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Effect of hydrolysis reaction time on the reducing sugar yield of Tacca (*Tacca involucrata*) and Tigernut (*Cyperus esculentus*) starch for Bioethanol production

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ABSTRACT

The effect of hydrolysis reaction time on the reducing sugar yield of Tacca (Tacca involucrata) and Tigernut (Cyperus esculentus) starch for Bioethanol production was studied. The starches were extracted from the feedstocks by wet and dry milling methods. They were gelatinized at slurry concentrations of 1.0, 2.0, 3.0 and 4.0 ml/g for Tacca and slurry concentrations of 3.5, 4.0, 4.5 and 5.0 ml/g for Tigernut. The starch content of the feedstocks determined the quantity of water used. They were hydrolyzed using malted barley as enzyme at enzyme concentrations of 0.1g/g to 0.3g/g at durations ranging from 1½ - 3½ h and even 4h. The results showed that for the two starches studied, none achieved highest reducing sugar yield at 1½ - 2½ h. The optimum fell largely between 3 to 3½ h. Overall results indicate that reducing sugar quantity is largely dependent on slurry concentration (ml/g) of starch, hydrolysis reaction time and even temperature.

Keywords: Hydrolysis reaction time, enzyme concentration, reducing sugar yield, bioethanol production, tacca, tigernut.

INTRODUCTION

Bioethanol is an environmentally friendly fuel for vehicles that normally run on petrol. As a renewable source of energy, it reduces demand on fossil fuels as a viable alternative while it burns more cleanly and with reduced emissions of CO_2 - a greenhouse gas. Bioethanol produced from renewable biomass such as starch, sugar or lignocellulosic materials, is believed to be one of these alternatives to non-renewable fossil fuels. It is expected to be one of the dominating renewable biofuels in the transport sector within the twenty years to come [1]. The hydrolysis of starch may be considered as a key step in the processing of starch-based feedstock for bioethanol production. The main role of this step is to effectively provide the conversion of two major starch polymer components: amylose, a mostly linear α-D-(1-4)-glucan and branched amylopectin, an α-D-(1-4)-glucan, which has α-D-(1-6) linkages at the branch points, to fermentable sugars that could subsequently be converted to ethanol by yeasts or bacteria. The hydrolysis may be performed by acids, an older process which is now mainly abandoned and replaced by more efficient enzymatic process. The starch-based bioethanol industry has been commercially viable for about 30 years; in that time, tremendous improvements have been made in enzyme efficiency, reducing process costs and time, and increasing hydrolysis and bioethanol yields [2]. *Cyperus esculentus* (also known as Chufa sedge, yellow nut sedge, Tigernut, Sedge, Earth almond, is a species of sedge native to warm temperate to subtropical regions of the Northern Hemisphere [3]. Zohary and Hopf [3] reported that this tuber ranks among the oldest cultivated plants in ancient

Egypt. Presently, they are cultivated mainly at least for extended and common commercial purposes in Spain. The nuts are also grown in Ghana, Nigeria, Burkina Faso and Mali. Since the tubers contain 20 – 36% oil, *Cyperus esculentus* has been suggested as a potential crop for the production of biodiesel [4, 5]. The nut was reported to be rich in sucrose (17.4 – 20.0%), fat (25.50%), protein (8%) [6]. One of the secondary and waste products of *Cyperus esculentus* is starch. Tigernut tubers are potentially a rich source of starch which may be extracted after the oil has been removed from the tubers. Chufa tubers can be used for the production of alcohol by fermentation. In Sicily, a cultivar with very high sucrose content is grown and used commercially for this purpose [7]. No reported research work has been cited so far on the use of the starch from *Cyperus esculentus* for bioethanol production. Most of the research focus have been on its application as a food and the oil for biodiesel production [8,4,5]. Since the tuber is rich in sucrose and has up to 38% starch, it is not widely consumed and does not constitute a staple food in most regions of Nigeria; it would be a very good supplement for existing bioethanol feedstock. Tacca also known with the common names such as East Indian arrow root, Fiji arrow root, Indian arrow root, Polynesian arrow root, Tacca, Tabiti arrow root, Williams arrow root has the botanical names *Tacca leontopetaloides*(L.) Kuntze. Syn. T. pinnatifida Forst, T. *involucrata schum* and Thonn. It is from the family of Taccaceae. It is also incorporated into the family of Dioscoreaceae (the yam family) [9]. The root tuber of *Tacca involucrata* has been known to contain large amount of starch as it is used locally as food in a variety of ways. Its subsidiary use includes; the wild plants are regarded as a famine food in parts of West Africa. The demand for East Indian arrow root (*Tacca involucrata*), has never been high and there seems no prospect of its expansion in the future. *Tacca involucrata* is another potential feedstock for bioethanol production. The crop is hardly cultivated (grows in the wild). It is consumed widely as a food material, hence its subsidiary uses as a famine food. Because the crop falls into the group of highly underutilized crops, information on the crop is very minimal and highly outdated. A few research works have been reported on the use of this crop mainly for food and pharmaceutical purposes [10,11,12]. No reported work has been cited so far on the use of tacca specie for bioethanol production. More interest is currently being focused as the possibilities of exploiting the vast numbers of less familiar plant sources existing in the wild [13]. This study was undertaken to explore the effect of hydrolysis reaction time on the reducing sugar yield for the two starch feedstock tacca (*Tacca involucrata*) and tigernut (*Cyperus esculentus*) in order to ascertain the optimum time for highest sugar yield which will in effect impact on bioethanol yields.

MATERIALS AND METHODS

The tacca and tigernut tubers were procured from local markets in Nigeria. The malted barley utilized as the source of enzyme was obtained from Nigerian Breweries Plc, in Enugu state. The chemicals utilized for the reducing sugar measurements were procured from a local supplier and were used without further purification.

Extraction of starch from Tacca and Tigernut

The extraction of the tacca starch from this feedstock was carried out by wet milling according to the method of Kunle *et al*., [14]. *Tacca involucrata* tubers (6.1 kg) were washed and peeled to remove the epiderm. The peeled bulks were washed with water, cut and sliced into small pieces. They were milled with mechanical grinder, thereby releasing the starch granules. The resultant paste was sieved with 0.25mm mesh to extract the starch using some quantity of water. The water was removed by allowing the starch to sediment by gravity and decanting of the water. The sedimented starch was squeezed in a muslin cloth bag to remove the water, leaving the starch in cakes. It was then dried by the use of solar dryer for a period of 4 days. The starch which was in caked form was dry milled with an electronic blender, a treatment that reduced it to a very fine powdery starch. The tigernut (4.5 kg) was threshed to remove the bad ones and other impurities. The nuts were milled to a coarse form. The resulting meal was dried in a solar dryer for a period of seven (7) days to remove the moisture in the nuts. Oil was extracted from the meal in batches with petroleum ether using an extraction column. The de-oiled meal was left in the open for two (2) days to dry off the residual solvent. The de-oiled, solvent-free meal was subsequently milled and sieved concomitantly using a laboratory mill equipped with 0.25 μm sieve. This gave a resulting fine powdery starch while leaving the husk/fibre behind [15].

Analyses of starch feedstock

Physicochemical analysis

Moisture, ash, crude fibre and calorific value were determined using AOAC method [16]. Crude fat by soxhlet extraction, crude nitrogen/protein by micro- Kjeldahl were all determined by Pearson method [17]. Reducing sugar was determined qualitatively by Benedicts test for reducing sugars and quantitatively by the Plummer method [18].

Amylose/amylopectin determination
For amylose and amylopectin analyses, the two starches were treated with n-hexane to remove any residual lipids present. The method of McReady *et al.*, reported in Adikwu [19] was used.

Data analyses
Statistical analysis was carried out on the data generated from the bioethanol hydrolysis using "Randomized Complete Block Design (RCBD)"; a two way analysis of variance (ANOVA) with repeated measures. This was carried out between parameters and between feedstocks using a combination of SPSS 17.0 version and Genstat 3.

Gellatinization of the starch samples
The gelatinization processes were carried out according to the method of Novellie and Shütte [20]. For the tacca, four sets of 100 g of tacca starch were weighed out and to each of them were added 100 ml, 200 ml, 300 ml and 400 ml of distilled water (representing slurry concentrations of 1.0, 2.0, 3.0 and 4.0 ml/g) respectively. For the tigernut, four sets of 100 g of tigernut starch were weighed and added to 350 ml, 400 ml, 450 ml and 500 ml of distilled water (representing slurry concentrations of 3.5, 4.0, 4.5 and 5.0 ml/g) respectively. Due to the high fibre content of the tigernut starch, less quantities of water could not adequately dissolve the starch.They were heated over water bath till gel formations took place and the gellation temperature noted.

Hydrolysis of the starches
The gelatinized tacca and tigernut starches using the different quantities of water were cooled and equilibrated at ambient temperature (30^oC). In each case 10 g, 20 g and 30 g of powdered malted barley were weighed into 250 ml conical flasks containing 50 ml, 100 ml and 150 ml of distilled water, respectively. These were stirred very well and added into the two separately gelatinized tacca and tigernut which were contained in 1000 ml beakers. The temperature of the water bath was increased and maintained constant between 40 -50^oC which is the temperature range for the activation of α-amylase. The temperature of the water bath was allowed to remain constant within this range for one hour. It was then raised to 65 – 70^oC range, another temperature range that helps the activation of β-amylase and glucosidase. The mixtures were stirred at intervals of 20 min for a total of 3½ h. Aliquots were withdrawn from the solution at 1½ h, 2 h, 2½ h, 3 h and 3½ h intervals and in some instance 4 h. They were tested for reducing sugar qualitatively and quantitatively. The quantities of reducing sugar produced at each interval were noted.

RESULTS AND DISCUSSION

Tacca
For the tacca starch, there was increase in reducing sugar yield with increased time across board for all the water contents. Among the different water contents, the sugar yield peaked at 3.0 ml/g with 0.2 g/g of enzyme concentration at 3½ h and 0.3 g/g at 3 h. Beyond the 3.0 ml/g the reducing sugar yield reduced (Fig. 1). For the 1.0 ml/g, there was significant difference ($P< 0.05\%$) in the reducing sugar yields between 1½ - 2 h for all the enzyme concentrations (0.1 g – 0.3 g/g) while there was no significant differences between the 2 and 2½ h ($P > 0.05\%$). The optimum sugar yield for this water content was obtained at 3½ h (0.1 g/g) and 3 h (0.3 g/g). For the 2.0 ml/g there was significant difference between the values of the reducing sugar for 0.1 g/g and 0.2 g/g. The optimum value for this water content was 4 h (0.1 g/g) and 3½ h (0.3 g/g). For the 3.0 ml/g, there was significant difference in the values obtained between 2 and 2½ h for all the enzyme concentrations. The optimum value of reducing sugar at this water content was obtained at 3½ h (0.2 g/g) and 3 h (0.3 g/g). For the 4.0 ml/g, there was no significant difference between 1½ - 2½ h in the sugar yields. The highest value was obtained at 4 h (0.3 g/g). General results for the tacca indicate that there was initial increase in reducing sugar yield with increase in water content up till 3.0 ml/g, above which the sugar yield reduced. Optimum conditions obtained for tacca starch were 3½ h (0.2 g/g) with 3.0 ml/g water content to give reducing sugar yield of 208.33 mg/ml.

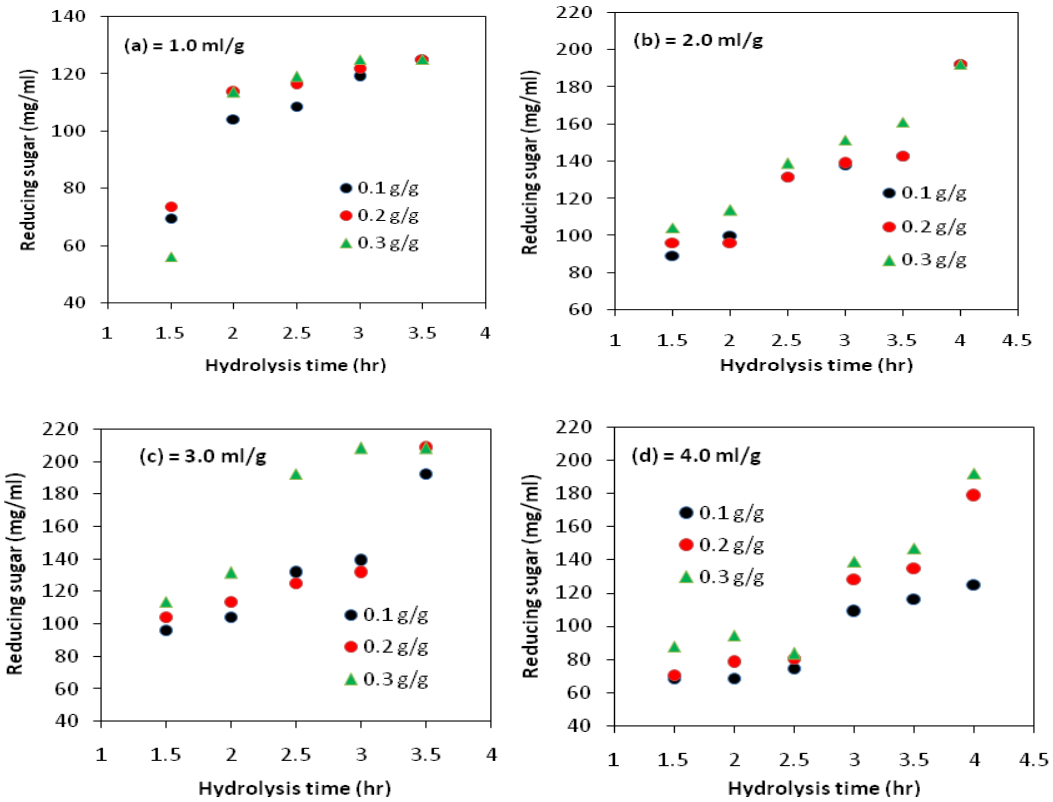

Fig. 1: Hydrolysis profile for tacca

Tigernut

For the tigernut starch, there was also general increase in the reducing sugar yield with increased reaction time across board for all the variants (enzyme concentration and water contents). Among the different water contents, the reducing sugar yield peaked at 4.5 ml/g with 0.3 g/g of enzyme concentration for 3 h (Fig. 2). Beyond that water content, the sugar yield reduced. For the 3.5 ml/g, there was no significant difference (P > 0.05%) between the reducing sugar yields for all the reaction times (1½ - 3½ h) and for the enzyme concentrations (0.1 – 0.3 g/g). However, the highest yield of sugar was obtained at 3½ h (0.2 g/g) and 3 h (0.3 g/g). For the 4.0 ml/g, the same trend followed and the highest value for the sugar yield was obtained at 3½ h (0.2 g/g) and 3 h (0.3 g/g). For the 4.5 ml/g, the difference in sugar yield for all the variants (enzyme concentration and reaction time), were also not significant and the highest sugar yield was obtained at 3 h (0.3 g/g). For the 5.0 ml/g, the observation of the trend was maintained and the highest value for the sugar yield was obtained at 3½ h (0.2 g/g) and 3 h (0.3 g/g). General results for the tigernut indicate that there was initial increase in the reducing sugar yields with increase in water content up till 4.5 ml/g, above which it reduced. Optimum conditions obtained for this starch were at 3 h (0.3 g/g) with 4.5 ml/g water content to give reducing sugar yield of 86.21 mg/ml.

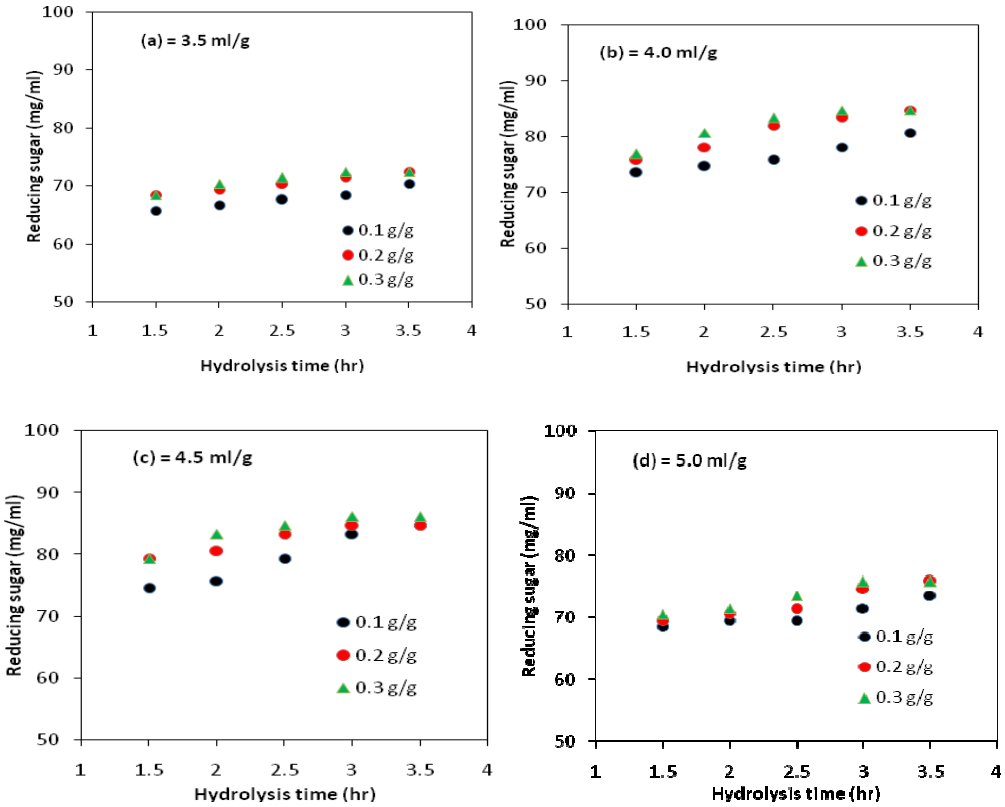

Fig. 2: Hydrolysis profile for tigernut

DISCUSSION

For the two starches studied, none achieved highest reducing sugar yield with 0.1 g/g of the enzyme concentration. They ranged between 0.2 g/g at longer times and 0.3 g/g at shorter times. Again none of the starches achieved optimum reducing sugar yield at 1½ - 2½ h. The optimum fell largely between 3 to 3½ h. Overall results indicate that reducing sugar quantity is largely dependent on slurry concentration (ml/g) of starch, enzyme concentration, reaction time and even temperature. The reasons for the little or no significant difference between 1½ - 2½ h reaction times for most of the variants (water content and enzyme concentration) can be attributed to the fact that the three enzymes for hydrolysis of starch are contained in the malted barley used as the enzyme for the hydrolysis reaction. The first enzyme α- amylase is activated at 40 – 50^{o}C for a period of 1 h. After 1 h, the second enzyme β-amylase is activated at 65 – 70^{o}C and later the third enzyme glucoamylase (glucosidase) at the same temperature. The results indicate that the third enzyme is not activated until after 2½ h reaction time, which could account for the lower yields of reducing sugar obtained at these reaction times (1½ - 2½ h).

The water content-dependence of starch for hydrolysis as observed in this study can be attributed to the origin and nature of starches. The biological origin of starch serves as a determining factor in the granules shape, size and morphology. As a result, these characteristics not only help to differentiate between various starches but also give an indication of the processing parameters. Moisture sorption by starch has been attributed to the interaction between the hydroxyl groups of the hexose moiety and water molecules [21]. Although water molecules form hydrogen bonds to both amylose and amylopectin, the amylopectin structures have been shown to physically trap water molecules. On this basis, it was hypothesized that starch granules high in amylopectin would have a higher moisture sorption potential [22]. Relative humidity is another factor in the sorption profile of starches. Once available sites are saturated at low humidity, the specific surface which should be relatively higher does not contribute to moisture sorption. The sorption process at high humidity is reduced to condensation of water molecules over the already existing molecules forming layers that have decreased interaction with the surface [12]. This principle may explain the reason why at certain water contents, reducing sugar yields reduced. The water

uptake of the starches at those water contents may be saturated and further increase in water content then leads to condensation of water molecules subsequently leading to decrease in interaction and reactions.

For the two starches, tacca gave a higher reducing sugar yield at the least water content. Tigernut starch gave the least reducing sugar yield at the higher water content. This can also be explained by the amylose and amylopectin ratios of the different starches. It has been severally reported that starches high in amylose contents would experience low ethanol yields [23, 24], it also follows that high amylose contents would lead to lower reducing sugar yields. The tigernut had the highest amylose content and therefore had the least reducing sugar yield.

CONCLUSION

The study has shown the effect of hydrolysis reaction time on the reducing sugar yield for tacca and tigernut. General results show that both feed stock had good reducing sugar yield, however the reducing sugar yield obtained for tacca was significantly higher than that for tigernut and therefore, tacca would be the preferred feedstock for the purpose of choice.

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