots (38.60%). High levels of protein were observed in above ground parts with leaves having the highest levels (12.3%) and the roots exhibiting the lowest (0.38%). The content of ash was considerably lower in leaves and roots (0.33 and 0.27%, respectively) compared to peels and stems. Lipid contents were comparable in dried peels (0.39%) and leaves (0.36%) and somewhat lower in stems and roots (<0.3%). Composition of plant bio-molecules did not follow a particular pattern in the plants and varied in most instances.

Production of Reducing Sugars

Hydrolysis of feedstocks, using acid, alkali as well as enzymes, resulted in production of various amounts of reducing sugars (glucose + C_n-sugars, where *n* = 3, 4, and 5, and other reducing saccharides), as illustrated in Fig. 1. Analysis of variance revealed significant differences in the total reducing sugars produced from the different plant parts (see Table 3). During initial hydrolysis, stems and peels produced the highest amount of

Fig. 1 Comparison of the amount of reducing sugars (RS) expressed as g hydrolyzed feedstock per 5 g raw feedstock, produced during the first 6 h of hydrolysis of the different plant parts, using acid, alkali

and enzymes. Bars display the levels of reducing sugars measured at different points of time. **a** 1st hour, **b** 2nd hour, **c** 3rd hour, **d** 4th hour, **e** 5th hour of hydrolysis, **f** 6th hour

Fig. 2 Comparison of the amount of reducing sugars produced after hydrolysis with enzymes (Cellulase + (amyloglycosidase and amy-lase), acid (HCl) or alkali (NaOH) after 5 days. *RS* Reducing sugars, given as gram total amount hydrolyzed per 5 g of the feedstock

reducing sugars compared to roots and leaves, the latter producing the least amount. In roots, the use of cellulase alone resulted in degradation of only cellulosic material, not affecting the starchy material. In leaves, hydrolysis with sodium hydroxide and hydrochloric acid produced larger amounts of reducing sugars than enzymes (Fig. 1). In all plant parts, the enzymes required more preparative stages and time before reducing sugars were produced (Fig. 2). The amount of reducing sugars produced from the leaves did not differ significantly between the different methods of hydrolysis. Hydrolysis continued after 8 h for 5 days, accompanied by a corresponding increase of reducing sugar levels, as illustrated by Fig. 2. After 5 days, the enzyme hydrolysis yielded almost the same amount of reducing sugars as acid and alkaline-hydrolyzed feedstocks (Fig. 2). Percentage relative increase in total reducing sugars ranged between 37% for the peels and 74.8% for the leaves compared to reference material (roots), suggesting that roots are more susceptible to hydrolysis compared to other plant parts.

Fermentation Efficiency

Fermentation efficiency was expressed as the amount of reducing sugars present in the fermenting solution at 1 h intervals for 8 h and thereafter at 24 h intervals for 6 days. No significant decrease in reducing sugar content was observed during the first 8 h of fermentation, after which a progressive decrease with time was observed (Table 1). After 24 h, the sugar content in the fermentation broth was significantly decreased ($P < 0.001$), while the number of yeast cells in the broth had increased. Fermentation efficiency varied between the different parts of the feedstocks, with peels utilizing only 78.5% of the sugars. Stems showed the most efficient fermentation; 90.41% of the sugars were utilized, leaving less than 10% unutilized. The decline in the total amount of reducing sugars from 0 to 5 days in the different plant parts is illustrated in Table 1.

Ethanol Yield and Quality

The ethanol produced after 6 days of fermentation was of a very low concentration, the first distillate held a concentration of ~11%. Three successive distillations increased the yield to a maximum concentration of ca. 60% of the azeotropic mixture with percentages of ethanol produced ranging from 59.5% acquired from the leaves to 61.4% from the roots. Properties of the ethanol produced are presented in Table 2. The total volume of ethanol produced from the different parts differed, with leaves yielding the lowest amount. The content of crude protein and ash affected the purity and clarity of the ethanol. The parts of the cassava plant with high protein and ash contents produced ethanol with a yellowish color. At low concentrations, (10–20%), the ethanol was partly hazy and cloudy, while clear and slightly yellowish to colorless at approximately 60%. The final ethanol extract was clear and its clarity was comparable to that of distilled water and commercial 96% ethanol (reference solutions), with an average light transmittance of 77%. The pH-levels of the ethanol produced did not show a large variation between the plant parts (roots and peels 2.87 and stems 2.82). No

Table 1 Reduction in total reducing sugars as fermentation progresses in different parts of the cassava plant

Part	Day 0 ^a	Day 1	Day 2	Day 3	Day 4	Day 5	LSD (5%)	CV %	RSU (%)
Roots	2.647	1.161	0.961	0.511	0.494	0.294	0.024	1.0	88.89
Peels	1.655	1.037	0.667	0.654	0.591	0.356	0.017	0.8	78.49
Stems	1.022	0.617	0.311	0.274	0.238	0.098	0.013	1.3	90.41
Leaves	0.667	0.605	0.442	0.148	0.103	0.075	0.005	0.6	88.76

^a Reducing sugar concentration is the total amount of reducing sugars immediately after preparation of the fermentation broth. *LSD* least significant difference, *CV* coefficient of variation, *RSU* percentage reducing sugar utilized after 5 days

Table 2 Characteristics of ~60% ethanol produced from different plant parts

Part	% EtOH	EtOH yield ^a (ml)	pH (25°C)	TA ^b (ml)	Color	Clarity (%)	Chlorides (±)	Sulfates (±)	Conductivity (mV)
Roots	61.4	55.8	2.87	0.5	Colorless	84.4	–	–	368.1
Peels	60.2	43.5	2.87	0.6	Colorless–Yellowish	74.7	–	–	371.1
Stems	59.8	52.4	2.82	0.5	Yellowish	74.6	–	–	368.9
Leaves	59.5	11.3	2.86	0.7	Yellowish	74.2	–	–	368.5
LSD	0.68	0.677	0.19	0.09	n/a	9.19	n/a	n/a	1.08
CV	0.6	9.0	2.3	14.2	n/a	4.3	n/a	n/a	14.2

^a Ethanol yield in ml/500 ml of original crude mixture of sugars. ^b Titratable acidity (TA) expressed as the volume of 0.1 M NaOH used to neutralize total acidity on titration of 2 ml ~60% ethanol. CV is in % and LSD is given for 5%

Table 3 Efficiency of ethanol production from the different parts of the cassava plant, compared to roots

Part	% EtOH	EtOH Yield (ml)	pH	TA (ml)	Clarity (%)	Conductivity (mV)	Total RS (units)	FE (%)
Roots ^a	61.4	55.8	2.87	0.5	84.40	368.10	2.647	88.89
Peels	60.2 (–1.95)	43.5 (–22.04)	2.87 (0.0)	0.6 (20.0)	74.70 (–11.49)	371.10 (0.82)	1.655 (–37.48)	78.49 (–11.7)
Stems	59.8 (–2.61)	52.4 (–6.09)	2.82 (–1.74)	0.5 (0.0)	74.60 (–11.61)	368.90 (0.217)	1.022 (–61.39)	90.41 (1.71)
Leaves	59.5 (–3.09)	11.30 (–79.74)	2.86 (–0.348)	0.7 (40.0)	74.20 (–12.09)	368.50 (0.11)	0.667 (–74.80)	88.76 (0.15)
Mean	60.23 (–2.55)	39.83 (–35.96)	2.85 (–0.69)	0.6 (20.0)	74.50 (–11.73)	369.15 (0.382)	1.498 (–57.89)	86.64 (–3.28)
<i>P</i> value (5%)	0.002	<0.001	0.849 ^b	0.121 ^b	0.095 ^b	0.005	<0.001	<0.001

% EtOH Ethanol concentration, TA titratable acidity, RS reducing sugar values, FE fermentation efficiency. ^a Values of the different properties of ethanol from roots were used for comparison; values in parentheses represent percentage of root values (used as denominator). ^b Differences not significant at 5%

precipitation was formed when adding barium (Ba²⁺) or silver (Ag⁺) ions, indicating no presence of chloride or sulfate. The conductivity of ethanol produced from different plant parts was >350 (mV). There were significant differences in ethanol yield (ml 500 l^{–1} of beer) as well as the ethanol percentage obtained, between the different plant parts used as feedstocks (see Table 3 for statistics).

DISCUSSION

The high content of dry matter observed in the different parts of cassava plants may be hydrolyzed into fermentable sugars. This has a bearing on the final yield of reducing sugars, since high contents of dry matter are desirable in ethanol production (Ziska et al. 2009). The variations in protein content between the different plant parts of the present study, leaves showing high levels of protein compared to other parts of the plants, were notable since a significant relationship between ethanol properties and total protein content in different feedstocks existed. High protein content resulted in production of lower quantities of ethanol and vice versa, suggesting that cassava, compared to highly proteinaceous cereal feedstocks (Wang et al.

2008), holds a potential of becoming an important feedstock for producing high quality ethanol, since it has low levels of micronutrients even in its roots (Nuwamanya et al. 2010). Compared to the composition of other important ethanol feedstocks like corn (Marshall and Sugg 2009; Agbogbo and Wenger 2007) and sugar cane (Hayes 1982), the cassava plant is favorable for production of ethanol of right quality and quantity (Ziska et al. 2009).

The low amount of reducing sugars obtained from roots during the initial hours of hydrolysis may be attributed to the preparation procedure since they were not crushed but chopped, further confirming the importance of the preparative steps for hydrolysis of the different feedstocks (Ziska et al. 2009; Mosier et al. 2005). Obtaining high initial levels of reducing sugars in peels and stems compared to roots, along with the stringent preparative procedures required, illustrates the difficulty of using cellulosic feedstocks for ethanol production. The observed differences in the amount of reducing sugars produced from roots and other plant parts, further confirms this fact. Thus, the choice of feedstocks should encompass the time and effort required as well as the cost of preparation and hydrolysis (Yoonan and Kongkiattikajorn 2005), which has a bearing on the fermentation as well as the final product

(Kristensen et al. 2008). The ability to produce sufficient amount of reducing sugars determines the importance of a particular feedstock for ethanol production (Hayes 1982; Agbogbo and Wenger 2007). Feedstocks with ability to produce high amounts of glucose using simple hydrolysis procedures are important alternatives for biomass fuel production (Londo et al. 2010). All the factors mentioned above will in the end determine the final cost of the ethanol produced (Marshall and Sugg 2009; Kristensen et al. 2008). The physiological state of the feedstock and the environmental conditions under which the feedstocks are grown, are also important factors to consider. In the present study, cassava was grown up to 12 months prior to 5 g being extracted from the different parts.

The comparison of the different hydrolysis methods revealed that acid and alkaline hydrolyses yielded higher amounts of reducing sugars than the enzymatic hydrolysis, suggesting that these two methods may be employed (Ziska et al. 2009). The use of enzymatic hydrolysis, on the other hand, is a slow but secure way of obtaining glucose for fermentation. It is important to note that enzymes are specific to particular substrates and give specific products, allowing employment of specific fermentation procedures and/or organisms (Patle and Lal 2009). Cellulases digest cellulose into glucose while amyloglycosidases and amylases digest existing starch in the different feedstocks. The ability to concentrate starch after cellulosic hydrolysis stresses the importance of complementation of enzymes when hydrolyzing different feedstocks, as each enzyme targets a particular substrate (Toran-Diaz et al. 2009). The low amount of reducing sugar obtained from leaves may be attributed to the low amount of dry matter associated with leaves as well as the low amounts of total carbohydrates coupled to a significant portion of proteinaceous and lipid matter in leaves (Ballesteros et al. 2000).

Fermentation efficiency depended on the ability of the yeast to utilize particular feedstocks based on their characteristics and compositional differences. The absence of differences in fermentation efficiency during the first 8 h was due to the yeast establishing itself in the fermenting solution, growing to a certain colony volume able to utilize the existing sugars. The variations in fermentation efficiency may be attributed to the type of sugars produced as well as substrate preferences by the fermenting organism, since different parts of the cassava plant produce different sugar types apart from glucose (Brauman et al. 1996). Different compositional characteristics of feedstocks affect their hydrolysis, consequently affecting the type of reducing sugars produced and hence moderating the type of metabolism carried out by yeast under such circumstances (Van Dijken et al. 1993). Other factors to consider are the different levels of cyanogenic glycosides and proteins in different parts of the cassava plants, which may have

affected establishment of yeast in the solution. These compounds affect the growth of yeast in fermenting broth depending on whether they are metabolized by yeast or not, which directly affects growth and metabolism of yeast (Boonnop et al. 2009; Klinker et al. 2004). In particular, lignocellulosic materials in hydrolyzed leaves, stems, and peels may result in production of small molecular weight compounds such as furan derivatives, phenolic compounds, and amine-based compounds such as vanillin, all inhibiting fermentation (Endo et al. 2008). The low ethanol percentage obtained with progressive fermentation of sugar is most likely due to the fact that yeast is affected by high concentrations of ethanol in the solution, which may inhibit its metabolism and hence reduce its efficiency (Adesanya et al. 2008).

The low volume of ethanol produced from leaves was due to low sugar contents obtained after hydrolysis of leaves, which in turn was attributed to low content of dry matter and the carbohydrate composition of this feedstock as shown in Fig. 1. The improvement of color and other aspects of the produced ethanol were most probably due to repeated distillations, removing colored and volatile compounds, thus increasing purity (Gryta et al. 2000). The differences observed in total titratable acidity were probably due to differences in feedstock composition. Earlier studies have showed that break down products of different feedstocks differ (Yu et al. 2007) and their effects on the fermentation broth may result in differences in its titratable acidity. Chloride and sulfate ions are the major impurities of crop-derived ethanol. These ions may reduce the quality of the ethanol, making it undesirable to apply in motor engines and other appliances (de Oliveira et al. 2009). Absence of chloride and sulfate ions in the ethanol produced in the present study, suggest high purity (Teixeira et al. 2009), thus making cassava highly competitive.

The protein and lipid contents are important components of dry matter, but their effect on ethanol volume and percentage seems to be linked to their inability to produce relevant sugars for ethanol production, thereby impinging on the overall outcome of sugar fermentation (Veljickovic et al. 2005). The presence of ash and lipids negatively affects the utilization of sugar, which consequently influences the fermentation efficiency of different feedstocks (Wu et al. 2006). These compounds appear to be important in determining the pH (positive correlation between pH and lipid contents) and conductivity of ethanol (positive correlations between ash and conductivity), thus having an impact on yeast growth regimes in the fermentation broth (Klinker et al. 2004). This most likely concerns the fermenting organisms, which are negatively affected by high contents of ash and lipids. The ash, lipid, and protein contents of feedstocks negatively affected the clarity and total volume of ethanol produced, but had no discernable

effect on its quality. Interestingly, ethanol clarity was positively correlated with the volumes of ethanol produced. Thus, feedstocks producing high quantities of ethanol also had ethanol of high clarity. The production of ethanol is affected by different parameters, mainly depending on the feedstock as well as the processing conditions.

CONCLUSIONS

The present study has demonstrated a potential for exploitation of cellulosic and starch ethanol from cassava non-food parts. Taking into account that the simultaneous need for energy and food need to be met without compromising the environment, they provide a viable option, holding great potential for acquiring a sustainable system. The use of cassava ethanol as a second-generation bio-fuel provides a starting-point for improvements in cultivation and adoption of cassava as well as improving food security. In addition, this may mitigate the human effects on climate change by producing efficient, clean, and renewable energy.

To achieve a sustainable food and energy system, it is important that investments be made in developing, improving, and making technologies available for feedstock preparation, hydrolysis, and fermentation. Comprehensive guiding policies on exploitation of the bio-fuel sector are urgently needed for this purpose. Policies would ensure the use of both edible and nonedible components of the plant rather than a competition between food and energy, thus facilitating dual plant utilization. In particular, seed availability in communities where the stems provide the bulk of cassava seeds, care should be taken not to generate a shortage of adequate planting material. This is particularly crucial in cassava-farming communities with poor households, mainly comprising women and children.

Acknowledgments The present study was financed by SIDA through the BIO-EARN program. We thank Dr Anton Bua of the Cassava Program, National Crops Resources Research Institute (NaCRRI) for providing the test material and associated logistics. The assistance of members of the NaCRRI biosciences laboratory, especially the biochemistry section, is gratefully acknowledged.

REFERENCES

Adesanya, O., K. Oluyemi, S. Josiah, R. Adesanya, L. Shittu, D. Ofusori, M. Bankole, and G. Babalola. 2008. Ethanol production by *Saccharomyces Cerevisiae* from Cassava Peel Hydrolysate. *Internet Journal of Microbiology* 5: 1.

Agbogbo, F., and K. Wenger. 2007. Production of ethanol from corn stover hemicellulose-hydrolysate using *Pichiastipis*. *Journal of Industrial Microbiology & Biotechnology* 34: 723–727.

Ahamefule, F.O. 2005. Evaluation of pigeon pea-cassava peel based diets for goat production in South-Eastern Nigeria. Ph.D. Thesis, Michael Okpara University of Agriculture, Umudike.

Akpan, I., N. Uraih, C.O. Obuekwe, and M.J. Ikenebomeh. 2004. Production of ethanol from cassava whey. *Acta Biotechnologica* 8: 39–45.

Amigun, B., R. Sigamoney, and H. von Blottnitz. 2008. Commercialisation of biofuel industry in Africa: A review. *Renewable and Sustainable Energy Reviews* 12: 690–711.

Ballesteros, I., J.M. Oliva, A. Navarro, J. Carrasco, and M. Ballesteros. 2000. Effect of chip size on steam explosion pretreatment of softwood. *Applied Biochemistry and Biotechnology* 84–86: 97–110.

Boonnop, K., M. Wanapat, N. Nontaso, and S. Wanapat. 2009. Enriching nutritive value of cassava root by yeast fermentation. *Scientia Agricola (Piracicaba, Braz.)* 66: 616–620.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.

Brauman, A.S., S. Ke'le'ke, M. Malonga, E. Miambi, and F. Ampe. 1996. Microbiological and biochemical characterization of Cassava Retting a traditional lactic acid fermentation for Foo-Foo (Cassava flour). *Production Applied and Environmental Microbiology* 62: 2854–2858.

Chandel, A., E. Chan, R. Rudravaram, L. Narasu, V. Rao, and P. Ravindra. 2007. Economics and environmental impact of bio-ethanol production technologies: An appraisal. *Biotechnology and Molecular Biology Review* 2(1): 014–032.

Chiwona-Karlton, L., J. Mkumbira, J. Saka, M. Bovin, N.M. Mahungu, and H. Rosling. 1998. The importance of being bitter—a qualitative study on cassava cultivar preference in Malawi. *Ecology of Food and Nutrition* 37(3): 219–245.

de Oliveira, M.F., A.A. Saczk, L.L. Okumura, and N.R. Stradiotto. 2009. Analytical methods employed at quality control of fuel ethanol. *Energy Fuels* 23: 4852–4859.

Dixon, A.G.O., J.M. Ngeve, and E.N. Nukene. 2002. Response of Cassava genotypes to four biotic constraints in three agro-ecologies of Nigeria. *African Crop Science Journal* 10: 11–21.

Dubois, M., K. Gilles, K. Hamilton, A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350–356.

Endo, A., T. Nakamura, A. Ando, K. Tokuyasu, and J. Shima. 2008. Genome-wide screening of the genes required for tolerance to Vanillin, which is a potential inhibitor of bio-ethanol fermentation, in *Saccharomyces cerevisiae*. *Biotechnology for Biofuels* 1: 3.

Fabro, M.A., H.V. Milanesio, L.M. Robert, J.L. Speranza, M. Murphy, G. Rodríguez, and R. Castañeda. 2006. Determination of acidity in whole raw milk. *Journal of Dairy Science* 89: 859–861.

FAO. 2005–2008. FAOSTAT statistics database-agriculture production statistics. Rome, Italy: FAO. <http://faostat.fao.org>. Retrieved 25 June 2010.

Fermont, A.M., J.A. van Asten, and K.E. Giller. 2008. Increasing land pressure in East Africa: The changing role of cassava and consequences for sustainability of farming systems. *Agriculture, Ecosystems & Environment* 128: 239–250.

Gryta, M., A. Waldemar, and Tomaszewska M. Morawski. 2000. Ethanol production in membrane distillation bioreactor. *Catalysis Today* 56: 159–165.

Guo, A., B. Webb, M. Miles, M. Zimmerman, K. Kendler, and Z. Zhao. 2008. ERGR: An ethanol related gene resource. *Nucleic Acid Research* 37(1): D840–D845.

Hall, J., S. Matos, L. Severino, and N. Beltrão. 2009. Brazilian biofuels and social exclusion: Established and concentrated ethanol versus emerging and dispersed biodiesel. *Journal of Cleaner Production* 17(Suppl. 1): S77–S85.

- Hayes, F.W. (1982). Production of ethanol from sugar cane (435/161 ed.). England. <http://www.freepatentsonline.com/4326036.html>. Retrieved 4 Oct 2011.
- Klinke, H.B., A.B. Thomsen, and B.K. Ahring. 2004. Inhibition of ethanol producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology* 66: 10–26.
- Kosugi, A., A. Kondo, M. Ueda, Y. Murata, P. Vaithanomsat, W. Thanapase, T. Arai, and T. Mori. 2009. Production of ethanol from cassava pulp via fermentation with a surface-engineered yeast strain displaying glucoamylase. *Renewable Energy* 34: 1354–1358.
- Kristensen, J., L. Thygesen, C. Felby, H. Jørgensen, and T. Elder. 2008. Cell-wall structural changes in wheat straw pre-treated for bioethanol production. *Biotechnology for Biofuels* 1: 29–32.
- Londo, M., E. Deurwaarder, G. Fischer, S. Prieler, H. van Velthuisen, M. de Wit, A. Faaij, et al. 2010. A roadmap for biofuels in Europe. *Biomass and Bioenergy* 34: 244–250.
- Marshall, L., and Z. Sugg. 2009. Corn stover for ethanol production, WRI policy notes, January 2009. No. 4: 1–10. www.csbp.org.
- Mosier, N., C. Wyman, B.E. Dale, R. Elander, Y.Y. Lee, M. Holtzapfel, and M. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Biore-source Technology* 96: 673–686.
- Nuwamanya, E., Y. Baguma, N. Emmambux, J. Taylor, and P. Rubaihayo. 2010. Physicochemical and functional characteristics of cassava starch in Ugandan varieties and their progenies. *Journal of Plant Breeding and Crop Science* 2: 001–011.
- Patle, S., and B. Lal. 2009. Ethanol production from hydrolyzed agricultural wastes using mixed culture of *Zymomonas mobilis* and *Candida tropicalis*. *Biotechnology Letters* 29: 1839–1843.
- Pattiya, A., J. Titiloye, and A. Bridgewater. 2007. Fast pyrolysis of agricultural residues from cassava plantations for bio-oil production. *Asian Journal on Energy and Environment* 08: 496–502.
- Rist, L., J. Ser, H. Lee, and L. Pin Koh. 2009. Biofuels: Social benefits. *Science* 326: 1344–a.
- Sassner, P., M. Galbe, and G. Zacchi. 2006. Bioethanol production based on simultaneous saccharification and fermentation of steam-pretreated *Salix* at high dry-matter content. *Enzyme and Microbial Technology* 39: 756–762.
- Teixeira, L., T. Chaves, P. Guimarães, L. Pontes, and J. Teixeira. 2009. Indirect determination of chloride and sulfate ions in ethanol fuel by X-ray fluorescence after a precipitation procedure. *Analytica Chimica Acta* 640: 29–32.
- Tillman, D., R. Socolow, J.A. Foley, D. Hill, E. Larson, L. Lynd, S. Pacala, J. Reilly, T. Searchinger, C. Somerville, and R. Williams. 2009. Beneficial biofuels, the food, energy and environment trilemma. *Science* 325: 270–271.
- Toran-Diaz, I., V.K. Jain, J.-J. Allais, and J. Baratti. 2009. Effect of acid or enzymatic hydrolysis on ethanol production by *Zymomonas mobilis* growing on Jerusalem artichoke juice. *Biotechnology Letters* 17: 527–530.
- Van Dijken, J.P., R.A. Westhuis, and J.T. Pronk. 1993. Kinetics of growth and sugar consumption in yeasts. *Antonie Van Leeuwenhoek* 63: 343–352.
- Van Hoek, P., J. Van Dijken, and J. Pronk. 1998. Effect of specific growth rate on fermentative capacity of baker's yeast. *Applied Environmental Microbiology* 64: 4226–4233.
- Wang, D., S. Bean, J. McLaren, P. Seib, Y. Shi, M. Lenz, X. Wu, and R. Zhao. 2008. Grain sorghum is a viable feedstock for ethanol production. *Journal of Industrial Microbiology Biotechnology* 35: 313–320.
- Wu, X., R. Zhao, D. Wang, S. Bean, P. Seib, M. Tuinstra, M. Campbell, and A. O'Brien. 2006. Effects of amylose, corn protein and corn fiber contents on production of ethanol from starch rich media. *Cereal Chemistry* 83: 569–575.
- Yoonan, K., and J. Kongkiattikajorn. 2005. A study of optimal conditions for reducing sugars production from cassava peels by diluted acid and enzymes. *Kasetsart Journal of Natural Science* 38: 29–35.
- Yu, J., X. Zhang, and T. Tan. 2007. A novel immobilization method of *Saccharomyces cerevisiae* to sorghum bagasse for ethanol production. *Journal of Biotechnology* 129: 415–420.
- Ziska, L., G. Runion, M. Tomecek, S. Prior, A. Torbet, and R. Sicher. 2009. An evaluation of cassava, sweet potato and field corn as potential carbohydrate sources for bio-ethanol production in Alabama and Maryland. *Biomass and Bioenergy* 33: 1503–1508.

AUTHOR BIOGRAPHIES

Ephraim Nuwamanya is a research scientist and provides laboratory technical support at the National Crops Resources Research Institute (NaCRRI), Uganda. His interests include carbohydrate chemistry, nanotechnology, biofuels, and plant physiology.

Address: National Agricultural Research organization, National Crops Resources Research Institute (NaCRRI), P.O. Box 7084, Kampala, Uganda.

Linley Chiwona-Karlton (✉) is a senior lecturer and research fellow at The Swedish University of Agricultural Sciences (SLU). Her expertise and publications focus on food security, food toxicology, and public health as well as natural resource management.

Address: Department of Urban and Rural Development, Swedish University of Agricultural Sciences, Box 7012, 750 07 Uppsala, Sweden.

e-mail: Linley.karlton@slu.se

Robert S. Kawuki is a cassava breeder based at the National Crops resources Research Institute, Uganda. He is keenly interested in biofuels and starch-related research.

Address: National Agricultural Research organization, National Crops Resources Research Institute (NaCRRI), P.O. Box 7084, Kampala, Uganda.

Yona Baguma (✉) is a principal researcher with the National Agricultural Research Organization (NARO), Uganda. His expertise lies within the field of crop physiology, plant biotechnology, molecular cell biology, and genomics.

Address: National Agricultural Research organization, National Crops Resources Research Institute (NaCRRI), P.O. Box 7084, Kampala, Uganda.

e-mail: bgmyn@yahoo.co.uk