

Characterization of Sisal Boles for Production of Polylactic Acid (PLA)

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Abstract Biobased biodegradable plastics (bioplastics) show a large range of properties which can compete with non-biodegradable thermoplastics in packaging, textile and biomedical applications. Polylactic acid (PLA) is one of the most promising bioplastics, which are synthesized by polymerization of Lactic Acid (LA). Traditionally LA is produced from pure sugars and food crop sources like potatoes, cassava, corn, wheat, rice, sugar beet, sugar cane, and others. However, because of competition with existing uses, alternatives raw materials are being researched. One such alternative is the sisal boles. Sisal bole is part of the 98% of the sisal biomass which is traditionally counted as waste. Sisal boles as raw material for PLA are advantageous since it is not competing with food. This work characterizes sisal boles juice and its potential to produce biodegradable plastics. The sisal boles juice was extracted from chopped autoclaved boles using a hydraulic pressing machine. The juice was hydrolyzed to allow the sugar polymers to break into monomer sugar. The boles have been found to comprise of up to 97.94% (w/v) organic matters and total sugar content in juice of up to 30% (w/v). The produced juice mineral content levels were within the recommended working fermentation range with necessary nutrients for LA producing microorganisms. Sisal boles can therefore be one of the good raw materials for lactic acid production.

Keywords Sisal Boles, Characterization, Lactic Acid Production, Biodegradable Plastics, PLA

1. Introduction

Tanzania has been earning foreign exchange through export of sisal products and in 2014 she was the second world's largest sisal producer (37,500 tonnes) after Brazil [1, 2]. Tanzania has a unique position to increase the sisal production as she has comparative and competitive advantages in terms of suitable weather, soil (soil surface temperatures often exceeding 55°C [3] and human capital which are essential catalysts to the growth of the industry. The Tanzanian government would like to ensure that the sisal subsector contributes to its agricultural sector policy objectives of improving food security; crop varieties; farming systems; production technologies and efficiencies, and crop diversification [4]. Sisal industry can improve food security to workers by assuring that they have economic access to sufficient, safe and nutritious food to meet their dietary needs and preferences for an active and healthy life. It is acknowledged that the recent revival of sisal industry in Tanzania is attributed to the smallholder farmers with 6 to 200 hectares. They contributed a third (12,500 tonnes) of the total sisal production in 2014 [1, 5].

In a typical sisal production process, a very small portion,

about 2%, of sisal plant (sisal fibres) is used to produce twine, packaging, carpets, mats, thread, fine yarns, ropes and roofing tiles [6]. The remaining 98% biomass which include leaves decortication waste, wastewater and sisal postharvest waste (sisal poles, core-sisal boles and stubs of leaves that remain on the boles after every cutting) is discarded as waste [2, 7]. Generally, fibre is only 4% of leaf weight and production of one tonne of fibre generate waste amounting to 24 tonnes of leaf residues, 100 m³ of wastewater and 4.7 tonnes of sisal boles [8]. The liquid wastes end up in stream and other water bodies while the solid waste is normally disposed of by burning or dumping on land and leaving it to rot thereby generating greenhouse gases such as methane.

It is an undisputed fact that the economical sustainability of the sisal industry, depends on utilization of this bulk waste material (98%). Although highly fibrous and poor in nitrogen, sisal waste has high contents of organic matter and rich in total sugars (boles), which makes it potentially suitable for production of a wide range of high value commodities [2, 9]. In efforts to increase sisal plant productivity, several studies have been undertaken to assess potential for production of other products: such as citric acid and ethanol [7, 9-11]. These researches demonstrate the viability of valorisation of sisal waste.

One of the potential products that has less or not been studied is the Lactic Acid (LA) which is a raw material for production of bioplastics products (PLA). PLA is a sustainable, environmentally friendly, non petroleum

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dependent and low-carbon bioplastics for future applications [12, 13]. This study investigated the viability of transforming sisal waste (sisal boles) into Polylactic Acid (PLA) or Polylactide, which is a bio-plastic. This is biobased biodegradable thermoplastic aliphatic polyester or so called biodegradable plastic.

2. Material and Methods

2.1. Sisal Boles Preparation and Handling

Samples of sisal boles from Agave (Hybrid 11648) plants of 10-12 years were randomly collected from Fatemi sisal estate, Morogoro and Katani Ltd, Tanga. Samples were collected from different sites to investigate if there is any impact on geographical location (weather and soil characteristics e.g. mineral content, fertility, etc., temperature and rainfall). Eight samples of boles were collected randomly from one-hectare farm area and transported to the laboratory.

The boles were weighed before and after removing the leaf stubs. They were then cleaned using raw municipal water. The moisture contents for each bole were measured following the standard procedures [14]. The samples for moisture content measurements were taken from top, centre and bottom part of the boles. The values were expressed as percentage of the boles weight. The boles were then stored in a refrigerator set at 4°C to minimize microbial activities. The sisal plant, boles and initial preparations are shown in Figure 1.



Figure 1. Sisal boles preparation: A-Sisal plant; B: Sisal bole with stubs; C: Leaves stubs removal; D: Bole cleaned with tap water; E: Boles weighing

2.2. Compositional Analysis of the Sisal Boles

The sisal boles were then chopped using panga and knives into small pieces (3-5 cm) for compositional analysis. Organic carbon content was measured following the procedure by Walkley- Black, [15]. The Nitrogen content

(total kjeldahl) and crude fibres were determined using standard procedures [14]. Mineral contents (Ca, K, Na, Cu, Zn, Mn, Mg, Fe, and Co) were measured following the Standard Methods for the Examination of Water and Wastewater [16]. Dried, digested and well filtered sisal bole samples were analysed using Atomic Absorption Spectroscopy (Varian AA240) with graphite tube atomizer model GTA120, A hollow cathode lamp for each mineral element was selected and set to its proper wavelength (ηm) such as Ca^{2+} (422.7), K^+ (766.5), Mg^{2+} (285.2), Na^+ (589.0), Cu^{2+} (324.8), Fe^{2+} (248.3), Mn^+ (279.5), Zn^{2+} (213.9) and Co^+ (240.7).

The Standard Methods for the Examination of Water and Wastewater [16] were used to determine the COD, the total solids (TS) and volatile solids (VS).

2.3. Compositional Analysis of Sisal Juice

Juice extraction process involved chopping of sisal bole into small pieces (about 5-15 mm³) to increase the surface area during pressing. The juice was extracted from 1 kg sample of chopped boles using hydraulic pressing machine operated by hydraulic jack with a capacity of 16 tonnes. The machine extraction housing is made of stainless steel housing. Three alternatives were tried to maximize juice extraction. The first option involved direct pressing sisal boles choppings without heating, the second involved heating them at higher temperature above 100°C this was done by autoclaving at 121°C for 5 minutes. The autoclaved chopped bole parts were then cooled to room temperature (30°C±3) before pressing.

The third option involved mixing the boles with 1L of boiling water (100°C) before pressing to increase solubility of soluble components in bole mass to bring about the easiness during extraction process. The biomass residue after the first juice collection in either option was tested for sugar concentrations. This was done by adding half a litre of hot water to enhance wet blending of the biomass residue, and pressed to extract the juice to be tested. The process was repeated until maximum juice (sugar) extraction was obtained. Each batch of juices was subjected to physical-chemical characterizations and average values were used and reported.

The extracted juice was hydrolyzed to allow the sugar polymers to break into monomer sugar before analysis. The hydrolysis method by Busairi, [17] was adapted. The method involved transferring 50 ml of the juice into volumetric flask (100 ml) followed by addition of 5 ml of 2M HCl, boiled for 5 minutes and left to cool to room temperatures (27±2°C). The product was neutralized using 2.5M NaOH and then the solution made up to 100 ml by addition of distilled water [17]. Calibration curve (CC) for sugar determination was drawn using glucose standard sugars supplied by Sigma Aldrich and the slope was used during total sugar determination.

The hydrolysates determination of total sugar was done using 3,5-Dinitrosalicylic Acid Method [18] using the calibrated curve already drawn. The absorbance of the

solution was measured by using digital UV/VIS spectrophotometer (Labtronics LT-31) at 540 nm and used to obtain the sugar concentrations. The total sugar concentrations were calculated using equation (1) taking into account the dilution done during absorbance reading.

$$\% \text{ Sugar} = \frac{\text{Absorbance}}{\text{Slope from CC}} \times \text{Dilution factor} \times 100 \quad (1)$$

To determine organic matter and ash content the method of Loss on ignition by Nyam *et al.*, [19] was adapted. A crucible was first weighed without and with samples before it was transferred to a muffle furnace, heated at 550-570°C to burn off all organic matter. The carbon chars then burn off as CO₂ leaving a white ash [19]. Determination of Nitrogen content, crude protein, total dissolved and total suspended solids in juice were done as per AOAC, [20]. The pH and conductivity of the sample were measured using pH meter (CH-9100 Metrohm) and conductivity meter (B36356-Thermo Scientific), respectively.

Chloride content and phosphorus were obtained according to standard methods for analysis of water and wastewater [16]. 1 ml of K₂Cr₂O₄ indicator was used during chloride determination and the titration was done using 0.1N AgNO₃. The titration ended when the colour changed from lemon yellow to red brown. Juice Viscosity and density was determined using falling ball viscometer (HAAKE™) and suchspindel hydrometer (0.7-2.0 g/ml) at 20°C, respectively. All the analyses were done three times and the mean and standard deviation are reported in Table 1.

3. Results and Discussion

3.1. Sisal Boles Preparation, Handling and Juice Extractions

The average weights of sisal boles before removal of leave stubs were 43.5±2.0 kg (Morogoro) and 57.3±1.5 kg (Tanga), and they were 11.55±1.0 kg (Morogoro) and 22.8±1.1 kg (Tanga) after the removal of leaves stubs. These shows that leave stubs cover more than 60% of whole sisal boles. It was important to remove the leave stubs from the core boles to avoid interference of chlorophyll with juice quality. The average moisture contents for boles from Morogoro and Tanga were 72.15±2.0% and 60.72±1.8% respectively which matches well with those of Muthangya, [21], who found that the moisture content was between 60.29±1.1 to 76.78±1.0 percent. Although the moisture contents of the boles from Morogoro is higher, their core boles size is smaller than that of Tanga. The difference in moisture content may be attributed to difference in soils fertility, rainfall pattern, harvesting season of the year and age of sisal plant. Samples from Tanga were harvested during dry season.

The study also found that the boles from Tanga comprises moisture content of 65.68±2.7 (%w/w), 57.04±1.9 (%w/w) and 52.68±3.5 (%w/w) corresponding to the top, centre and bottom section of the sisal bole respectively. The higher amount of moisture at the top section can be attributed to fact

that, as the plant grows, the cells become matured upwards as new cells are generated. Young plant cells have more water content than earlier cells. Juice extraction results were 567±2.9, 791±0.6 and 653±2.9 volumes of juice per kg of sisal bole for chopped and pressed, autoclaved and mixed with boiling water, respectively. Thus, the autoclaved sisal boles gave the highest volume of juice compared to other methods. This can be attributed to higher temperature (121°C) used under autoclaving pressure of 1.5 bar, which led to rupture of sisal boles providing more surface area for juice extraction.

3.2. Compositional Analysis

The calibration curve for the standard sugar gave a linear relationship with R² of 0.9971. Boles from Morogoro gave a total sugar of between 8-15% (m/v) which is similar to what was obtained by Elisante & Msemwa, [9] who used samples from Morogoro. Boles from Tanga produced 10-30% (m/v) total sugars which are similar to what was obtained by Ngonyani, [7] who used samples from Tanga. The differences in amount of sugars for the samples from the two sites, Morogoro and Tanga, might be due age of sisal plant used, soil characteristics and the geographical locations which need to be further researched. Tanga has the largest sisal plantation in Tanzania with more than five estates with land area 3,635 hectares. Table 1 gives the compositional analysis of the boles and the juice extracted from these boles.

Table 1 clearly shows that sisal boles comprise of high organic matter content of up to 97.94±1.0% (biomass) and 99.97±0.2% (juice) and is found to characterize the large part of the resource. The organic matter represents the amount of nutrients available. Amino acids in form of organic and inorganic nitrogen show the promising level of available nutrients hence their availability for microbial growth. This is an indication that the bioresources can serve as a possible alternative substrates for lactic acid production because it contains recommended nutrients. These values are similar to those obtained by Mshandete *et al.*, [2] who used the same hybrid specie in his study.

The boles were also found to contain high total sugar content in juice of up to 30% (m/v). On average one bole weighs 40 kg, if one kg of bole contains almost 791 ml of juice, it is clear that 31.64 L of juice can be obtained from one bole. The juice can provide 9.2 kg of total sugar that can be hydrolysed and used to produce LA. Msuya *et al.*, [22] reported that it was possible to produce 24 g of LA from sisal bole juice with 12% (w/v) sugar concentration. This is almost 91 g of LA from one sisal bole. Sisal boles are available in Tanzania in plentiful amount, it is estimated that 176,250 tonnes of sisal boles can be produced per year which could produce more than 1.6 million tonnes of sugar that can be fermented to produce 16 million kg of LA. LA can be polymerized to PLA which has a huge market potential for packaging material especial short life food packaging.

The recommended initial juice sugar content for fermentation is between 8-15% [23-25]. The extracted juice

concentration level therefore will require dilution and hence will assure more raw materials for fermentation. The juice sugar content for all juice extraction options was found to be less than 1% before hydrolysis; therefore autoclaving had no impact on sugar yield without acid hydrolysis. In all juice extraction options sugar in biomass residue was less than 5%, hence residual biomass was discarded to avoid high juice operational costs with only small increase in total extracted sugar yield.

Table 1. Compositional Analysis of the Fresh Biomass and the Extracted Juice (Mean±SD, n=3)

Parameters	Biomass	Juice
Total sugars% (g/l)	-	30±0.5
Organic matter% (g/l)	97.94±1.0	99.94±0.2
Organic carbon (%v/w)	35.1±0.3	-
COD (O ₂ g/L)	-	28.88±0.5
C:N ratio	-	34.41:1
Ash content (%w/w)	4.28±0.6	3.97±0.5
Total Nitrogen content (%mg/l)	0.85±0.1	1.02±0.02
Crude protein (mg/L)	5.31±0.4	6.38±0.4
Total dissolved solids (ppm)	-	5,245±2
Total suspended solids (ppm)	-	908±2
Calcium (mg/L)	-	45.96±0.05
Magnesium (mg/L)	-	103.56±0.1
Iron (mg/L)	-	0.422±0.02
Potassium (mg/L)	-	534.88±0.01
Sodium (mg/L)	-	1.498±0.3
Manganese (mg/L)	-	NIL
Cobalt (mg/L)	-	NIL
Zinc (mg/L)	-	0.15±0.05
Copper (mg/L)	-	0.09±0.01
Chloride (mg/L)	-	3,722±0.10
Nitrate (mg/L)	-	1.061±0.01
Phosphorus (mg/L)	-	22.94±0.31
pH	-	5.23±0.20
Density (g/cc)	-	1.02±0.01
Conductivity (µS/cm)	-	1415±12
Viscosity (mPaS)	-	2.00±0.01

Minerals elements like Mg and Fe in the form of salts (MgSO₄ and FeSO₄) and vitamins belonging to B group are very important during fermentation [24], as they increase the strength and power of Lactic Acid Bacteria (LAB). From Table 1, all the fraction of light metals content are low compared to the recommended levels hence safe level of metals required for microbial growth during lactic acid fermentation. The recommended levels for mineral elements during fermentation are (g/l): 0.5-1.0 phosphorus as KH₂PO₄, 0.5 potassium in form of KCl, 0.5 magnesium in form of MgSO₄.7H₂O, 0.005 iron as FeSO₄.7H₂O, 0.0066 zinc in form of hydrous zinc sulphate and 0.001 copper as copper sulphate [7, 24, 26].

Phosphorus is present in the microbial cell as phosphate sugar, nucleic acids and nucleotides. This is normally added as nutrients. The presence of phosphorus in juice (22.94±0.31 mg/L) will reduce the amount of phosphorus nutrients to be added hence saving cost of nutrient needed. The average available potassium in the extracted sisal bole juice was found to be 534.88±0.01 mg/L. Potassium is required for maintenance of ionic balance across the cell membranes, and as stabilising component RNA [17, 24, 25]. Potassium content is within the required range for microbial growth therefore its addition will not be necessary. Magnesium is an essential enzyme activator and component of the cell membrane [17]. The available magnesium in the sisal bole juice in the range of 103.56±0.1 mg/L is sufficient to serve as activator or cofactor of LAB during growth.

Any trace amount of manganese will negatively affect the microbial growth [26]. The upper level that is tolerable is 3-10 (ppb) for manganese as manganese sulphate [7, 25]. Therefore since the juice was found to have no traces of manganese, microbial growth will not be hindered. In her research, Ngonyani, [7] found traces of manganese in juice of around 8 mg/L which was attributed to equipment used during juice extraction. Most of equipments that are not made out of steel can contribute on adding iron and manganese metal in the processed product. The iron level of 0.422±0.02 mg/l obtained in sisal boles juice is within the required range and can play important role as enzyme activator and stabilizer during fermentation.

Heavy metals like copper, zinc and cobalt are necessary inhibitors to microbial activity during fermentation process [26]. The extracted juice from sisal bole was found to contain no cobalt. It has traces of copper 0.09±0.01 (mg/l) and zinc 0.15±0.05 (mg/l) which are within the acceptable range. Most of the LAB can grow well at the pH level of 3-7. The juice extracted had an average pH of 5.23±0.20 which is within the required range for microbial growth. Other factors like conductivity, density and viscosity were within the range that will enhance microbial environment for growth and production during fermentation. The sisal bole juice extraction equipment used in this for this study produced juice with satisfactory quality therefore it can be deployed for juice extraction.

4. Conclusions

Sisal boles are available in Tanzania in plentiful amount, it is estimated that 176,250 tonnes of sisal boles can be produced per year. About 1.6 million tonnes of sugars can be produced from the sisal boles and when fermented can produce 16 million kg of LA. LA can be polymerized to PLA which has a huge market potential for packaging material especial short life food packaging. This study has shown that, sisal boles which have been traditionally treated as waste can be utilized to produce products with significant positive environmental impacts. Production of Lactic acid from sisal boles and PLA from LA produced is discussed in other

papers [6, 22]. The produced sisal boles juice had sugar concentration of 30% which is higher than the recommended juice fermentation range of 8-15% (w/v). The produced juice mineral content levels were within the recommended working fermentation range with necessary nutrients for microorganisms. Sisal boles can therefore be one of the good raw materials for lactic acid production.

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