



## Integrated process standardisation as zero-based approach to bitter cassava waste elimination and widely-applicable industrial biomaterial derivatives



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### ABSTRACT

Integrated standardised methodology for biopolymer derivatives (BPD) production from novel intact bitter cassava was demonstrated by desirability optimisation of simultaneous release, recovery, cyanogenesis (SRRC) process. BPD were evaluated for yield and colour using buffer (0, 2, 4% v/v), cassava waste solids (15, 23, 30% w/w), and extraction time (4, 7, 10 min). Nearly all the root was transformed into BPD, with higher yield and colour in comparison to starch extrinsically processed. Maximum global desirability, predicted efficient material balance, buffer 4.0% w/v, cassava waste solids 23% w/w and extraction time, 10 min, producing BPD yield, 38.8% wb Validation using buffer, 3.3% w/v, cassava waste solids, 30% w/w and extraction time, 10 min, produced 40.7% wb BPD. SEM, DSC, TGA, FTIR and moisture barrier analyses revealed a uniform microstructure and high thermal stability of BPD and film, thus demonstrating efficient performance of the standardised integrated methodology. Hence, processing intact cassava root as a standardised integrated methodology could be used to produce sustainable low cost BPD for a broad range of applications.

Methodologies designed around standard integrated procedures, matching zero-based approach to contamination, are novel strategies, and if used effectively can eliminate cassava wastes and recover BPD resources as sustainable biomaterials.

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## 1. Introduction

Environment-borne cassava wastes represent a potential economic source of biopolymer derivative cellulose, hemicellulose, pectin, lignin and starch [6,21]. Cassava waste solids could support the sustainable production of low-cost industrial bioproducts such as food and non-food added value products. The global turnaround concept of regarding waste as a worthless material to the idea of a high demand secondary material resource has widened value-added waste research. Currently, research emphasis is focused more on waste minimisation than waste recycling [18].

Among the cassava varieties, bitter cassava contributes a greater amount of disposed environmental waste, >16% [36] as compared to sweet cassava, 0.5% [16]. Unfortunately, bitter cassava (BC) waste minimisation, has not yet received much attention, therefore the environmental accumulation of BC wastes has been inevitable. Traditionally, there have been initiatives to transform BC into food and other low-value products such as fermented crude ethanol and flour [35], gari [1]. However, total cyanogens, inherent in these varieties and poor processing methodologies have impeded the efforts with negative environment impacts. Meanwhile, sweet cassava starch production, using reinforcements and modifications of biopolymer derivatives (SC-BPD) for various applications have also been studied [27,38]. Although, such procedures require high energy leading to higher production costs. Moreover, starch processing using added cellulose materials, have been limited by non-uniformity and less compatibility [5,25], requiring further additional chemical and physical modifications and costs. Recently, a novel methodology, using simultaneous

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release, recovery and cyanogenesis (SRRC), to transform intact BC wastes into safe (total cyanogens, <1.0 ppm) BPD with significant high yields, has been reported [36]. Due to these findings, SRRC methodology could be successfully employed to produce safe intact BC-BPD.

Standardising methods of producing materials could ease the choice and cost of formulations, by defining the design space [8,14], processing parameters and material functional properties, which could lead to the engineering design of tailored food and non-food added value products. Design of experiments (DOE) has been successful in simultaneous investigation of the effect of multiple variables, to determine the most efficient and economic matrix formulations needed for optimal formulations [30,2,33]. Robust production processes provide methodologies for balancing desired material properties with processing parameters, marginal costs and maximum functionality. Desirability function approach is extensively employed in the optimization of multiple response processes, in which the operating parameters provide the “most desirable” responses [20,22]. In this study “Pareto front approach” was explored to determine possible optimal solutions known as Pareto solutions or Pareto fronts. According to [34], allocations to parameter choices are made in such a way that tradeoffs ensure unequal distribution, in which one factor is constrained in place of the alternative so as to find feasible choices that lie on the Pareto front. It is worth noting that choices on a Pareto frontier are Pareto efficient and non-dominated by any other choice.

In order to broaden ways of modifying native polysaccharides and produce new materials for the utilisation of cassava in food packaging and other potential industries, customised methods that ensure combined release of BPD are necessary.

To the best of our knowledge SRRC, integrated process, optimisation of BC-BPD production, to minimise production costs, and standardisation of design methodology have not been studied. Thus, it was justifiable to develop a standardised integrated sustainable low-cost methodology to produce BPD from novel intact BC root.

The purpose of this study was to develop an integrated standardised process methodology for novel intact BC-BPD recovery, and evaluate its impact on BPD. The optimum processing parameters necessary to obtain maximum yield and colour as defined by maximum global desirability, and development of a standardised methodology by validation of optimal BPD were investigated. The potential of applying BC-BPD as food and non-food material was determined by assessing the effect of standardised methodology on BPD's quality (physical, chemical and safety), and film moisture barrier characteristics.

**Table 1**

Box-Behnken design matrix: Actual/(coded) variables of processing parameters and biopolymer derivatives (BPD) yield and colour.

Runs	Actual/coded independent variables			Thermoplastic derivatives	
	Buffer concentration, w/v%	Cassava waste solids, w/w%	Extraction time, Min.	Yield, %	Colour change, ΔE
1	0.0 (−1)	23.0 (0)	4.0 (−1)	28.0 ± 0.1 a	6.3 ± 0.1 a
2	2.0 (0)	23.0 (0)	7.0 (0)	32.9 ± 0.2 b	4.3 ± 0.0 b
3	4.0 (1)	30.0 (1)	7.0 (0)	38.0 ± 0.3 c	5.6 ± 0.1 c
4	2.0 (0)	15.0 (−1)	10.0 (1)	27.9 ± 0.3 a	4.8 ± 0.0 d,b
5	4.0 (1)	23.0 (0)	4.0 (−1)	27.9 ± 0.4 a	5.8 ± 0.0 a,c,e,g,l
6	2.0 (0)	23.0 (0)	7.0 (0)	26.7 ± 0.5 a	5.5 ± 0.0 e,c
7	0.0 (−1)	23.0 (0)	10.0 (1)	27.0 ± 0.4 a	5.2 ± 0.1 f,c,d,e
8	2.0 (0)	30.0 (1)	10.0 (1)	27.9 ± 0.4 a,j	6.3 ± 0.1 a
9	0.0 (−1)	15.0 (91)	7.0 (0)	27.8 ± 0.4 a,k	6.1 ± 0.0 a,g
10	2.0 (0)	23.0 (0)	7.00 (0)	33.8 ± 0.2 d,b	5.7 ± 0.0 g,c,e,f
11	2.0 (0)	15.0 (−1)	4.0 (−1)	37.1 ± 0.3 e,b,c	5.1 ± 0.0 h,c,d,e,f
12	4.0 (1)	15.0 (−1)	7.0 (0)	31.1 ± 0.3 f,d	4.9 ± 0.1 i,d,h,f,j
13	0.0 (−1)	30.0 (1)	7.0 (0)	23.0 ± 0.4 g	4.7 ± 0.1 j,b,d,f,h
14	4.0 (1)	23.0 (0)	10.0 (1)	38.8 ± 0.1 h,c,e	4.1 ± 0.0 k,b
15	2.0 (0)	30.0 (1)	4.0 (−1)	23.1 ± 0.3 i,g	5.4 ± 0.0 l,f,g,h,j

Marked differences (subscripts) are significant at  $p < 0.05$ .

## 2. Materials and methods

### 2.1. Integrated process methodology for production of novel intact BC-BPD

#### 2.1.1. Intact bitter cassava preparation

Bitter cassava, Tongolo, procured from farmers' fields, Northern Uganda was separated from soil debris, placed in ice boxes, transported to the laboratory and kept at  $-20^{\circ}\text{C}$  for further use.

#### 2.1.2. Integrated process methodology

Production of biopolymer derivatives followed the procedure developed earlier for peeled and intact roots via the simultaneous release, recovery and cyanogenesis (SRRC) process [36] with slight modifications. Mechanical tissue rupture and cell disruption of intact bitter cassava (BC) roots were performed using motorised method in a high-speed grating pulper (6000g).

SRRC was found to be crucial in the biopolymer derivatives (BPD) production as the process release stage and processing parameters influenced positively the yield and properties of BPD [36]. Effects of various parameters at three levels, buffer, 0, 2, 4% v/v, cassava waste solids, 15, 23, 30% w/w, and extraction time, 4, 7, 10 min on biopolymers yield and colour were evaluated using a pre-designed experiment (Subsection 2.3.1). During biopolymers' release stage, 100 g of pulp mass was mixed with 100 ml of various extraction buffers in a commercial blender (500 W Breville IHB086 Hand Blender), and the mixtures homogenised according to set time (Table 1) at ambient temperature ( $20\text{--}23^{\circ}\text{C}$ ). The buffers used in release and recovery, i.e., sodium chloride ( $\geq 99\%$  AR) and conc. sulphuric acid (99.9%) were analytical grade from Sigma Aldrich (Ireland).

Recovery was achieved by centrifuging the slurry at 8000g,  $5\text{--}7^{\circ}\text{C}$  for 10 min, washing in deionised water ( $3\times$ ) and drying semi-dehydrated coarse pulp in an air-circulating, temperature ( $30 \pm 5^{\circ}\text{C}$ ), relative humidity (30–40% RH), and constant weights obtained after 9 h. The dried samples were weighed, colour measured and milled to a fine powder using an analytical grinder (IKA Yellowline-RA 10, Germany) and kept refrigerated ( $4\text{--}7^{\circ}\text{C}$ ) between tests and further use.

### 2.2. Optimisation via desirability approach and statistical analysis

#### 2.2.1. Factorial and box-behnken response surface experimental design

The BPD production was performed using a factorial and Box-Behnken response surface experimental design methodology. The individual factors and levels chosen for Box-Behnken design are shown in Table 1.

A factorial analysis was defined to determine the functional relationship between processing parameters and yield, colour responses, and their combined interaction effects.

Yield was defined as the percentage of constant weight dried powder recovered from initial mass of 100 g roots, and determined in triplicates.

Colour difference ( $\Delta E$ ) was determined according to [28] using CR-400 Chroma Meter, Konica Minolta Sensing Japan without major changes. Measurements were taken, in triplicates, on derivative powders and BI each, and mean values used in CIELAB  $L^*$ ,  $a^*$ ,  $b^*$  using the Eq. (1) as described [29].

$$\Delta E = \sqrt{[(\Delta L'/S_L)^2 + (\Delta C'/S_C)^2 + (\Delta H'/S_H)^2 + R_T(\Delta C'/S_C)(\Delta H'/S_H)]} \quad (1)$$

where  $\Delta E$ , differences between sample and standard (S) colour parameters; S is background colour reference parameters;  $\sqrt{\quad}$ , square root symbol;  $\Delta L'$   $\Delta C'$   $\Delta H'$ , lightness, chroma and hue differences.

A statistical analysis and individual parameter empirical model equations for yield and colour were performed as reported [39] with slight modifications. The design matrix for both processing parameters (actual/coded independent variables) and responses (yield and colour), with a total of 15 experimental runs are presented in Table 1. Each run was an average of 3 replicates. Data was fitted to four models (linear, combined two factor interaction, quadratic) in order to describe the adequacy of the experimental linear models and determine the second order polynomial equations, their regression coefficients and  $R^2$  values. The aliased cubic model was not considered for analysis. Analysis of variance (ANOVA) was used for regression coefficient determination, lack-of-fit test and significance of curvature effect. The model adequacy was determined by lack-of-fit test, residual analysis and coefficient of determination ( $R^2$ ) and illustrated visually by contour plots. Following, processing parameters were harmonised with properties to determine significant effects for optimisation purposes.

2.2.2. Simultaneous optimisation using desirability function

Response surface methodology was applied to quantify factor-response associations in simultaneous determination of optimal processing parameters considering yield and colour by desirability function. Consideration of waste solids reinforced starch was important in order to gauge intact bitter cassava ability to produce biopolymer derivatives with desirable yield and colour (towards brighter colours). The lower and upper limits were set for buffer (2 and 4% v/v), cassava-rich waste solids (15 and 30% w/w) and extraction time (4 and 10 min).

The simultaneous optimisation (desired functional combination) of buffer concentration, waste solids, extraction time (material balance) and biopolymer derivatives yield and colour were achieved by a desirability (D) approach (Eq. (2)) and suggested by Derringer [13]. The method is reported widely in literature but also common for multiple responses optimisation in many industrial applications.

$$D = [d_y(Y) \times d_c(C)]^{1/n} \quad (2)$$

where, D, desirability; Y, yield (%);  $d_y$  (Y), yield desirability function, C, colour difference ( $\Delta E$ );  $d_c$  (C), colour desirability function; n, responses ( $n=2$ );  $d_y$  (Y) and  $d_c$  (C)=0, perfectly undesirable;  $d_y$  (Y) and  $d_c$  (C) = 1, perfectly desirable.

Desirability function methodology determines the working (active) parameters (independent variables), which provide the best desirable responses (dependent variables). For a given dependent variable  $Y_i(x)$ , a desirability (D) function  $d_i(Y_i)$  assigns “0–1” numbers to possible values of  $Y_i$ , with  $d_i(Y_i)=0$  (entirely undesirable  $Y_i$  value) and  $d_i(Y_i)=1$  (absolutely desirable  $Y_i$  value). Individual desirabilities (Ds) by geometric mean give a global desirability (GD), such that,  $= [d_1(Y_1) * d_2(Y_2) * d_3(Y_3) \dots \dots d_\alpha(Y_\alpha)]^{1/\alpha}$ , where,  $\alpha$ , number of dependent variables (responses). Practically, if any response is completely undesirable, i.e.  $d_i(Y_i)=0$ , then  $GD=0$ . Thus, according to [13], a response can be assigned a target, minimised or maximised (optimised). The whole concept is illustrated in Plate 1.

Power (exponent), s and t, determine how essential it is to hit the target value. For  $s=t=1$ , the desirability function increases linearly towards  $T_i$ ; for  $s < 1, t < 1$ , the function is convex, and for  $s > 1, t > 1$ , the function is concave.

In this study, for biopolymer yield, the desirability function demanded maximisation while colour difference minimisation. To achieve the preceding situation, the criteria set for material balance was: buffer, maximise concentration (range 0–4% w/v); waste solids, in range content (15–30% w/w); extraction time, maximise frequency (range 4–10 min).

Statistica 7.1 software (StatSoft Inc., Tulsa, USA) was used for experimental design, quadratic model buildings, response surfaces and charts generation, and numerical and graphical optimization.

2.3. Standardisation of integrated process methodology by validation of optimal models'

Standardised methodology by validation of optimisation was accomplished by running experiments using optimal formulations.

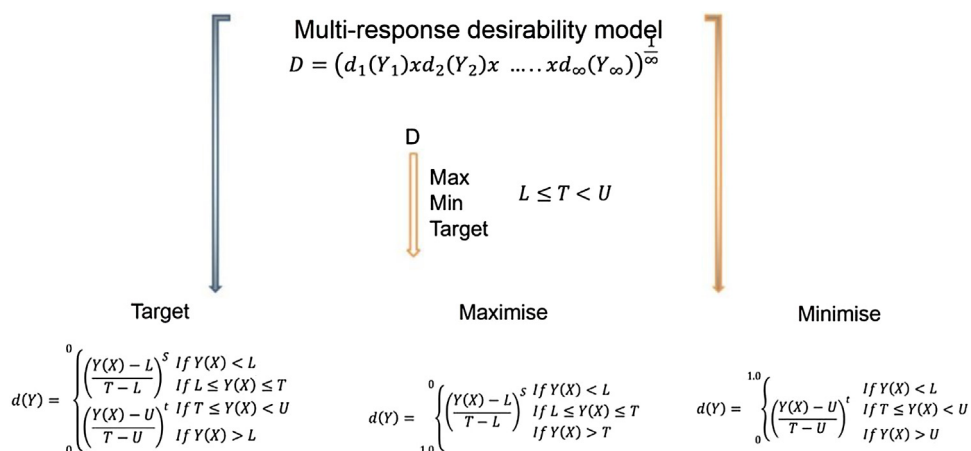


Plate 1. Desirability function concept for using in multiple variable optimisations.

Results thereof were compared with optimal values in order to set standards for intact BC-BPD production.

Scenarios were developed based on set objectives i.e. ‘minimise’, ‘in the range’ and ‘maximise’, and using Design Expert (Version 9.0.4.1, State-Ease, Inc. 2015, Minneapolis, USA) and non-repetitive permutation approach, the most promising scenarios with the highest desirability (D) were found to better describe optimisation process.

In addition, a maximum global desirability (GD) was achieved for both parameters and responses, and predicted the optimal processing parameters from the material balance.

#### 2.4. Impact of standardisation on biopolymer derivatives and packaging film moisture barrier properties

##### 2.4.1. Effect on total cyanide decontamination and BPD appearance

Biopolymer derivatives safety and appearance were determined by running two related experiments: (i) testing the effect of ionic buffer (0, 2, 4% w/v), waste solids (15, 23, 30% w/v), and sodium bisulphite (1, 2, 3% w/w of waste solids) on total cyanogens (TC) and colour difference ( $\Delta E$ ); and (ii) sodium chloride (0, 1.5, 3 M), sulphuric acid (0, 25, 50 mM), and peeling (peeled, intact) on TC. The TC was determined using the cyanide kit developed by Bradbury et al. [9] while  $\Delta E$  was measured as described in Section 2.3.1.

##### 2.4.2. Microstructure and chemical

Biopolymer derivatives microstructural properties were examined using Scanning Electron Microscope (SEM), JSM-5510 (Jeol Ltd., Tokyo, Japan). A small amount of derivatives powder was placed on stubs using double-sided carbon tape to form a very thin layer and leaving a space on either side of the strip to allow clear observation of surfaces and cross section. Prior to capturing SEM images, powder stubs were spluttered with a thin layer of gold. Powder stubs were subjected to a focus magnifications as high as 20,000 $\times$  and images capture between 200 $\times$  and 30,000 $\times$  magnification and intensity of 5 kV.

Biopolymer derivatives were analysed for their chemical composition and their possible interactions resulting from use of intact root and modification by SRRC using Fourier transform infrared spectroscopy (FTIR). A small sample of the derivative powder was mixed with potassium bromide in a mortar while under the lamp heater, converted into thin pellets using a timed pneumatic press for 20 s and placed in the sample holder. The spectra were recorded with an UV/Vis spectrum one FTIR spectrometer (Perkin Elmer Lambda 35, USA), frequency range of 4000–400  $\text{cm}^{-1}$  and 4  $\text{cm}^{-1}$  resolution in the transmittance and absorbance modes for individual spectrum with 30 scans at room temperature.

##### 2.4.3. Thermal

Biopolymer derivatives thermal analysis, glass transition ( $T_g$ ) and melting ( $T_m$ ) temperatures and crystallinity (C), was conducted using a differential scanning calorimeter (DSC 200 F3) equipped with a thermal analysis data station. A hermetically sealed DSC pan with fresh derivative powder (10 mg), together with a reference empty pan were heated from 20 to 250  $^{\circ}\text{C}$  at a rate of 10  $^{\circ}\text{C}/\text{min}$ , cooled back rapidly to 20  $^{\circ}\text{C}$  and reheated at a rate of 5  $^{\circ}\text{C}/\text{min}$  to 250  $^{\circ}\text{C}$  to give them thermal history.  $T_g$ ,  $T_m$ ,  $\Delta H$  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 derivatives using TG Analyser (Spectrum 500) and analysed by the Universal Analysis 2000 (New Castle USA) between 30  $^{\circ}\text{C}$  and 500  $^{\circ}\text{C}$ , heating rate of 20  $^{\circ}\text{C}/\text{min}$ , nitrogen

flow of 60 mm/min. Prior to analysis, each sample was corrected against a background scan. All samples were evaluated in triplicate and mean measurements reported.

##### 2.4.4. Moisture adsorption

Thermoplastic derivative powder (100 g) was dried (48 h) in a vacuum oven (60  $^{\circ}\text{C}$ ) and cooled in a desiccator (2 h). Its moisture content was 8% dry weight basis. The sample was placed in different relative humidity (10–90% RH) using different saturated salt solutions (LiCl,  $\text{CH}_3\text{COOK}$ ,  $\text{MgCl}_2$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{Mg}(\text{NO}_3)_2$ , NaBr, NaCl, KCl,  $\text{K}_2\text{SO}_4$ ) at 20  $^{\circ}\text{C}$ . Weight gains by the moisture-adsorbed powder, at regular interval (every 48 h), were obtained using an analytical balance (precision, 0.0001 g). Moisture adsorbed was reported as a percentage on dry weight basis. Three replicates were run.

##### 2.4.5. Film preparation

Films were produced by solution casting using the procedure reported by Tumwesigye et al. [36]. Mixtures of BPD (3% w/v) and glycerol (30% w/w) were heated at 70  $^{\circ}\text{C}$  for 25 min. Prior to moisture barrier characterisation, films were conditioned at  $23 \pm 2^{\circ}\text{C}$  and 54% RH. Thickness was measured in six different locations with using an absolute digital Calliper (Digmatic, Mitutoyo UK Ltd).

##### 2.4.6. Film moisture barrier characterisation

Determination of moisture adsorption (MA) characteristics followed the method described for BPD in Section 2.4.4 with slight modifications. Film strips (3  $\times$  1.5 cm) were pre-dried until constant weight for 9 h at 90  $^{\circ}\text{C}$ .

Water vapour permeability (WVP) of the films was determined according to ASTM E96-05 (2005) method at 10, 20, 30, 40  $^{\circ}\text{C}$  and at a gradient from 0 to 75, 85 and 95% relative humidity. Results were expressed in  $\text{gmm}/(\text{m}^2 \text{ s kPa})$ .

##### 2.4.7. Moisture barrier modelling

The MA and WVP data were fitted to different models (Table 4) in order to correlate equilibrium moisture content and water activity. Model parameters were estimated by the non-linear regression procedure using Excel (2010) solver and goodness of fit evaluated as mean relative percentage deviation in modulus,  $\rho$ , % (Eq. (3)) and regression variance, VR (Eq. (4)). Accurate mathematical description of isothermal was considered when  $\rho \leq 5\%$ ,  $\text{VR} \leq 5$  and  $R^2 \leq 0.97$ .

$$\rho, \% = \frac{100 \sum_{i=1}^n |\varepsilon_i|}{n \sum_{i=1}^n X_{i(o)}} \quad (3)$$

$$\text{VR} = \sum_{i=1}^n \frac{|X_{i(p)} - X_{i(o)}|^2}{n - 1} \quad (4)$$

where, n, number of experimental points;  $\varepsilon_i$ , absolute value; (p) and (o), predicted and observed.

The effect of standardisation on the water uptake and diffusion characteristics of intact bitter cassava films was determined using Eq. (5) following the procedure described by Sultana and Khan [32].

$$\frac{M_t}{M_e} = 2 \left( \frac{D_t}{\pi l^2} \right)^{\frac{1}{2}} \quad (5)$$

Where,  $M_t$ , moisture weight uptake at time (t);  $M_e$ , moisture weight uptake at equilibrium (e); l, film thickness; and D, diffusion coefficient, which was calculated from the slope by plotting  $M_t/M_e$  against  $t^{1/2}$ .

To predict long-term behaviour of intact bitter cassava materials, the temperature-time dependence of WVP was determined by fitting experimental data to Arrhenius and time-temperature superposition of William-landel-Ferry (WLF) models.

### 3. Results and discussion

#### 3.1. Integrated process methodology for production of novel intact bitter cassava biopolymer derivatives (BC-BPD)

Process integration methodologies are fundamental techniques for designing sustainable methodologies, processes and materials for broad range applications. In this study, a process integration methodology was developed, optimised, and standardised to provide important means of achieving environmental-resilient approaches for production of intact bitter cassava biopolymer derivatives suitable for both food and non-food applications.

The standardised methodology demonstrates an integrated process that uses cheap novel intact bitter cassava and SRRC approach for sustainable biopolymer derivatives production and cassava waste reduction.

#### 3.2. Optimisation of BPD production by desirability

Yield is an indicator of any process efficiency and economy [24]. Biopolymer derivatives (BPD) yield is shown in Fig. 1 & Table 1, revealing that parameter main effects and their interactions had a significant ( $p < 0.05$ ) impact on yield. Except for buffer-waste solid and buffer-extraction time interactions with linear and quadratic negative impacts, all main effects and their sole interactions with time had linear positive effects on yield. The increased yield signalled the intact BC-SRRC process efficiency. Unlike sweet cassava (SC) which yielded 17–26% w.b., intact BC-BPD presented higher yields (23–39% w.b.) when SRRC was employed [36], signifying that BC could be the future sustainable source of BPD production for industrial applications.

Colour difference ( $\Delta E$ ) results are presented in Fig. 1 & Table 1. Extraction time had a highly significant positive linear effect as compared to linear buffer positive, buffer-extraction time positive and buffer-waste solid interaction negative effects on  $\Delta E$ . In this study, major parameter interactions presented negative effects on  $\Delta E$ . Similarly, it was shown that increasing extraction time reduced the BPD brightness, due to high positive effects. Conversely, negative parameter interactions (I) impacts on  $\Delta E$  implied brighter colours. Thus, optimising the formulation was necessary to obtain trade-offs and produce industrial-appealing BPD.

Production of intact BC-BPD through integrated methodology showed, 0–1.3, 92, 3.6 for  $\Delta E$ , luminosity parameter ( $L^*$ ) and chroma ( $C^*$ ), respectively. Production of SC-BPD from peels and bagasse [38], showed,  $\Delta E$ , values ranged from 11 to 28, 10–22 and 78–90 for  $\Delta E$ ,  $L^*$ , and  $C^*$ , respectively. Comparably, previous work using intact SC root under similar SRRC processing conditions produced BPD with 1.1–4.6, 88–92 and 0.5–1.0 for  $\Delta E$ ,  $L^*$ , and  $C^*$ , respectively [36].

Therefore, an integrated process methodology and SRRC produced BPD higher yields and lower colour difference ( $\Delta E$ ) than previously reported, providing positive evidence for future production of sustainable low-cost BPD.

Variations in processing parameters led to significant changes in BPD yield and colour (Table 2). The ANOVA regression coefficients,  $p$ -values,  $R^2$ , significant curvature and lack of fit test (Table 2) showed that the quadratic models were highly significant ( $p < 0.05$ ) for all responses while an aliased condition occurred for cubic models. Therefore, the second order polynomial model equations (Eqs. (6) & (7)) adequately described the association between buffer concentration, waste solid content, extraction time

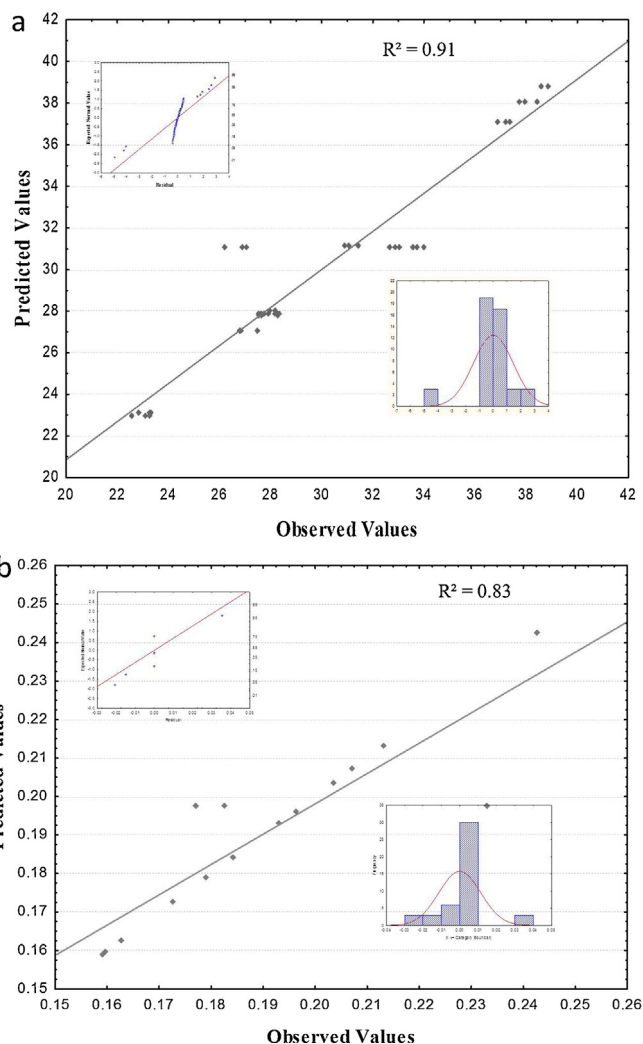


Fig. 1. Model fitting, residual plots and normal distribution for effect of processing parameters on biopolymer derivatives (BPD) (a) yield and (b) colour change,  $\Delta E$ .

and BPD yield and colour. Additionally, yield and colour  $R^2$  of greater than 90% and 82% respectively suggested that data explained the adequacy and significance of the models.

$$\begin{aligned} \text{Yield, \%} = & 31.110 \pm 0.56 + 2.901 \pm 0.49 \mathbf{B} - 3.483 \pm 0.49 \mathbf{W} - 1.123 \\ & \pm 0.49 \mathbf{T} + 2.939 \pm 0.49 \mathbf{BW} + 2.974 \pm 0.49 \mathbf{BT} + 3.515 \pm 0.51 \mathbf{WT} \\ & + 0.152 \pm 0.51 \mathbf{B}^2 - 1.276 \pm 0.51 \mathbf{W}^2 - 0.825 \pm 0.69 \mathbf{T}^2 + 1.696 \pm 0.69 \\ & \mathbf{BW}^2 + 3.991 \pm 0.69 \mathbf{B}^2\mathbf{W} + 3.590 \pm \mathbf{BT}^2 \quad (R^2 = 0.91) \end{aligned} \quad (6)$$

$$\begin{aligned} \text{Colour, } \Delta E, \% = & 5.142 \pm 0.11 - 0.388 \pm 0.09 \mathbf{B} + 0.442 \pm 0.09 \mathbf{W} \\ & + 0.142 \pm 0.09 \mathbf{T} + 0.534 \pm 0.09 \mathbf{BW} - 0.140 \pm 0.09 \mathbf{BT} + 0.276 \\ & \pm 0.09 \mathbf{WT} + 0.068 \pm 0.10 \mathbf{B}^2 + 0.127 \pm 0.10 \mathbf{W}^2 + 0.136 \pm 0.10 \mathbf{T}^2 \\ & + 0.306 \pm 0.13 \mathbf{BW}^2 - 0.638 \pm 0.13 \mathbf{B}^2\mathbf{W} - 0.837 \\ & \pm 0.13 \mathbf{BT}^2 \quad (R^2 = 0.83) \end{aligned} \quad (7)$$

where, B, buffer concentration (% w/v); W, waste solid (% w/w); T, extraction time.

Processing parameters had a higher influence on yield than colour difference ( $\Delta E$ ), as shown by results from Eqs. (6) and (7). However, based on the importance of both yield and colour of BPD, a trade-off was necessary in order to balance their production, leading to acquisition of an optimal formulation. Thus, the criteria were set to establish the maximum yield and minimum  $\Delta E$  with the best desirability function (D). Accordingly, 21 scenarios were

**Table 2**

Analysis of variance of effects of processing conditions on (a) total cyanogen and colour, and (b) peeling on total cyanogen.

a)		
Parameter	Sum of Squares (SS)	
	Total cyanogens	Colour difference, $\Delta E$
(1) Buffer L+Q	0.0164 <sup>b</sup>	0.9214 <sup>ns</sup>
(2) Waste L+Q	9.1808 <sup>ns</sup>	0.20632 <sup>a</sup>
(3) Sodium bisulphite L+Q	16.8033 <sup>a</sup>	4.22208 <sup>a</sup>
1*2	23.0613 <sup>a</sup>	6.42021 <sup>a</sup>
1*3	68.5222 <sup>a</sup>	4.62008 <sup>a</sup>
2*3	4.2222 <sup>a</sup>	0.9148 <sup>a</sup>
Error	4.334	3.29029
Total SS	118.5975	18.9811
R <sup>2</sup>	0.97	0.83

b)	
Parameter	Sum of Squares (SS)
	Total cyanogens
(1) Sodium chloride L+Q	39.7139 <sup>a</sup>
(2) Sulphuric acid L+Q	4.8668 <sup>a</sup>
(3) Peeling L	74.8370 <sup>a</sup>
1*2	4.2133 <sup>a</sup>
1*3	7.1268 <sup>a</sup>
2*3	5.5200 <sup>a</sup>
Error	4.5923
Total SS	140.8700
R <sup>2</sup>	0.97

ns, Not significant at 5% level.

<sup>a</sup> Significant at 1% level.

<sup>b</sup> Significant at 5% level.

developed based on set objectives, and using Design Expert and non-repetitive permutation approach, 11 promising scenarios with the highest D were found to better describe optimisation process (Table 3a). In addition, a maximum global desirability (GD) of 1.0 was achieved for both parameters and responses, and predicted material balance of buffer concentration, 4.0% w/v, cassava waste solids, 23% w/w and extraction time of 10 min. Meanwhile, the

processing parameter balance simultaneously predicted yield and colour difference of 38.8% and 4.2 (reciprocal, 0.24) respectively.

### 3.3. Standardisation of integrated process methodology by validation of optimal models'

#### 3.3.1. Optimal model's validation

The best formulations were predicted at the highest waste solid content (10% w/w) and extraction time (10 min), as shown in Table 3a, and the best D did not predict highest yields and lowest  $\Delta E$ . Thus, applying zero-based standardisation approach [3] and sustainability [17], within the experimental scope, most important desirability values and corresponding parameters and responses were selected (Table 3a, asterisk).

Validation of the optimisation process in order to develop a standardised methodology, BPD were produced using the 4 optimal formulations (Table 3a, asterisk) including GD analysed for yield, and the results thereof compared with optimal values using mean relative deviation modulus ( $\rho$ ) (Table 3b). Although there were significant colour differences in the 15 formulations, biopolymer derivatives did not show any differences visually. Applying the ASTM's E313 yellowness index measure, BPD whiteness did not show much deviation from perfect white (100% lightness). This, coupled with BDP similar visual appearance, colour was not considered in the validation process.

#### 3.3.2. Standardisation of integrated process methodology

As shown in Table 3b, it was evident that  $\rho$  values less than 10% illustrated a good agreement of optimal values with experimental validation values. Furthermore, the  $\rho$  value of less than 5% showed a stronger association, indicating that parameter adjustment sufficiently responded to higher BPD yields. The BPD yield slightly above 40%, corresponding to the dry solids range of cassava roots, showed the capability for the developed method to use most waste solids.

**Table 3**

Optimised intact bitter cassava biopolymer derivatives (BPD) a) individual response desirability using non-repetitive permutation approach, and b) validated optimal yield for global standardised methodology.

a)						
Permutation	Buffer, % w/v	Waste solids, w/w%	Extraction time, Min.	Yield, %	Colour change, $\Delta E$	Desirability, D
A	3.6	30	10	43	4.8	0.87
B	3.7	30	10	43	4.8	0.89*
C	3.7	30	10	43	4.8	0.86
D	3.4	30	10	41	4.5	0.87
E	3.4	30	10	41	4.5	0.87
F	3.3	30	10	41	4.5	0.87*
G	3.2	30	10	40	4.5	0.87
H	2.9	30	10	39	4.5	0.92
I	2.9	30	10	39	4.5	0.94*
J	2.9	30	10	39	4.5	0.93
K	3.1	30	10	39	4.5	0.93*

b)						
Permutation	Buffer, % w/v	Waste solids, w/w%	Extraction time, Min.	Optimal yield, %	Experimental validated yield, %	Mean relative deviation modulus $\rho$ , %
B	3.7	30.0	10	43.0	42.3	24.5
F	3.3	30.0	10	41.0	40.7	4.5
I	2.9	30.0	10	39.0	37.8	72.0
K	3.1	30.0	10	39.0	38.4	18.0
GD formulation	4.0	23.0	10	38.8	38.4	8.0

Thus, using an integral methodology, incorporating intact root with 23–30% w/w waste solids, 4% v/v ionic buffer concentration and 10 min extraction time, could be applied for sustainable production of BPD. This integral methodology is a systematic approach for the production of BPD from intact (whole) cassava root in three major steps. It intended to support production of high yield and bright coloured BPD in an integrated economic, relatively short time production process and to impart some level of modification necessary in their applications.

### 3.4. Impact of standardisation on biopolymer derivative properties and packaging film moisture barrier properties

#### 3.4.1. Appearance

The produced BPD exhibited bright powders with uniformly distributed and semi-flowing particles (Fig. 2), demonstrating the potential of standardisation to turn wastes into value added bioproducts. Transparency is always a requirement in packaging foods in which their identities and visibilities are highly valued. Thus, BPD total cyanogen, colour, microstructure and chemical

properties, thermal and adsorption behaviour were further investigated. Transparent films are generally used in food packaging when other properties such as barrier, mechanical or thermal are adequate. Therefore, BPD moisture barrier properties were determined together with the developed films in order to determine impact of standardisation on these vital properties. As pointed out earlier, the higher BC yield compared to the yield of analogue SC could point to the unique BC-BPD. Thus, the impact of standardised methodology of optimal BC-BPD, i.e., GD was compared with SC-BPD.

#### 3.4.2. BPD dull colour and total cyanogen decontamination

Total cyanogens (TC) and colour were found to reduce and brighten, corresponding to increase in the biopolymer derivatives yield, when intact (whole) bitter cassava was processed following a previously developed SRRC methodology [36]. TC and colour decontamination is achieved through functionalising bisulphite and acid reaction during SRRC. When compared with the conventional cassava processing methods, the SRRC produced biopolymer derivatives with far less TC [36]. Therefore, to determine the impact of integrated process methodology on

**Table 4**

Fitted sorption models and estimated parameters for intact bitter cassava BPD (a) and film diffusion coefficients ( $m^2s^{-1}$ ) (b) at various temperatures and relative humidity.

(a)												
Model name	Equation	Parameters at various temperatures ( $^{\circ}C$ )										
		10			20			30				
Modified BET, [10]	$M_{eq} = \frac{M_0 C a_w}{(1-a_w)(1-C \ln(1-a_w))}$	MMC, % d.b.	9.90–67.44			2.07–53.24			2.00–50.46			
		$M_0$	17.98			16.18			15.46			
		C	10.38			2.73 $\pm$ 0.14			2.66			
		P, %	4.08 $\pm$ 0.16			4.29 $\pm$ 0.22			3.42 $\pm$ 0.01			
		VR, %	1.13 $\pm$ 0.36			0.41			0.28 $\pm$ 0.04			
		R <sup>2</sup>	0.99631			0.99851			0.99885			
Oswin, [11]	$MC_{eq} = k \left[ \frac{a_w}{1-a_w} \right]^C$	MMC, % d.b.	7.98–65.59			3.81–54.55			3.57–51.95			
		K	0.48			14.9			14.2			
		C	22.88			0.59			0.59			
		P, %	9.58 $\pm$ 0.17			4.01 $\pm$ 0.15			4.50 $\pm$ 0.01			
		VR, %	5.03 $\pm$ 0.36			0.73 $\pm$ 0.22			0.38 $\pm$ 0.07			
		R <sup>2</sup>	0.98756			0.99818			0.99955			
Peleg, [26]	$MC_{eq} = A a_w^B + C a_w^D$	MMC, % d.b.	10.92–67.49			4.10–54.12			3.92–51.42			
		A	28.04			29.32			27.35			
		B	0.41			0.91			0.92			
		C	83.53			81.2			67.55			
		D	6.84			10.05			8.78			
		P, %	3.26 $\pm$ 0.01			5.58 $\pm$ 0.00			3.13 $\pm$ 0.00			
		VR, %	0.59 $\pm$ 0.04			0.53 $\pm$ 0.02			0.12 $\pm$ 0.02			
		R <sup>2</sup>	0.99854			0.99818			0.99955			
Arrhenius, [4]	$P = P_0 \exp^{-E_p/RT}$	$a_w$	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	
			$E_p$	43106.6	303887.7	22983.8	17893.7	14414	12472.6	11859.8	11462.9	9609.9
		$P_0$	1.03E-07	3.21E-05	9.10E-04	8.03E-29	0.05	8.22	0.12	0.32	1.11	
		R <sup>2</sup>	0.92	0.93	0.95	0.97	0.98	0.98	0.94	0.92	0.99	
		P, mean relative deviation modulus; VR, standard error of estimate; R <sup>2</sup> , coefficient of determination; $M_{eq}$ , equilibrium moisture content; MMC, modelled moisture content; $M_0$ , monolayer moisture content; d.b., dry basis; $a_w$ , water activity; A, B, C, D, K, models' coefficients; P, water vapour permeability; $P_0$ , pre-exponential factor; $E_p$ , activation energy for permeation; R, gas constant (8.314 J/mol K); T, temperature (expressed in K).										
		(b)										
Temp, $^{\circ}C$	Relative humidity, %											
	75			85			95					
10	4.02051E-12			4.04668E-12			4.03098E-12					
20	4.01528E-12			4.03883E-12			4.04798E-12					
30	4.03621E-12			4.03752E-12			4.05322E-12					
40	4.11994E-12			4.1448E-12			4.90538E-12					

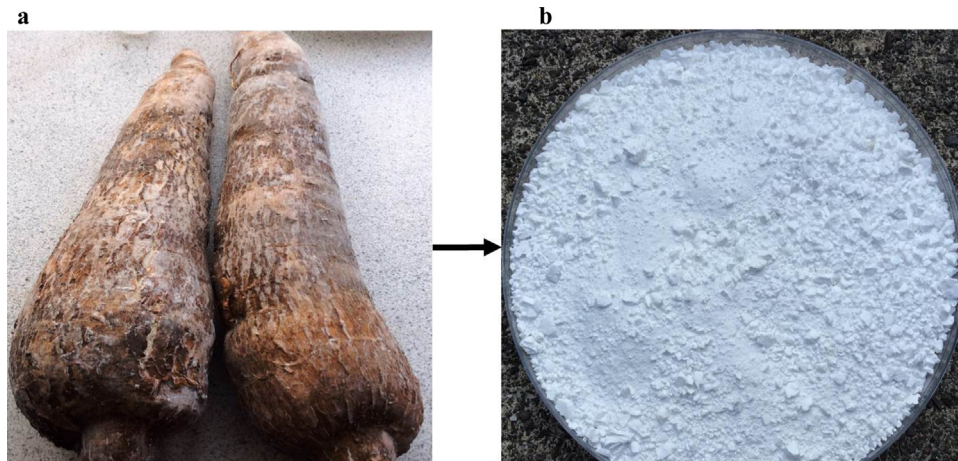
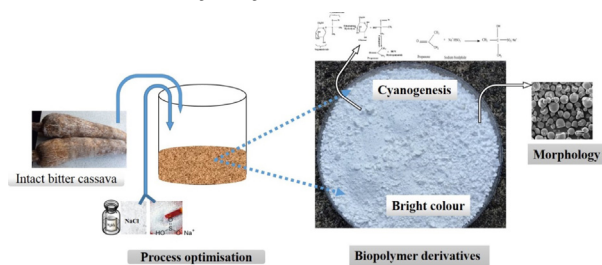


Fig. 2. Illustration of (a) intact bitter cassava root and (b) biopolymer derivatives (BPD) example.

safety and appearance of derivatives, TC reduction and/or possible elimination, and colour brightening were established. The analysis of variance results are shown in Table 2a. As observed in Table 2a showing the sum of squares, the combined effects of ionic buffer and bisulphite, ionic buffer and wastes, and sole bisulphite presented the highest ( $p < 0.01$ ) TC reduction potential. Similarly, their impact on colour difference ( $\Delta E$ ) did not deviate much from that of TC. These results can be attributed to: (i) the enhanced TC intrinsic hydrolysis by the intact root (Eq. (8)) during SRRC process; (ii) reductive effect of the food grade sodium bisulphite (Eq. (9)); and (iii) ionic buffers providing acid environment for hydrolysis.

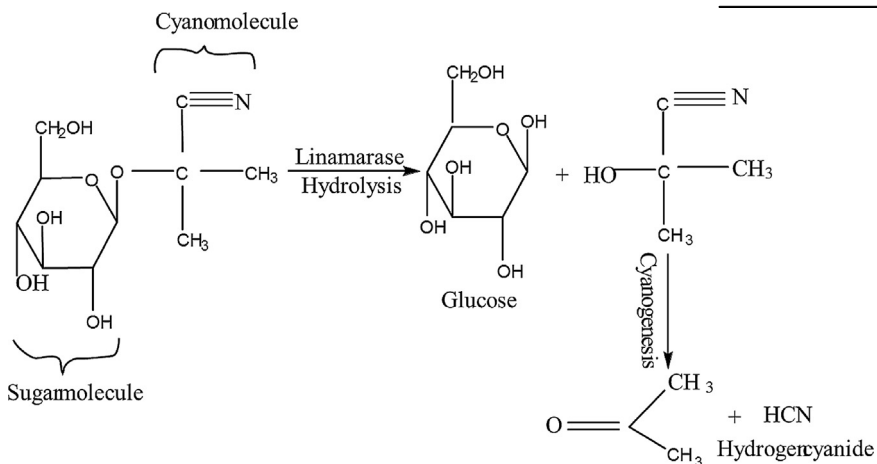


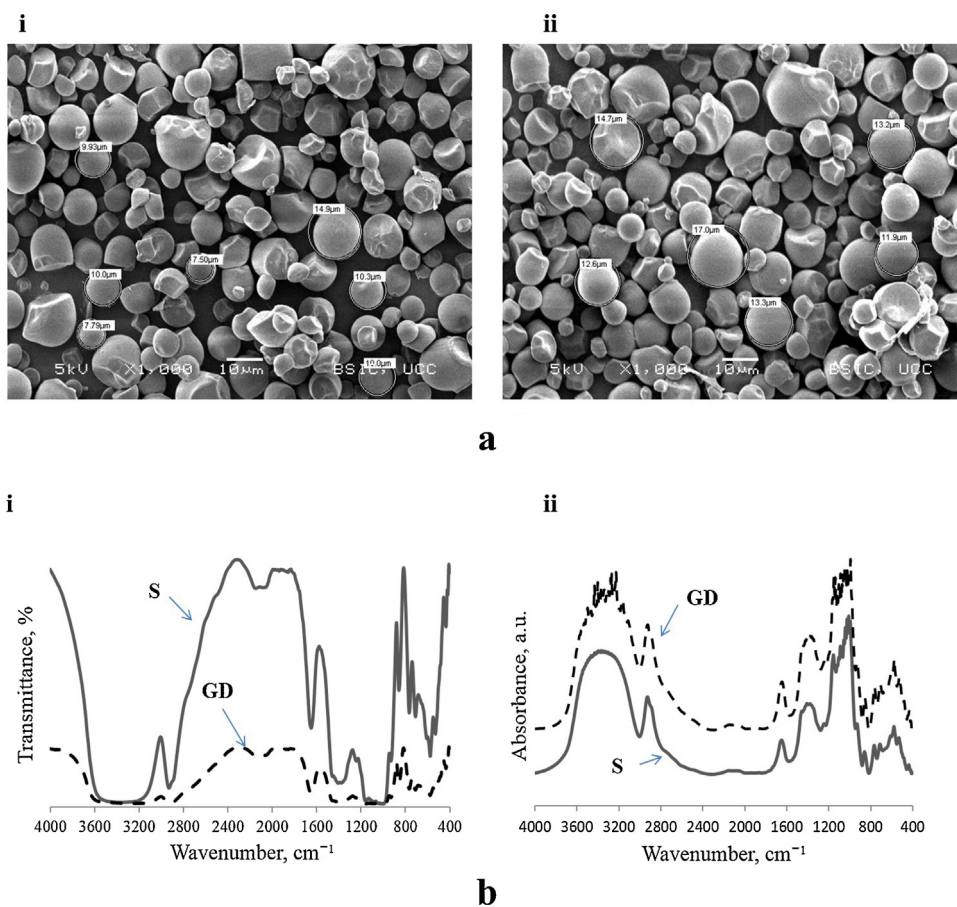
The effect of peeling factor on TC, practiced in traditional processing, was also determined and results shown in Table 2b. The peeling factor showed a much higher ( $p < 0.01$ ) TC reductive effect, that using whole root is more important in reducing cyanide from bitter cassava.

### 3.4.3. Microstructure and chemical

The scanning electron micrographs (SEM) of BPD are presented in Fig. 3a, showing heterogeneous particle sizes for SC (Fig. 3ai) and optimal GD (Fig. 3a ii with round and polygonal shapes [15]). Although slightly bigger round granule size range occurred in GD (11.79–17.00  $\mu\text{m}$ ) than in SC (7.50–14.9  $\mu\text{m}$ ), over all particle size distribution did not differ, suggesting that the optimal GD formulation produced near similar BPDs. Unlike in the externally reinforced starch biocomposites, whereby cellulosic and other fibrous materials were observed surrounding the starch granules [37,38], SRRC allowed a more homogenous mixture with minimal material surrounding starch granules (Fig. 3ai & ii).

Fourier transform infra-red (FTIR) spectroscopy applied to gain insight into structural alterations and possible physical and chemical interactions in SC and optimal –GD BPD as a function of optimisation are illustrated in Fig. 3b. Despite the SC and GD spectra looked similar, manifesting clear patterns, GD exhibited





**Fig. 3.** Scanning electron micrograph (a) and Fourier transmission infra-red spectra, (b) analysis of biopolymer derivatives at optimum conditions using sweet cassava (SC) (ai & bi) or intact bitter cassava global desirability (GD) (aii & bii).

high absorption intensity than SC. The differential absorption intensities could be explained by the differences in chemical composition [36,38], and perhaps might suggest that SRRC seems to release more chemical components in BC than in the SC analogues. This result is supported by the higher BC yields attributed to more BPD compounds by SRRC. In addition, the peak band gap dissimilarities might be due to differences in the structure, with the broader band of GD revealing the more amorphous regions in BC-BPD.

Apparent interactions largely depended between hydrogen bonding of hydroxyl functional groups in starch, cellulose, hemicellulose, pectin and some polyphenols (tannin) [7,35] due to an observed broad peak between 3000 and 3500  $\text{cm}^{-1}$ , manifesting alcohol O–H, alkyne C–H stretching vibrations. Additionally, the peak at around 1700  $\text{cm}^{-1}$  is an indication of associations between carbonyl groups among the compounds mentioned above. However, the interaction phenomenon was non-existent beyond 3500  $\text{cm}^{-1}$  with absence of amine C–H stretching vibrations indicating that there were insignificant total cyanide and protein contents. The low protein content could be explained by the protein-tannin complexing in BPD.

#### 3.4.4. Thermal

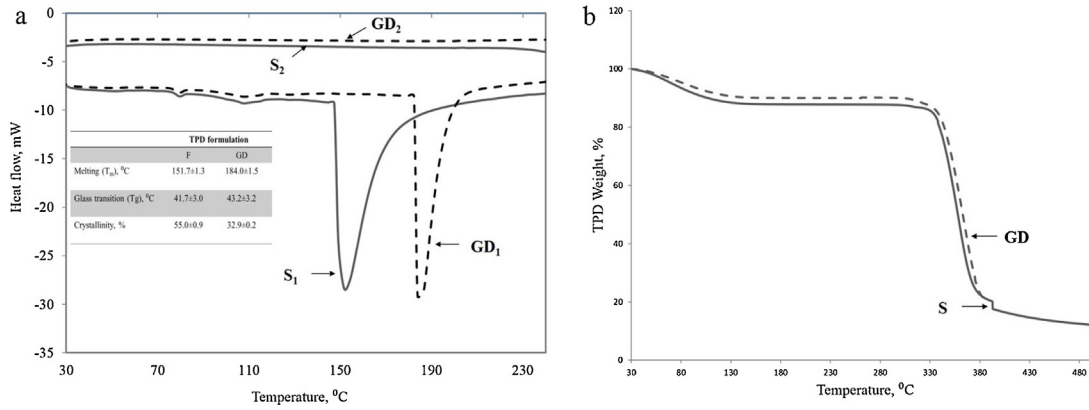
The differential scanning calorimetric (DSC) thermograms of BPD demonstrated that the processing history was similar for SC and GD (Fig. 4a) despite the higher GD melting ( $T_m$ ) and glass transitional ( $T_g$ ) temperatures and lower crystallinity ( $C$ ) than SC. Both SC and GD presented sharp and narrower melting endotherms in temperature ranges, 150–205 °C for SC and 180–205 °C

for GD, than earlier reported [23]. Additionally, SC and GD exhibited bimodal endotherms at about 70 and 110 °C as reported by De Meuter et al. [12]. The differences in melting transitions between SC and GD could be due to more component hydrogen bond interactions in GD than SC caused by waste solids incorporation. Both SC and GD did not show any water crystallisation due to amylopectin since there were no visible endotherm changes between 50 and 120 °C [23] rather a shift to above 150 °C  $T_m$ , possibly due to incorporation of waste solids.

A similar pattern of SC and GD due to thermal effect is exhibited by thermogravimetric analysis (TGA) (Fig. 4b). TGA revealed that BPD thermal stability of SC and GD followed same pattern during the decomposition period (30–400 °C), with highest weight loss observed at 335–380 °C. In both cases, the almost linear decomposition stability up to 300 °C could be due to stability of BPD resisting fast decomposition as exemplified by DSC analysis (Fig. 4a).

#### 3.4.5. BPD moisture adsorption behaviour

Isotherms of BPD powders require a lot more time than BPD films, and thus SC was not considered in the adsorption studies. Fitting of sorption models to experimental data of the equilibrium moisture contents of BPD is shown in Table 4. The three models (modified BET, Oswin, Peleg) followed type II isotherm [19] and demonstrated an increase of moisture content corresponding to increasing water activity. The models' estimated parameters for BPD and  $R^2$ , mean relative deviation modulus ( $\rho$ ) and variance of regression (VR) constants are presented in Table 4. The higher  $R^2$  and the lower  $\rho$  and VR signify the goodness of fit. While all the



**Fig. 4.** DSC (a) and TGA (b) analysis of intact bitter cassava (BC) and sweet cassava (SC) biopolymer derivatives (BPD). S<sub>1</sub> & S<sub>2</sub>, DSC thermograms of SC BPD for first & second heating; S, TGA decomposition curve of SC BPD; GD<sub>1</sub> & GD<sub>2</sub> global desirability, DSC thermograms of BC BPD for first & second heating; and GD global desirability, TGA decomposition curve of BC BPD.

three models presented low  $\rho$  and VR, Peleg model provided the best fit when the BPD was subjected to the whole range of water activity and temperatures of 10, 20 and 30 °C. Monolayer moisture contents (mo) of BPD using modified BET model decreased with increase in temperature. The low mo at high temperatures coincided with absence of visible mould at high relative humidity above 70% RH within 6 weeks of storage.

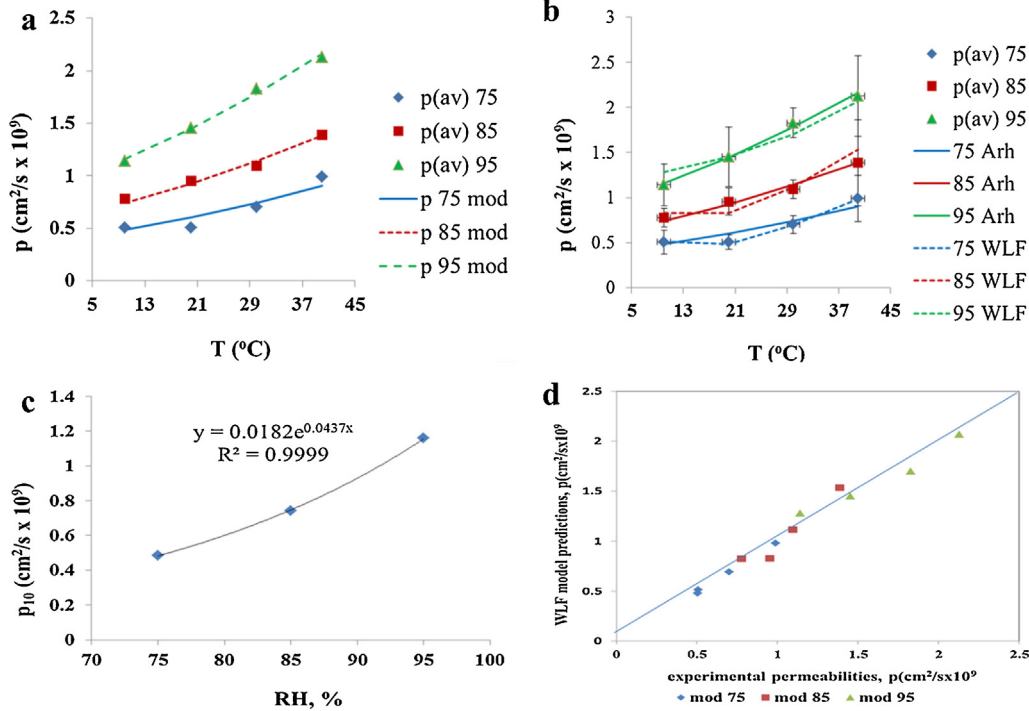
Like any other food material, BPD moisture adsorption decreased with increasing temperature as shown by mo values (Table 4).

3.4.6. Film moisture barrier properties

The moisture barrier property of materials is essential to approximation and prediction of the product-package shelf-life, with a precise package system barrier requirement governed by

the product characteristics and the targeted applications. Moisture regulation in packaging can cause negative changes in product quality and shelf-life, and change package material characteristics.

As shown in Table 4a, there was a decrease in activation energy ( $E_p$ ) of permeation and an increase in pre-exponential factor ( $P_0$ ), which proves a usual pattern of global (Arrhenius-type) model-dependency of temperature, and an activation energy independent of RH, with a pre-exponential factor varying exponentially. The diffusion coefficients (D) (Table 4b) showed insignificant increased trends with increases in both temperature (T) relative humidity (RH), which suggest that there were minimal physical and chemical changes in film matrix. The minimal structural changes might be due to the compact packing density in the structural matrix. As compared to previously tested materials for thin films and scaffolds [32], intact bitter cassava film D for the entire RH are



**Fig. 5.** Standardisation effect on film moisture barrier properties as influenced by (a and b) temperature. Points show average moisture permeability experimental data at 75 RH [P(av) 75]; 85 RH [P(av) 85]; 95 RH [P(av) 95]. Dotted and bold lines represent corresponding permeability fitted by Arrhenius model (a) and combined Arrhenius and William-Landel-Ferry (WLF) models (b); (c) relative humidity showing exponential increase of moisture permeability; (d) time-temperature superposition of William-Landel-Ferry (WLF) model showing best to the data.

much smaller than polyhydroxy-butyrates-co-hydroxyvalerate (PHBV) (593E–12 m<sup>2</sup>/s), PHBV + polylactic acid (PLLA) (598E–12 m<sup>2</sup>/s) and PHBV + PLLA + hydroxyapatite (229E–12 m<sup>2</sup>/s) films. Therefore, the film provides interesting potential barrier properties that can be used in developing films, not only for food use but also there is a possibility of their wide application.

The temperature-time dependence of WVP as predicted by Arrhenius and William-Landel-Ferry models is presented in Fig. 5, showing linearity of the plots and best fits. The linearity confirms that water uptake is regulated by the diffusion process [32]. It has been reported that WLF model allows for estimation of temperature shift factors by extrapolation, thereby being able to predict the life-span of products [31]. Together with Arrhenius model, the shelf-life of materials with temperatures close to and above their glass transition (T<sub>g</sub>) can be estimated. In this study, BPD and films with T<sub>g</sub> of around 40 °C (Fig. 4a), corresponding to WVP test temperatures of 40 °C, were produced from intact bitter cassava, suggesting that the above models are applicable to this polymer.

#### 4. Conclusion

A standardised simple, integrated methodology which allowed efficient and low-cost production of biopolymer derivatives (BPD) from novel intact BC was developed. Employing an integrated methodology, incorporating intact BC root with 23–30% w/w waste solids, 4% v/v ionic buffer and 10 min extraction time, allowed production of more BPD compared to a commercial method using SC.

The BPD yield of 41% showed that it is possible to utilise nearly the whole root during processing since the root dry matter contents of most cassava varieties lie between 40 and 45%. It can be concluded that the standardised methodology is an efficient process and could be used as a simple, low cost and sustainable method in production of BPD. This has implications in eliminating the triple BPD preparation steps, production, reinforcements and modifications, as separate entities that increase energy in industrial production of materials.

SEM, DSC, TGA, FTIR and moisture barrier analyses revealed a uniform microstructure, high thermal stability of BPD, and promising good barrier properties, thus demonstrating efficient performance of the integrated standardised methodology.

Standardised integrated methodology for production of biopolymer derivatives (BPD) from novel intact bitter cassava was demonstrated by desirability optimisation, allowing sustainable low cost production of BPD for a broad range of applications.

Methodologies designed around standard integrated procedures, matching zero-based approach to contamination elimination, are novel strategies, and if they are used effectively and widely can provide better avenues to eliminate cassava wastes and recover BPD resources as sustainable biomaterials.

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