New sustainable approach to reduce cassava borne environmental waste and develop biodegradable materials for food packaging applications

K.S. Tumwesigye[a,b], J.C. Oliveira[a], M.J. Sousa-Gallagher[a,*]

[a] Process & Chemical Engineering, School of Engineering, University College Cork, Ireland
[b] National Agricultural Research Laboratories, NARO-Kawanda, Uganda

ABSTRACT

Transforming waste cassava into a sustainable resource requires a new approach and redesign of the current processing methodologies. Bitter cassava cultivars have been employed mainly as an emergency famine food, but could also be used as a value added material for packaging. Processing of intact bitter cassava can minimize waste, and produce low-cost added value biopolymer packaging films for targeted applications. This study developed an improved simultaneous release, recovery and cyanogenesis (SRRC) downstream processing methodology for sustainable reduction of waste and development of film packaging material using intact bitter cassava.

SRRC approach produced peeled (BP) and intact (BI) bitter cassava biopolymer derivatives. BI showed significantly higher yields ensuring 16% waste decrease with no environmental impact caused by discard residues. SRRC was very effective in reducing the total cyanogen content to within Codex minimum safety limits, demonstrating that the peeling of bitter cassava process can be avoided. Transparent films were produced using the casting method from both BP and BI derivatives. BI films were more transparent and homogeneous, less soluble, less permeable to moisture, less hydrophilic, more permeable to oxygen and carbon-dioxide, sealable, lower cost, than the BP.

Hence, intact bitter cassava and SRRC can be used as sustainable, safe, integrative process solution for high value-added product (e.g., packaging film) production from low-cost biobased materials.

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1. Introduction

The environmental problems caused by food supply chain waste and by-product streams have triggered increased demand for research into biobased value added products and efficient sustainable renewable resources. Thus far, there is a growing realization of the requirement to increase value products that are made out of secondary raw materials. Among these, waste is considered a valuable resource to provide sustainable feedstock and concurrently contribute to circular-based approach of energy recovery (Essel & Carus, 2014).

Cassava (Manihot esculenta Crantz) crop is considered among the highest generators of huge amount of wastes in the form of peel, pulp, wastewater and leaves during postharvest processing (FAO, 2013). With increased population, production and consumption of cassava has increased consistently and thus waste disposal in the environment has increased tremendously due to a linear and irreversible behavioral pattern that follows a produce–consume and dispose model. According to FAO (2013), starch roots, mainly cassava contributes over 700 MT wastes in the global upstream food wastes, requiring conversion into valuable products and energy in an environmentally friendly manner.

Apart from direct food wastes, other sectors such as foods, beverages and consumer goods packaging generate more non-eco-friendly plastic wastes and this has resulted into huge impact on the environment. With insufficient prioritization of packaging source reduction, recyclability, compostability, recycled content and recycling policies (MacKerron & Hoover, 2015), wastes are likely to increase in the years ahead. It is estimated that less than 14% of plastic packaging materials are recyclable (MacKerron & Hoover, 2015), and as plastic commands the greatest proportion of food packaging industry, the need to design biobased material is a priority.

With increased devotion to research into packaging sustainability, it is highly likely that non-commercial and non-food

plants-derived feedstock will anchor packaging industry. Besides, the finite and dwindling natural material sources and competition for food supply amidst population growth, justify the need to invest into efficient utilization of unexploited resources. Thus, addressing waste minimization and developing packaging materials, in tandem, could result into a more competitive resource efficient economy.

An alternative strategy is to use a circular utilization model whereby cassava waste could be transformed into resource for development of value-added flexible packaging materials resulting in waste minimization due to the biodegradable nature of the materials. If used efficiently, cassava borne environmental wastes have the potential commercial viability in better eco-designing of materials for food and non-food applications. Semi-commercial crops such as bitter cassava (BC) have the potential to sustain the growth of food plastic packaging industry. Like sweet cassava, BC-based films have potential biodegradability and could form excellent film forming properties. However, their demands for processing prior to use due the high total cyanogens (TC) (containing 900–2000 ppm) (Cardoso et al., 2005), has limited their commercial potential. Essentially, when bitter cassava is used as food, the peels are extensively removed resulting in huge contribution to waste generation and even a negative environmental impact. Traditionally, processing is achieved by peel removal, generating great amounts of waste. Nonetheless, elimination of the peelings does not guarantee its safety, reasonable lethal amounts of TC still remain and the roots have to be further processed. Traditional soaking and heaping fermentation methods produce high levels of unspecified total cyanogens combined in waste waters and peels, and deliberate burning of peel wastes contributes to carbon dioxide emission and strong offensive smells to the environment (Heuzé et al., 2015). Using bitter cassava for waste minimization and package development could be done in an efficient manner, which is compatible with increased income and improved safety of cassava dependants and reduced environmental impact, while providing a sustainable feedstock for packaging applications.

Transforming waste cassava into a sustainable resource requires a new approach and redesign of the current processing methodologies. Simultaneous release, recovery and cyanogenesis (SRRC) could be a sustainable approach in processing and has been explored and piloted with success to ease downstream extraction of biopolymers from whole root cassava (Tumwesigye, Oliveira, & Sousa-Gallagher, 2014). Safe and clear biopolymer derivatives have been produced, with success, from the whole root of cassava for potential food and non-food industrial use. The SRRC processing methodology, utilizing semi-commercial intact bitter cassava, could be explored in production of flexible food packaging film materials with improved properties. Moreover, for production of low-cost packages but also by obtaining sustainable feedstock because bitter cassava has no competition with food supply since sweet cassava has normally been utilized as food and non-food materials. Additionally, SRRC is an intrinsic processing methodology which re-enforces starch with compounds from the peel and other waste solids, and there is no generation of wastes and waste streams. This could reduce the cost of film package production and ensure process economy.

The objective of this study was to (i) use intact bitter cassava to reduce waste, (ii) apply an improved systematic downstream processing approach to improve biopolymer derivatives physico-chemical properties, and (iii) development of film packaging material. If biopolymer derivatives and films produced out of intact bitter cassava presented comparable or better properties than those made from peeled equivalent, then it would be possible to eliminate the peeling, and its environmental impact, with additional production of flexible packaging material as added value product.

2. Methodology

2.1. Source material

Decisions to source for a sustainable raw material was based on many factors taking into account the renewable resources, no competition with food supply, minimising waste and environmental impact, and cost-effective option. Accordingly, bitter cassava (Tongolo) was the preferred material used for package development.

2.1.1. Cassava preparation

Bitter cassava roots (Tongolo) at 12–18 months were picked from farmers' fields (Northern Uganda) according to recommended harvesting practices (CODEX, 2013). The roots were separated from soil debris, placed in ice boxes, transported to the laboratory and kept at −20 °C for further treatment. Fresh bitter cassava was assessed for total cyanogen content immediately after harvesting and prior to pulp preparation. The cyanide kit A from Australian National University (Canberra) was used for determination of hydrogen cyanide in fresh cassava as described (Bradbury, Egan, & Bradbury, 1999).

2.1.2. Waste solid analysis

Bitter cassava was used intact (I) or peeled (control), washed thoroughly, rinsed 3 times with deionized water and kept at −20 °C between sample extractions.

Waste solids quantification was done by randomly selecting 12 intact roots from the bulk bitter cassava. The wastes (peel, cambium, phloem, central xylem fiber) were carefully removed from the parenchyma, and both peel and peeled root portions were separately weighed. Each measurement was taken from 100 g intact roots that were correspondingly assigned to 9 different treatments during subsequent tests. The weight of the waste solid was calculated by dividing the weight of waste by the weight of intact root and expressed as a percentage.

2.2. Simultaneous release, recovery and cyanogenesis (SRRC) of biopolymer derivatives

The downstream processing procedure is schematically presented in Fig. 1. Both intact and peeled roots were processed in two-stages, the mechanical tissue rupture and the biopolymer release, in order to obtain biopolymer powders. In elucidating the function of simultaneous release, recovery and cyanogenesis (SRRC) in downstream processing, intact (I) (periderm-free) roots were scrubbed, while in the peeled (P) (cortex-free) roots, the peel was manually and carefully detached from the edible portion (parenchyma). Intact and peeled bitter cassava roots were fed into an automated grating machine and the resulting pulp mass obtained after mechanical tissue rupture and cell disruption. The machine was equipped with a feeding hopper, a constant speed rotating perforated spiked drum and an inclined delivery channel. This initial action serves the dual purpose of activating cyanogens hydrolysis into release of volatile hydrogen cyanide and bringing together different polymer components for possible modification.

Biopolymer derivatives release and recovery was performed by adding 100 g of pulp mass into 100 ml of extraction buffers in a commercial blender (500 W Breville IHB086Hand Blender). Full factorial design of 9 different solutions, i.e., 2 extraction buffers at 3 levels, NaCl (0, 1.5, 3.0 M) and H_2SO_4 (0, 25, 50 mM) were used. A total of 18 different samples (9 Intact and 9 peeled cassava roots) were then homogenized, filtered, centrifuged and washed in deionized water (Fig. 1). The chemicals used in release and recovery, i. e., sodium chloride (≥99% AR), conc. sulphuric acid
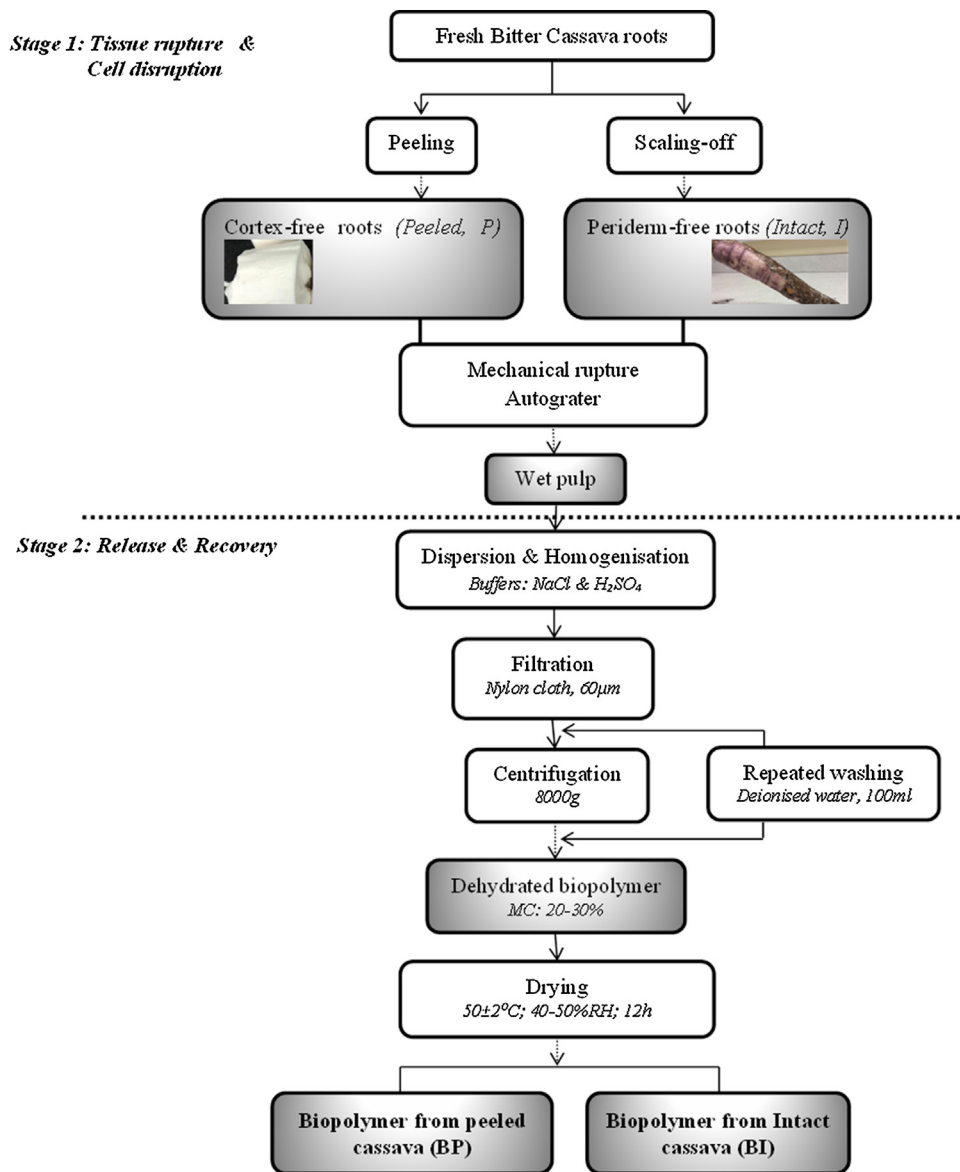
Fig. 1. Schematic flow of downstream processing for peeled (P) and intact (I) bitter cassava and derived biopolymer products from peeled (BP) and intact (BI) roots.

(99.9%), food grade sodium bisulphite (ACS 58.5% SO_2), and glycerol (99.5%) were analytical grade from Sigma–Aldrich (Ireland).

The recovery was achieved by drying semi-dehydrated coarse pulp in an air-circulating oven at uniform conditions, temperature ($50 \pm 5\,^\circ$C) and relative humidity (30–40%) until constant weights were obtained. The dried samples were milled to a fine powder using an Analytical Grinder (IKA Yellowline-R A 10, Germany) and kept refrigerated (4–7 $^\circ$C) between tests and further use.

Release, recovery and drying of the biopolymer derivatives were performed at NARO-Kawanda laboratory (Uganda), and samples properly packed in air-tight bags and shipped to the labs in Process & Chemical Engineering, School of Engineering, University College Cork, Ireland for further experimental analysis.

2.2.1. Yield determination

Yield was defined as the percentage of constant weight dried powder recovered from initial mass of 100 g roots, and determined in triplicate for the 18 derivative products, totalling 54 samples per analysis.

2.2.2. Total cyanogens analysis

The total cyanogens (TC) content were quantified by the cyanide kit from Australian National University (Canberra). The quantification entailed grounding samples into 53 μm particle size powders and their total cyanide contents (ppm) measured based on the color chart and spectrophotometric-derived absorbance kit protocol B2 method as described by Bradbury et al., 1999.

2.2.3. Amylose measurement

Amylose contents of biopolymer derivatives and their corresponding proportions were determined by the assay kit from Megazyme International, Ireland. The determination involved grounding samples into 53 μm particle size powders and their amylose contents assayed in a 25 ± 0.1 mg sample as described in the kit (Megazyme International Ireland, 2014).

2.3. Film preparation

The films were manufactured using the casting method, either with peeled (BP) or intact (BI) bitter cassava derivatives (3 w/v%)

and glycerol (30 w/w%). The solution was placed in agitating water bath at 70 °C, heated to 65 °C with constant stirring until a viscous transparent gel was observed; and held for 20 min. Casting was done by pouring film-forming solution (30 ml) onto a previously lubricant sprayed 14 cm diameter flat glass plate using a dropper. The film solution was measured to ensure production of uniform thickness films (30 ± 5 μm) for different samples, and the dry PTFE spray was used to ease peeling of films after drying. The plates were left at 25 ± 1 °C for 3 h to allow stabilization and bleeding of trapped bubbles, and then dried in a ventilated oven 40 ± 1 °C for 4 h. The dried films were peeled off the plates and equilibrated (54% RH, 23 ± 2°C, at least 48 h) for further use.

2.3.1. Optical properties

Film optical properties were assessed using transparency and color parameters. Film transparency was determined with film strips (3 × 2 cm) as described (Muo et al., 2012) with slight changes. Films strips were carefully inserted into cuvettes and placed inside a spectrophotometer cell. Transmission was measured using a spectrophotometer (Biochrom Libra S22 UV/vis) at 700 nm. Three replicates of each film were tested. The percent transparency was calculated as described (Muo et al., 2012). For each value, the lower T implies that more light passed through a film, thus described as transparent.

Color difference (ΔE) was determined according to Ramírez-Navas and Rodríguez de Stouvenel (2012) using CR-400 Chroma Meter, Konica Minolta Sensing Japan without major changes. Measurements were taken on six different equal positions on a circular film piece for 3 samples of BP and BI each, and mean values used in CIELAB L^*, a^*, b^* using the Eq. (1) as described (Sharma, Wu, & Dalal, 2005).

$$\Delta E = \sqrt{[(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]} \tag{1}$$

where $\sqrt{}$, square root symbol; ΔL^*, Δa^* and Δb^*, differences between sample and standard (S) color parameters. S is film background color parameters.

2.3.2. Solubility

Film solubility (FS) in water was measured as described (Belibi et al., 2014) with minimal modifications. Previously oven-dried film strips (3 × 2 cm) were weighed on an aluminum foil, submerged in a beaker with 50 ml of distilled water and tightly covered with parafilm to minimize water loss and airborne contaminants. The contents were kept at 23 °C for 24 h, intermittently agitated to allow dissolution, partially dehydrated (where necessary filtered) on filter paper and dried in an air-circulating oven at 70 °C until the sample weight was constant. Total soluble matter of the sample was calculated as described (Belibi et al., 2014). Sample tests were performed in triplicate, and mean values were used for computing FS in water.

2.4. Analysis of film performance properties

2.4.1. Surface

Film surface properties were analyzed in terms of its morphology and energy.

To supplement optical properties and gain better understanding of the film homogeneity and microstructure, film was examined using Scanning Electron Microscope (SEM), JSM-5510 (Jeol Ltd., Tokyo, Japan). Small film strips were fixed on stubs using double-sided carbon tape and leaving a space on either side of the strip to allow clear observation of film surfaces and cross section. Prior to capturing SEM images, stub-fixed strips were coated with a thin layer of gold. The films were subjected to a focus magnifications as

high as 20,000× and images capture between 200− and 30,000× magnification and intensity of 5 kV.

Gaining insight of surface energy properties (surface hydrophobicity, hydrophilicity and wettability) was through measurement of contact angle (CA) using the sessile drop (SD) method by Optical tensiometer (Theta, BiolinScientific Finland) at 20 °C. SD determines the contact angle between drop baseline and drop boundary tangent. The Theta was equipped with a liquid dispenser holder fitted with a 0.5 mm diameter precision microsyringe steel needle, and OneAttension software for drop shape analysis and live measurements. A rectangular film strip (2 cm × 2 cm) was mounted on a pre-cleaned glass slide covered with double-sided tape, then placed on a horizontal holder at 90 °C to enable clear observation of film surface and cross-section. The syringe was positioned vertically 10 mm from the film surface and 2 μL deionized water drop automatically dispensed (1.0 μl s^{-1}) on the film. The measurements lasted 180 s and data was analyzed for contact angle (θ). All films were conditioned (23 ± 2 °C, 50% RH, 48 h) prior to measurements and five measurements were carried out. Drop wetting, spreading and beading/shrinking away gives CA of 0, 0–90° and >90° respectively.

2.4.2. Chemical

Film chemical properties were analyzed using Fourier transform infrared spectroscopy (FTIR). The films were prepared into thin round discs while under the lamp heater and mounted directly on the sample holder. The spectra were recorded with an UV/vis spectrum one FTIR spectrometer (PerkinElmer Lambda 35, USA), frequency range of 4000–400 cm^{-1} and 4 cm^{-1} resolution in the transmittance and absorbance modes for individual spectrum with 30 scans at room temperature.

2.4.3. Barrier

Film barrier properties were assessed by determining its water vapor transmission rate (WVTR) as well as oxygen transmission rate (OTR) and carbon dioxide transmission rate (CTR). WVTR was determined gravimetrically according to ASTM E96-05 (2005a,b), whereas OTR and CTR were determined using a method described by Abdellatief and Welt (2013) with deviations from steady state to dynamic oxygen accumulation procedure. Films for WVTR, OTR and CTR were formulated and cast on 8.4 cm and 14.0 cm diameter dishes to maximize uniformity and permeation cell fitting specificity. For WVTR, each previously conditioned (54% RH, 23 ± 2 °C, at least 48 h) film was carefully positioned between acrylic permeation cells containing CaCl$_2$ (0% RH) and enclosed in a humidity-controlled plastic containers partially filled with 1000 ml of salts solution, corresponding to relative humidity of 95%. The containers were put in temperature controlled incubators at 38 °C, and changes in weight of the cell were recorded every 2 h for 10 h and data obtained was used for WVTR calculation. This was intended to elucidate the differential flux gradient as a deviation from the normally applied conditions (38 °C, 90% RH), and also have results comparable to previous research on films. Similar procedure for film preparation was followed for OTR and CTR, but changes to the method by Abdellatief and Welt, 2013) were made in terms of (i) flushing (0% O$_2$, 20% CO$_2$ and 80% N$_2$) and measurement (23 °C, 50% RH) conditions, using a PBI Dansensor (CheckMate 9900, USA). It was assumed that O$_2$/CO$_2$/N$_2$ concentration gradient was maintained across the film throughout experimental period. All tests were conducted in triplicate and mean values were used for calculating WVTR, OTR and CTR, expressed as g/(m^2 day) and cm^3/(m^2 day), respectively.

2.4.4. Mechanical

Mechanical properties, i.e., tensile strength (TS), elongation at break (E) and elastic modulus (EM) were tested using TA HD Plus

Texture Analyzer (Stable Microsystems, UK), fitted with a 50 kg load cell, according to ASTM 882-09 method (2009). Ten previously conditioned (23 ± 2 °C, 54% RH, at least 48 h) films were cut into 25 × 100 mm strips and their thickness measured with an absolute digital Caliper (Digmatic, Mitutoyo, UK Ltd.) prior to the tests. Measurements were taken for all the strips and at least 5 close values were used in estimating the cross-sectional area (thickness × initial grip distance). The initial grip separation (50 mm) and cross head speed (1 mm/s) were used. TS (MPa) was calculated by ratio of the force necessary to break a sample to the cross-sectional area, E (%) as a change in the sample original length between grips at break, and EM (MPa) by ratio of TS to the extensional strain. All 10 strips were tested and at least 5 with closest values were used in calculations of TS, E and EM.

2.4.5. Seal integrity

Seal integrity (SI) is used as an indicator of seal quality (ASTM, 2005a,b). The film SI was determined by measuring its seal strength (ST). A manual impulse sealer, UK with adjustable temperature control was used for optimal seal quality. Two film strips (100 × 150 mm) were placed on top of each other between two jaw pads, and the upper jaw held against the lower jaw. A temperature of 135 °C, pressure of 69 kPa and dwell time of 1 s were used as the film sealing conditions. The sealed strips were removed from the sealer and cooled at ambient temperature (23 ± 2 °C) for 10 min to allow stabilization. The ST was determined using a TA HD Plus Texture Analyzer (Stable Microsystems, UK), fitted with a 50 kg load cell. The film non-heat sealed end was held perpendicular to the direction of the pull, with the distance between the clamps of 20 mm and a test speed of 1.0 mm/min. The maximum force breaking the seal and causing seal failure was recorded as the seal strength in gram–force/millimeter (g(f)/25 mm). Ten pair film strips measured and the means of at least 5 strips were used in computing seal strength.

2.4.6. Thermal

Thermal testing, glass transition (T_g) and melting (T_m) temperatures, heat of fusion (ΔH) and crystallinity (C), was conducted using a differential scanning calorimeter (DSC 200 F3) equipped with a thermal analysis data station. A previously conditioned (54% RH, 23 ± 2 °C, at least 48 h) hermetically sealed DSC pan with film, together with a reference empty pan were heated from 20 to 220 °C at a rate of 10 °C/min, cooled back rapidly to below its glass transitional temperature (25 °C) and reheated at a rate of 5 °C/min to 220 °C to give them thermal history. T_g, T_m, ΔH and C were calculated using the built in software and determined by considering the heat capacity change observed on the second heating.

Thermogravimetric analysis was carried out to establish thermal stability of films using TG Analyzer (Spectrum 500) and analyzed by the Universal Analysis 2000 (New Castle, USA) between 25 °C and 300 °C, heating rate of 10 °C/min. Prior to analysis, each sample was corrected against a background scan.

All samples were evaluated in triplicate and mean measurements reported.

2.5. Statistical analysis

A factorial analysis of variance (ANOVA) was performed, pooling the 3rd level interaction with the error and testing all main effects and 2-way interactions for significance at a 95% confidence level. The main effects were further decomposed in a linear and a quadratic component, drawing P and R^2 to analyze statistical significance at a 95% confidence level. Statistica 7.1 software (StatSoft Inc., Tulsa, USA) was used. Color was recorded in the CIE $L^*a^*b^*$ system and analyzed using Microsoft Excel, version 2010.

3. Results and discussion

3.1. Waste solids

Intact bitter cassava produced an average 16% waste decrease, due to incorporation of the waste solids, and no environmental impact caused by discard residues. It is clearly shown that the use of intact roots reduces the waste yield when compared to the peeled roots. Thus, it's possible to avoid 160 kg waste peel per 1 t bitter cassava processed when the SRRC process is deployed by small-to-medium enterprises to develop added value products such as biobased films. It has been shown that a 100 t cassava starch production unit releases up to 47 t fresh waste and can have a huge impact on the environment (Aro, Aletor, Tewe & Agbede, 2010; Heuzé et al., 2015). Moreover, it is well established that cassava processing wastes produces strong offensive smell and their decomposing or incinerated disposal heaps immensely emit carbon dioxide (Aro, Aletor, Tewe & Agbede, 2010; Heuzé et al.,

Table 1

Analysis of the influence of peeling and extraction conditions on biopolymer derivatives and film properties. Statistically significant effects are denoted in bold and shown by a higher value regardless of direction (positive or negative). Asterisk shows highly significant effect.

Factor	Biopolymer derivatives						Polymeric films					
	Yield		Total cyanogens		Amylose		Transparency		Color		Solubility	
	Effect	p-value	Effect	p-value	Effect	p-value	Effect	p-value	Effect	p-value	Effect	p-value
Mean/interc	**26.710***	0.000	**2.257***	0.000	**28.512***	0.000	**16.556***	0.000	**15.249***	0.000	**29.675***	0.000
(1) NacL (L)	**5.052***	0.000	**−2.891***	0.000	−2.739	0.006	2.642	0.509	**−0.717***	0.000	**6.672***	0.000
NacL (Q)	**−1.202***	0.000	**−0.389***	0.000	0.5007	0.527	−0.858	0.799	−0.070	0.476	0.667	0.541
(2) H₂SO₄ (L)	0.605	0.090	0.068	0.208	−0.432	0.636	**−10.258**	0.048	−0.156	0.174	−0.081	0.949
H₂SO₄(Q)	−0.293	0.338	**0.241***	0.000	−0.4128	0.601	0.794	0.814	−0.034	0.724	0.141	0.897
(3) Peeling (L)	**1.086***	0.000	**−1.575***	0.000	**−12.2522***	0.000	**−26.945***	0.001	**−12.423***	0.000	**−22.184***	0.000
1L by 2L	0.036	0.933	**−0.211**	0.002	−0.7782	0.487	6.390	0.226	−0.175	0.210	0.058	0.970
1L by 2Q	−0.043	0.907	−0.059	0.299	0.4651	0.630	4.229	0.336	0.114	0.342	0.077	0.954
1Q by 2L	−0.184	0.621	−0.007	0.908	−0.3401	0.725	−1.771	0.671	−0.222	0.072	−0.110	0.934
1Q by 2Q	−0.148	0.646	−0.030	0.547	0.6205	0.460	−2.863	0.441	0.001	0.992	−0.081	0.944
1L by 3L	0.560	0.116	**1.043**	0.000	**2.1617**	0.025	−2.461	0.537	−0.195	0.093	**−8.852***	0.000
1Q by 3L	−0.164	0.590	**0.350**	0.000	1.2038	0.136	0.979	0.772	−0.086	0.378	−1.518	0.168
2L by 3L	0.280	0.426	−0.037	0.487	0.8639	0.347	**10.308**	0.047	−0.021	0.850	−0.003	0.998
2Q by 3L	0.296	0.332	**−0.102**	0.032	0.4669	0.555	−0.937	0.781	−0.080	0.416	−0.060	0.956
Total effects	**32.59**		**147.73**		**17.84**		**5.98**		**1.19**		**4.29**	
Total std error	**4.51**		**0.69**		**11.64**		**47.22**		**1.44**		**16.17**	
R^2	**0.86**		**0.99**		**0.93**		**0.96**		**1.00**		**0.93**	

2015). Taken together, these findings and the current study suggest that incorporating waste solids through use of intact bitter cassava can be a sustainable waste reduction process that involves cutting down costs of waste treatment and legislation.

3.2. Effect of SRRC on biopolymer derivative products

The biopolymer derivative powders were evaluated in terms of total cyanogen and amylose contents. The Analysis of Variance of 6 responses are summarized in Table 1, showing the portion of the effect estimates explained by each factor and 2-way interaction, as well as the p-values. Statistically significant effects are denoted by bold typeface, whereas the highly significant ones are denoted with an asterisk mark. For each response the effects are shown by a value, irrespective of its sign (positive or negative), to facilitate the analysis of which is more influential in the response.

3.2.1. Biopolymer powder yields

Sodium chloride (NaCl) showed the most statistically significant ($p < 0.05$) effect on yield (Table 1). NaCl had a statistically significant linear (L) and quadratic (Q) effect (Table 1), with higher yields the greater its content (Fig. 2). Peeling also showed a

statistically significant ($p < 0.05$) linear effect (Table 1), and the yields of intact (BI) bitter cassava (Fig. 2a$_1$) were significantly higher than for the peeled (BP) (Fig. 2a$_2$). This difference could be attributed to a differential water holding capacity (WHC) of intact versus peeled material, with the former having lower WHC probably due to their reduced hydration capacity (Sannino, Demitri, & Madaghiele, 2009) and strong chemical bonds. Furthermore, the increased BI yield was due to incorporation of the waste solids. Therefore, it can be concluded that using an optimized SRRC will be more relevant to increase the yields when using intact bitter cassava.

3.2.2. Total cyanogens content

The total cyanogen (TC) content was significantly ($p < 0.05$) reduced in all samples (Peeled, 1.1–4.8 ppm and Intact, 0.4–2.5 ppm) compared to TC content in fresh roots (400–900 ppm) or in defectively processed bitter cultivars (50–135 ppm). The Codex standards recommend 0.02 ppm TC per kg body weight in the daily diets of individuals, which is equivalent to less than 10 ppm in cassava product (FAO/WHO Codex, 2013).

The SRRC process had a greater degradation influence on TC; sodium chloride (NaCl), sulphuric acid (H$_2$SO$_4$) and peeling and
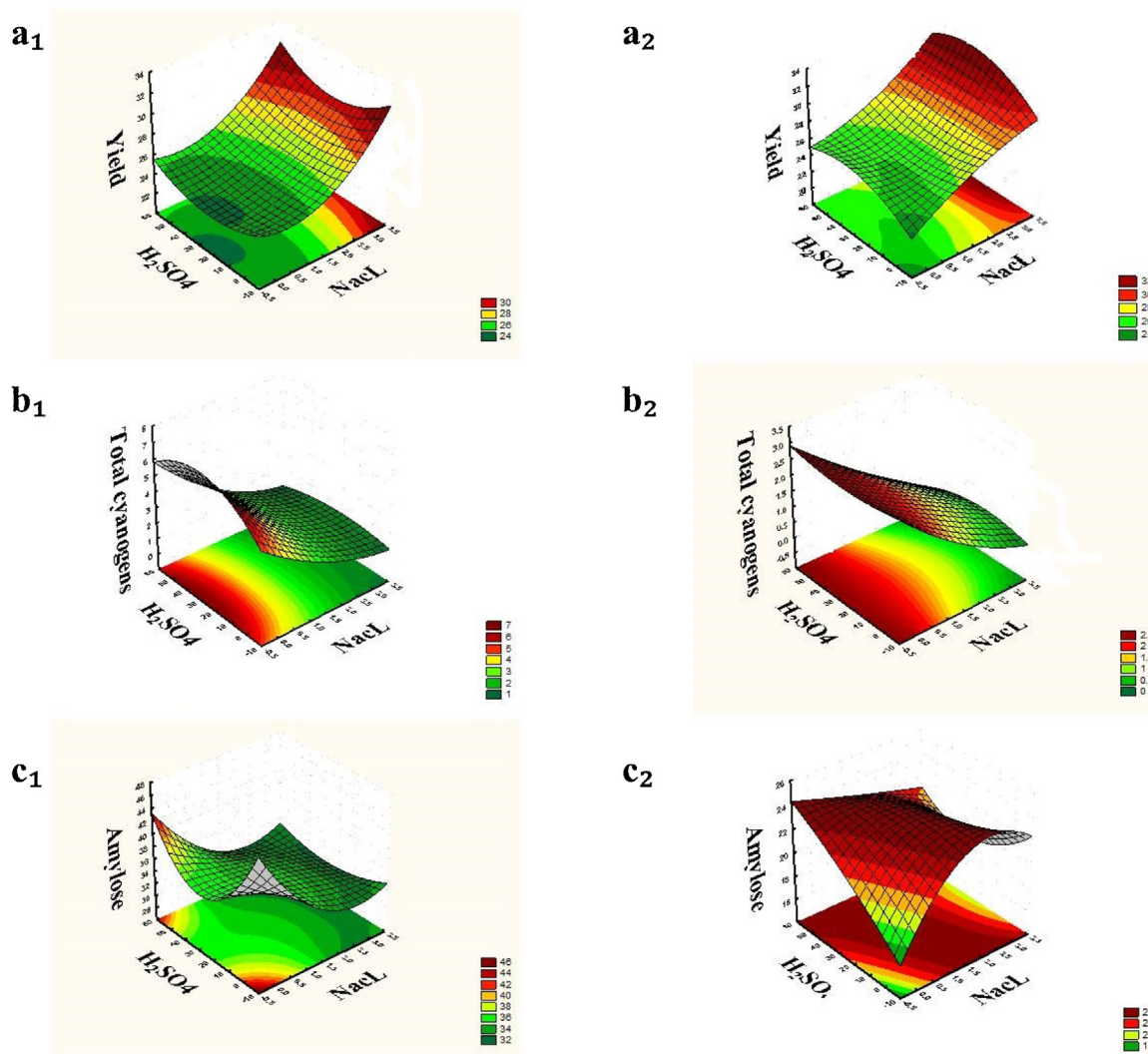

Fig. 2. Yield of BP (a$_1$) and BI (a$_2$), total cyanogens of BP (b$_1$) and BI (b$_2$) and amylose content of BP (c$_1$) and BI (c$_2$) bitter cassava biopolymer derivatives as affected by extraction conditions (NaCl, H$_2$SO$_4$).

their interaction showed highly significant ($p < 0.05$) effects on total cyanogen loss (Table 1). NaCl had linear and quadratic reduction effects on TC (Table 1). Peeling also showed a significant linear effect on TC, both individually and combined with the linear and quadratic NaCl effect (Table 1). H_2SO_4 showed significant quadratic effect on TC, and combined linear effect with NaCl and combined effect with peeling (Table 1); these effects were less pronounced for the intact (BP) (Fig. 2b_1) than for the peeled (BI) (Fig. 2b_2) bitter cassava.

SRRC processing was very effective in reducing the total cyanogen content well below the Codex minimum safety standard in all cassava samples, in comparison to fresh bitter cassava, demonstrating that there is no need for peeling bitter cassava.

3.2.3. Amylose content

Intact bitter cassava showed lower amylose content (18–24%) than peeled (31.4–39%). The peeling showed the strongest significant ($p < 0.05$) effect on the amylose content (Table 1). NaCl showed a significant effect individually and combined with peeling (Table 1). Peeled bitter cassava showed slightly higher amylose content (Fig. 2c_1) than previously reported for sweet cassava varieties ($\sim$15–30% amylose content) (Alves, Mali, Beléia, & Grossmann, 2007; Nuwamanya, Baguma, Wembabazi, & Rubai-hayo, 2011; Souza et al., 2012), whereas, amylose content (%) for intact bitter cassava were within this range (Fig. 2c_2). The amylose content (%) reduction in intact bitter cassava biopolymers could be explained due to the enzymatic hydrolysis during the SRRC process because of the presence of the waste solids. The lower amylose content (%) of the biopolymers produced with the intact roots can explain the more noticeable transparency of these types of films (see Fig. 3).

3.3. Film prototypes

The biopolymer packaging film prototypes from peeled (BP) and intact (BI) bitter cassava powders are shown in Fig. 3. Eighteen formulations produced transparent and flexible films and further characterization was performed in terms of optical, solubility, barrier, mechanical, and thermal properties.

3.3.1. Optical properties

Both peeled (BP) and intact (BI) bitter cassava films were transparent (Fig. 3). The BI films showed lower values of transparency (3.64%) when compared to BP (11.94%) equivalent, indicating that BI films were more transparent (lower transparency %) than BP equivalents. When subjected to the same experimental testing methods and conditions, the BI transparency was found to be comparable to the commercial NatureFlex NVS (NVS) (4.55%) and polylactide (PLA) (3.39%) but much lower than orientated polypropylene (OPP) (13.55%) films. Therefore, BI films were more transparent than OPP and with comparable transparency to other biobased films.

Analysis of the effect of peeling and extraction conditions (NaCl, H_2SO_4) on transparency is presented in Table 1 and Fig. 4a_1,a_2, showing that peeling had a significant ($p < 0.05$) effect on transparency. H_2SO_4 also showed a significant effect individually and combined with peeling (Table 1). The implication of this result is that more transparent films can be produced at low cost from intact bitter cassava by applying an intrinsic modification during the SRRC process.

To further corroborate the transparency results, film color was determined and the analysis for the color difference ($\Delta E_{a^*b^*}$) is shown in Table 1 while for their degree of lightness (L^*) is presented in Fig. 4b. Unlike transparency, peeling and NaCl showed a higher significant ($p < 0.05$) impact on film color, thus confirming that BI (Fig. 4b_2) films were more transparent than those of BP (Fig. 4b_1). The higher BI L^* value is a manifest of better visual characters (minimum haze) of these films when compared to BP.

3.3.2. Solubility of bitter cassava films

Water solubility, as a function of processing conditions (buffer and root type) is shown in Table 1 and Fig. 4c_1,$4c_2$. Generally, films from the peeled (BP) bitter cassava were more soluble compared to the intact (BI) equivalent as shown by a higher significant ($p < 0.05$) effect of peeling on solubility (Table 1). This could be due to adding the waste solids that resulted in slightly higher water-resistant films. An increase in NaCl concentration increased film solubility in all treatment categories. Moreover, highly significant ($p < 0.05$) effect was observed in BP films when NaCl was increased. Although the effect H_2SO_4 was not significant, as the H_2SO_4 concentrations increased, the BP and BI films were difficult to recover, maybe due to fast acidic cellulose depolymerization forming hydrophilic oligomers that were easily lost in water. Nonetheless, the aforementioned solubility effect in the BI films could not match the higher effect in the BP categories, possibly due to water-resistance advantage induced in the film by the waste solids in BI films. The lower BI film solubility, at higher buffer concentrations (NaCl−3 M: H_2SO_4−50 mM), could be due to amorphized cellulose structure caused by enhanced high enzymatic digestibility at high acid concentrations (Ioelovich, 2012) leading to higher solvent mobility. Therefore, BP films produced with higher buffer concentrations (NaCl−3 M: H_2SO_4−50 mM) cannot be used in their

Fig. 3. Film visual images: Transparency of peeled (BP-A) and intact (BI-B) bitter cassava films as affected by peeling and extraction conditions (NaCl, H_2SO_4) I, as potential printable material (II) and bag manufacturing capability (III).
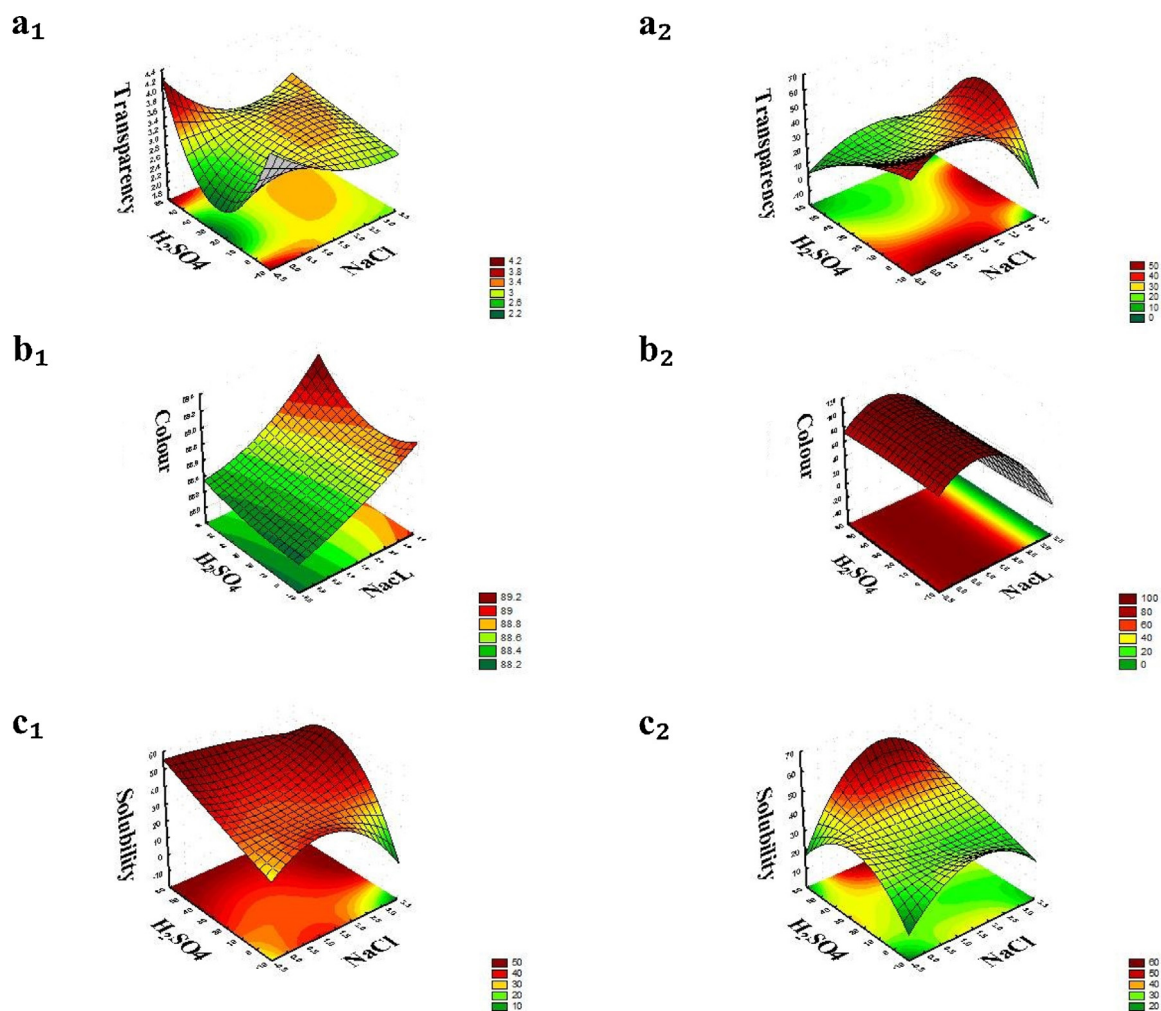
Fig. 4. Transparency of BP (a_1) and BI (a_2), color of BP (b_1) and BI (b_2) and solubility of BP (c_1) and BI (c_2) bitter cassava biopolymer derivatives as affected by extraction conditions (NaCl, H_2SO_4).

Table 2
Comparison of barrier and mechanical properties of intact bitter cassava (BI), commercial (PLA, NVS, OPP) and sweet cassava films.

Property	Film type						
	Intact cassava	Commercial				From previous studies	
	BI	PLA	NVS	OPP	Reference	sweet cassava	Reference
Barrier							
Water vapor transmission rate (g/(m^2 day))	438.6	375.0[ii]	600.0[ii]	20.0[ii]	[ii]Innovia films	648.0	Polnaya, Talahatu, Haryadi, & Marseno (2012)
Oxygen transmission rate (cc/(m^2 day))	812.9	524.9[ii]	20.0[ii]	1693.3[ii]	[ii]Innovia films	21.5×10^{-9a}	Souza, Goto, Mainardi, Coelho, & Tadini (2013)
Carbon-dioxide transmission rate (cc/(m^2 day))	822.3	3080.0[iii]	Nil	Nil	[iii]Curtzwiler et al. (2008)	Nil	Belibi et al. (2014)
Mechanical							
Tensile strength (MPa)	41.1	48.8[i]	70.0[ii]	81.6[i]	[i]ASTM D638-10 (2010) [ii]Innovia films	1.2–2.4	Belibi et al. (2014)
Elongation at break (%)	17.4	9.2[i]	70.0[ii]	65.3[i]	[i]ASTM D638-10 (2010) [ii]Innovia films	26.8–49.4	Belibi et al. (2014)
Elastic modulus (MPa)	200.0	2020.2[i]	>1500[ii]	13.2[i]	[i]ASTM D638-10 (2010) [ii]Innovia films	14.0–63.0	
Seal strength (g(f)/25 mm)	323.0	815[iv]	200[ii]	900[v]	[iv]Hishinuma (2009) [v]Jindal films		

NIL, not found in literature.

[i] International standard methods which are proven, accredited and used widely in packaging film tests. Their use provided the authenticity of the study results.

[ii & v] Test methods which have been used in packaging films produced by recognised manufacturing companies and whose products are applied in packaging a wide range of foods visible on the markets. Their use provided better comparison tests of the commercial films and films in this study, with potential food applications.

[iii & iv] Test methods used in peer reviewed and published articles of recognised and widely cited journals. Their use provided a better comparison of literature and current results.
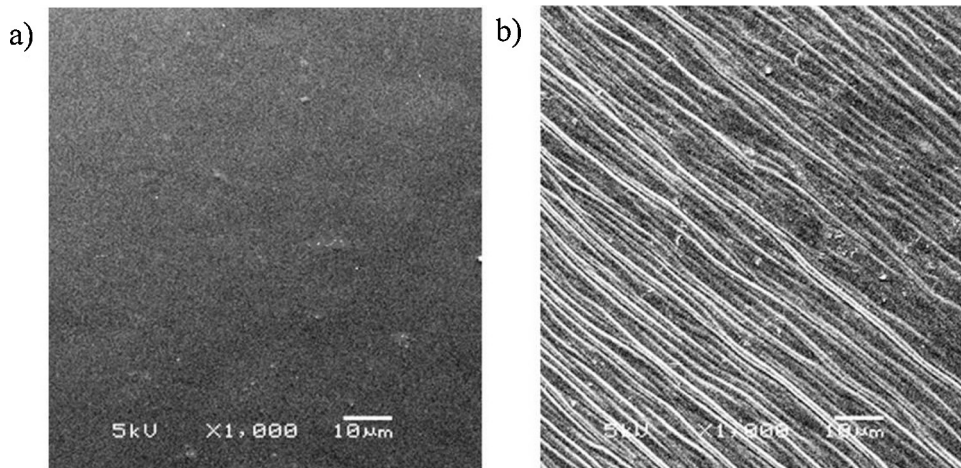
[a] Units given in cm^3/(m day Pa).

Fig. 5. Scanning electron micrographs of intact bitter cassava film surface (a) and cross section (b) morphology.

current form in an environment where a high water resistance is required, and would need further enhancement to reduce their solubility. In summary less soluble films can be produced with intact bitter (BI) cassava, whereas BP films cannot be used on high humidity systems.

Knowledge of film properties is important in assessing package performance. Therefore assessment of BI films in comparison to commercial (OPP, PLA and NVS) films was done and is shown in Table 2, in terms of their barrier, mechanical and thermal properties.

3.4. Film performance properties

3.4.1. Surface

The scanning electron micrograph (SEM) of surface and cross-section is presented in Fig. 1. The film surface homogeneity is evident in Fig. 5a, suggesting that there was complete solubility of biopolymer derivatives in the polymer matrix but also this could have been a result of limited solvent migration at the interface. This result is also supported by the cross-section micrograph (Fig. 5b), suggesting a strong and uniform adhesion of material additives leading to homogenous mesh network structures in the film. Additionally, the result could explain the flexibility which was conspicuously evident in the films.

Knowledge of surface energy is important in determining the printability and adhesion of flexible films. Intact bitter (BI) cassava

films presented contact angles (CA) of 72.7–87.6° between 0 and 180 s, suggesting the presence of polar functional groups in the film structure leading to less hydrophobicity. The polar functional groups were confirmed by FTIR analysis. Despite the low film hydrophobicity but less hydrophilicity tendencies, films exhibited some degree of printability (Fig. 3). The BI cassava films presented the CA within the range of sweet cassava, 73.5–85.2° (de Moraes, Scheibe, Sereno, & Laurindo, 2013), PLA, 73.4–81.0° (Navarro et al., 2008) but lower than OPP, 111.0° (Gourianova, Willenbacher, & Kutschera, 2005) (Fig. 6).

3.4.2. Chemical

The infrared (IR) spectra peak analysis of intact bitter cassava films is shown in Fig. 7. It can be seen that there is peak absence at the 4000–3600 cm^{-1} position, indicating that there were no N—H stretching vibrations of amines or amides. The result meant that the film contained insignificant protein and total cyanogen contents. This is consistent with 0.4–2.5 ppm total cyanogens of the powder derivative used in film formulation. The 3700–3000 cm^{-1} broad spectrum peak due to O—H stretching vibrations (Hinterstoisser & Salmén, 2000), the aromatic CC peaks at 1600–1500 cm^{-1} could be an indication of cellulose, hemicellulose, pectin and lignin (Liang & Marchessault, 1959) in addition to starch incorporation in the film by solid wastes. This result agrees with the cassava waste chemical composition obtained in earlier studies (Babayemi, Ifut, Inyang, & Isaac, 2010).
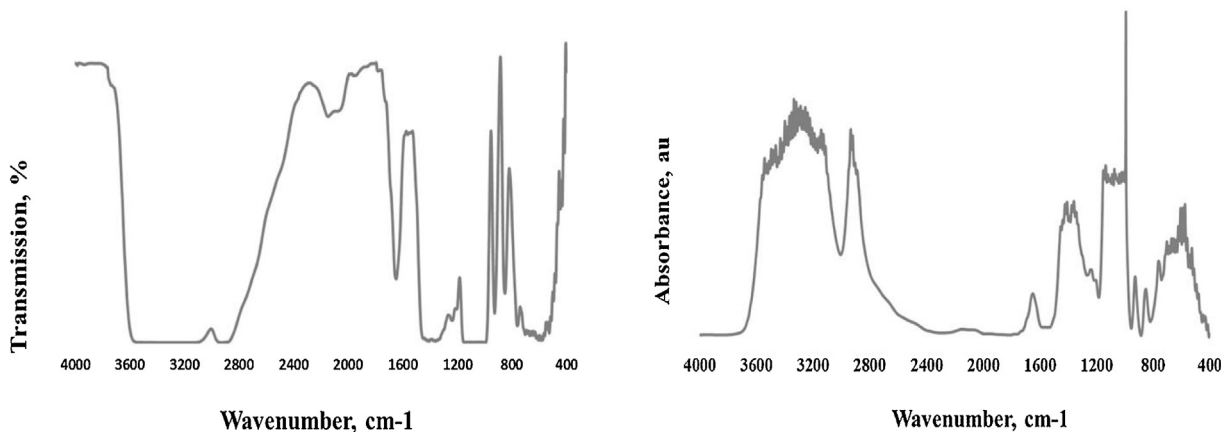

Fig. 6. FTIR spectra of intact bitter cassava films prepared with derivative powder (3 w/v%) and glycerol (30 w/w%).
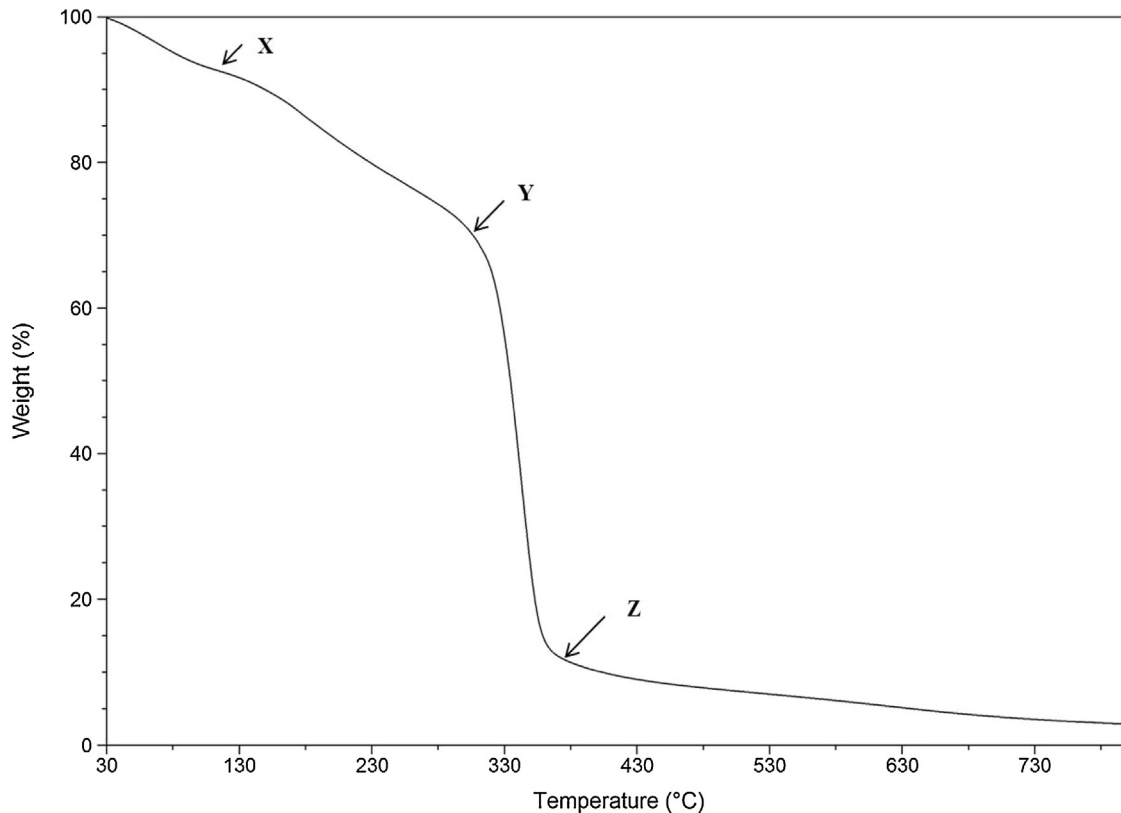
Fig. 7. Thermogravimetric analysis of intact bitter cassava films prepared with derivative powder (3 w/v%) and glycerol (30 w/w%).

3.4.3. Barrier

Intact bitter (BI) cassava films, produced from powders with NaCl: H_2SO_4, 1.5 M: 25 mM and cassava: glycerol: drying temperature, 3 w/v%: 30 w/w%: 50 °C conditions, exhibited different barrier behavior in moisture and gas environments (Table 2). The water vapor permeability of BI cassava films was within the range of commercial polylactic (PLA), Natureflex (NVS) and sweet cassava, and less than that of commercial oriented polypropylene (OPP). Similarly, the BI oxygen transmission rate (OTR) was comparable to commercial PLA but higher than NVS and lower than OPP (Table 2). Conversely, the BI carbon-dioxide transmission rate (CTR) was reasonably lower than PLA, suggesting that the BI structure provided less solubility to carbon-dioxide than that of PLA. Comparably, bitter cassava produced films with lower OTR than commercially available OPP. These results could be an indication of highly amylopectin amorphous network structure of BI imparting less oxygen transfer in the films when compared to OPP and sweet cassava. Regardless of CO_2 importance in modified atmosphere packaging, the noteworthy feature is that the CTR data on the cassava films and its relations to the barrier properties is conspicuously lacking in literature.

3.4.4. Mechanical properties

Intact bitter (BI) cassava film tensile strength (TS), elongation at break (E) and elastic modulus (EM) are shown in Table 2. As can be seen, TS of BI is comparable to commercial PLA, slightly lower than commercial NVS and OPP but higher than sweet cassava. To gain further insight, the E and EM of BI were compared with PLA, NVS and OPP. It was found that BI films yielded lower E than for NVS, OPP and sweet cassava but higher than the E of PLA. Conversely, the EM of BI was lower than that of PLA and NVS but higher than the EM of OPP and sweet cassava.

Thus, it can be concluded that the comparative nature of BI films with commercial films could suggest that they have potential commercial application.

3.4.5. Seal integrity

Intact bitter (BI) cassava films presented comparable seal strength with commercial Natureflex, NVS but lower strengths than PLA and OPP (Table 2). The BI films exhibited lasting seal strengths when left exposed to ambient conditions (15–20 °C; 50–60% RH) for 12 h beyond which the seals showed a gradual separation. However, in all the replicates tested, there was no evidence of instant separation, suggesting that they were less brittle and the film surfaces adhered adequately. The gradual loss of strength suggests that the films could be applied in moderately high temperatures and low humidity to form the internal lining of food bags.

3.4.6. Thermal

Like barrier and mechanical properties, glass transition (T_g) temperature, melting (T_m) temperatures, heat of fusion (ΔH) and crystallinity (C) of intact bitter (BI) cassava were compared to commercial films. BI cassava produced films with slightly lower T_g (54.3 °C) than commercially available polylactic acid (PLA) (60.0 °C), and comparable T_m (210.0 °C) to PLA (170–230 °C) (Curtzwiler, Vorst, Palmer, & Brown, 2008). The BI film ΔH (67.0 J/g) was in a similar range with LLDPE (61.97 J/g), but higher than PLA (23.4 J/g), whereas, the C of BI (55.7%) exhibited higher values than those of PLA (12.9%) and LLDPE (45.2%) (Imai & Ametani, 1996). The comparable T_g and higher C could be due to the influence of SRCC on BI films, turning them into semi-crystalline state and falling into other semi-crystalline polymer category e.g., linear polyethylene, PE (Ehrenstein & Theriault, 2001). Conversely,

the higher T_m could be due to higher structural regularity of films imparted by incorporation of the waste solids. These findings suggest that intact bitter cassava films can withstand thermal conditions similar to the other commercial films.

Thermogravimetric analysis (TGA) of intact bitter (BI) cassava films is shown in Fig. 7. As shown at point *X*, the film weight loss of slightly less than 10% over 180 °C was due to bound water. This behavior is similar to the one observed for most polymer networks (Gonsior, Mohr, & Ritter, 2012), although the loss is not much pronounced as shown by the linearity in the graph (Fig. 7). The uniform decomposition in film structure (region XY) could be attributed to other volatile compounds in film contributed by inclusion of the waste solids. However, BI cassava films showed high thermal stability up to Z, corresponding to 373.06 °C, the onset of total degradation (OTD). Although BI cassava film total weight loss (84.52%) is slightly higher than values found for other polymers (>30%), the thermal stability loss to OTD point (373.06 °C) of BI cassava films is higher than previously reported range (340–360 °C) for most polymer networks (Gonsior et al., 2012).

4. Conclusion

Intact bitter cassava showed significantly ($p < 0.05$) higher yields than peeled equivalent, and increasing with an increase on both extraction buffers. Intact bitter cassava produced an average 16% waste decrease, showing higher yield due to incorporation of the waste solids, and no environmental impact caused by discard residues. The amylose content was significantly ($p < 0.05$) lower for the intact bitter cassava than for the peeled equivalent. Simultaneous release recovery cyanogenesis (SRRC) processing of bitter cassava was very effective in reducing the total cyanogen content well below the Codex minimum safety standard level in all cassava samples, in comparison to fresh bitter cassava. This outcome demonstrates that there is no need for peeling bitter cassava, and presents promising results for its application to food and non-food applications.

The films were produced using either peeled (BP) or intact (BI) bitter cassava. BI films showed to be more transparent, homogeneous, exhibited lower solubility and hydrophilicity, higher WVTR, moderately OTR and CTR, with higher strength (higher TS, and EM) and thermal stability (higher T_m, sealable) than BP and commercial PLA, but with lower strength than NVS and OPP. The study showed that BI films can be produced at lower cost than the peeled (BP) equivalents.

Therefore, safe, high value-added biopolymers, with potential application for food packaging can be produced from intact bitter cassava, minimizing waste and environmental impact, generating practical applicability and contributing to a sustainable system. Furthermore, it is noteworthy that although the goal of this study was a proof of concept to develop film prototypes from intact bitter cassava, the film production could be potentially scale up using tape casting as reported by de Moraes et al. (2013).

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