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ORIGINAL PAPER

Novel Intact Bitter Cassava: Sustainable Development and Desirability Optimisation of Packaging Films

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Abstract Novel biomaterials and optimal processing conditions are fundamental in low-cost packaging material production. Recently, a novel biobased intact bitter cassava derivative was developed using an intrinsic, high-throughput downstream processing methodology (simultaneous release recovery cyanogenesis). Processing of intact bitter cassava can minimise waste and produce low-cost added value biopolymer packaging films. The objective of this study was to (i) develop and characterise intact bitter cassava biobased films and (ii) determine the optimal processing conditions, which define the most desirable film properties. Films were developed following a Box-Behnken design considering cassava (2, 3, 4 % *w/v*), glycerol (20, 30, 40 % *w/w*) and drying temperature (30, 40, 50 °C) and optimised using multi-response desirability. Processing conditions produced films with highly significant ($p < 0.05$) differences. Developed models predicted impact of processing conditions on film properties. Desirable film properties for food packaging were produced using the optimised processing conditions, 2 % *w/v* cassava, 40.0 % *w/w* glycerol and 50 °C drying temperature. These processing conditions produced films with 0.3 %; transparency, 3.4 %; solubility, 21.8 %; water-vapour-permeability, 4.2 gmm/m^2/day/kPa; glass transition, 56 °C; melting temperature, 212.6 °C; tensile strength, 16.3 MPa; elongation, 133.3 %; elastic modulus, 5.1 MPa and puncture resistance, 57.9 J, which are adequate for packaging applications. Therefore, intact bitter cassava is a viable material to produce packaging films that can be tailored for specific sustainable, low-cost applications.

Keywords Bitter cassava · Biobased film · Sustainability · Optimization desirability

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Introduction

Natural bioresources have drawn packaging research interest due to rising environmental sustainability awareness and demand for economic food packages. This demand is due to growing negative impact of the fossil-based non-biodegradable plastic packaging materials on the environment (Souza et al. 2011) and perhaps concerns of escalating costs of package production. Among the natural materials, sweet cassava has been progressively used in film formulations due to abundance, biodegradability and low cost of its polysaccharide derivatives, but this makes it unsustainable due to competition with food supply. Its derived polysaccharide starch is by far evaluated as a main component in the formulation of biobased films (The et al. 2009; Flores et al. 2010; Souza et al. 2012; Maran et al. 2013a). Biobased packaging films have been reported to maintain quality and improve shelf life of fresh and processed foods especially when they exhibit good mechanical properties and selective barriers (Cerqueira et al. 2010). Sweet cassava has been successful in the development of biobased films with wide range of properties (Embuscado and Huber 2012), but these films have not been used in many food packages possibly due to lack of standardisation and systematic approach. Bitter cassava cultivars, regarded traditionally as a famine-reserve crop (Essers 1988; Chiwona-Karltun et al. 1998; Line 2006; Burns et al. 2010; Vuong et al. 2012), are being transformed into resourceful commercial crops but many issues regarding their full value as

industrial materials are yet to be resolved. Recently, a novel biobased intact bitter cassava derivative was developed using an intrinsic, high-throughput downstream processing methodology known as simultaneous release recovery cyanogenesis (SRRC) (Tumwesigye et al. 2014, 2015).

Novel materials and optimal processing conditions are fundamental in low-cost packaging material production.

Optimisation of formulations is dependent of material balance and processing conditions. Optimisation of formulations has been accomplished mainly for various compositional blends of starch/flour of any botanical crop, other additives and different plasticisers. Examples that have been reported include optimisation of amaranth flour/glycerol matrix (Tapia-Blácido et al. 2011), optimisation of starch/polyester film properties (Olivato et al. 2013) and optimisation of xanthan gum/tapioca starch/potassium sorbate edible matrices (Arismendi et al. 2013). Few studies have used banana flour to determine the optimal processing conditions of films (Pelissari et al. 2013). These studies allude to the fact that better films can be produced when initial material additives and plasticisers are balanced. Optimisation of a good film requires precise control of the processing conditions and careful examinations of the fluctuations that occur in film properties. However, no systematic optimisation study, integrating biobased film production by combining material balance, processing conditions and low-cost processing has been performed for intact bitter cassava. Nowadays, experimental design methodology tools have been employed to determine the most efficient and economic matrix formulations needed for optimal formulations (Steele et al. 2012). Robust packaging design provides approaches for fine-tuning processing conditions to the desired possible package properties with marginal costs and maximum functional presentation. For each property-condition match, there is a need to tailor the formulation that best suites a specific product. Desirable properties could be achieved by process robustness incorporating low-cost base materials and formulations. Unfortunately, the common current approach is characterised by piecemeal evaluations whereby there is a tendency to improve the properties of films by evaluating parameters on individual basis.

The objective of this study was to (i) develop and characterise intact bitter cassava biobased films; (ii) define the parameters and conditions, which relate formulation to film development properties and iii) optimise processing conditions and properties in order to obtain films with desirable characteristics for tailor food packaging.

Methodology

Material Source: Intact Bitter Cassava

Intact (whole) bitter cassava (Tongolo), with a total cyanogen content between 900 and 2000 ppm, were processed using an intrinsic extraction procedure known as SRRC into biopolymer derivative with a total cyanogen around 0.5 ppm according to the method described by Tumwesigye et al. (2014, 2015). The biobased intact bitter cassava derivative was used for film formulation.

Development of Intact Bitter Cassava Films

The development and production of semi-commercial intact bitter cassava biobased films was performed based on Box-Behnken design. The design matrix for both processing conditions (actual/coded independent variables) and responses (film properties), with a total of 15 experimental runs are presented in Table 1. Each run was an average of three replicates. The criteria for selection of processing conditions that have a significant impact on the film development were set considering previously reported range values of cassava starch/flour and their reinforced films (de Moraes et al. 2013; Tumwesigye et al. 2014, 2015).

Biobased intact bitter cassava derivative (2, 3, 4 % *w*/*v*) and glycerol (20, 30, 40 % *w*/*w*) were mixed in 100 mL deionised water and homogenised using a magnetic stirrer (100 rpm, 20 °C, 5 min). The mixture was transferred to a bath (Huber Ministat 240 Heating Recirculating Unit, UK) and heated (70 °C, 2 °C/min, during 5 min) until a viscous transparent gel was observed and held for 20 min.

Film casting was done by pouring 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 film release spray (Ambersil Formula 5 non-silicone, UK) was used to ease peeling of films after drying. The plate was left at 25 ± 1 °C for 3 h to allow stabilisation and bleeding of trapped bubbles and then dried in a ventilated oven (30, 40 and 50) ± 1 °C for 4–8 h. The dried film was peeled off the plate and equilibrated at 23 ± 2 °C and 54 % relative humidity for at least 48 h prior to experimental analysis.

Characterisation of Intact Bitter Cassava Films

Thickness Measurement

For each experiment (Table 1), film thickness (mm) was measured using an absolute digital calliper (Digmatic, Mitutoyo UK Ltd). Measurements were taken at six different random sites and the average values were calculated for film surface area that was intended only for use in each test. The purpose of measurement was to ensure that films' thickness was maintained within the same range during characterisation.

Table 1 Box-Behnken design matrix: actual/coded variables of processing conditions and film properties

Runs	Actual/ coded independent variables			Film properties								
	Cassava (% w/v)	Glycerol (% w/w)	Temp (°C)	Moisture content (%)	Optical (%)	Solubility (%)	Water vapour permeability (gmm/m^2/day/kPa)	Glass transition (°C)	Melting temperature (°C)	Tensile strength (MPa)	Elongation at break (%)	Elastic modulus (MPa)	Puncture resistance (J)
1	4 (1)	30 (0)	30 (−1)	0.6 ± 0.0^{a}	$8.4^{a} \pm 0.2$	$19.6^{a} \pm 0.1$	$6.5^{a} \pm 0.0$	$44.4^{ab} \pm 0.0$	$190.7^{a} \pm 1.2$	$23.0^{a} \pm 0.1$	$45.1^{a} \pm 0.5$	$9.4^{a} \pm 0.0$	$56.3^{a} \pm 0.5$
2	2 (−1)	30 (0)	30 (−1)	0.2 ± 0.0^{b}	$10.0^{b} \pm 0.0$	$17.1^{b} \pm 0.5$	$5.2^{b} \pm 0.0$	$41.7^{cd} \pm 0.1$	$203.8^{b} \pm 1.0$	$20.0^{ba} \pm 0.4$	$5.2^{b} \pm 0.4$	$5.7^{b} \pm 0.0$	$2.1^{b} \pm 0.0$
3	3 (0)	20 (−1)	30 (−1)	0.2 ± 0.0^{c}	7.1 ± 0.1^{c}	$18.3^{c} \pm 0.0$	$4.1^{c} \pm 0.0$	$42.9^{d} \pm 0.0$	$188.0^{ca} \pm 1.0$	$21.2^{ba} \pm 1.7$	$3.0^{b} \pm 0.5$	$10.6^{c} \pm 0.0$	$2.5^{b} \pm 0.5$
4	3 (0)	40 (1)	30 (−1)	0.7 ± 0.0^{d}	$10.9^{d} \pm 0.1$	$23.1^{d} \pm 0.4$	$7.7^{d} \pm 0.1$	$39.7^{acc} \pm 0.1$	$185.0^{dc} \pm 1.0$	$4.7^{cd} \pm 1.1$	$74.6^{c} \pm 13.4$	$0.7^{d} \pm 0.0$	$63.9^{e} \pm 0.8$
5	2 (−1)	20 (−1)	40 (0)	0.2 ± 0.0^{e}	5.6 ± 0.2^{e}	$18.1^{e} \pm 0.3$	$3.4^{e} \pm 0.0$	$49.5^{fg} \pm 0.1$	$202.7^{db} \pm 2.1$	$11.1^{e} \pm 0.7$	$2.8^{b} \pm 0.6$	$5.9^{b} \pm 0.0$	$1.7^{b} \pm 0.1$
6	4 (1)	20 (−1)	40 (0)	0.2 ± 0.0^{f}	9.9 ± 0.2^{f}	$21.9^{f} \pm 0.4$	$4.4^{f} \pm 0.0$	$50.7^{gh} \pm 0.1$	$196.7^{e} \pm 1.2$	$37.6^{f} \pm 3.0$	$4.1^{b} \pm 0.8$	$9.7^{e} \pm 0.0$	$29.0^{d} \pm 1.5$
7	2 (−1)	40 (1)	40 (0)	0.3 ± 0.0^{g}	3.5 ± 0.5^{g}	$20.1^{g} \pm 0.3$	$4.8^{g} \pm 0.0$	$46.8^{gf} \pm 0.0$	$208.0^{f} \pm 0.0$	$1.9^{e} \pm 0.1$	$189.3^{d} \pm 9.5$	$0.1^{f} \pm 0.0$	$51.6^{c} \pm 0.1$
8	4 (1)	40 (1)	40 (0)	0.5 ± 0.0^{h}	5.3 ± 0.5^{h}	$30.5^{h} \pm 0.1$	$4.5^{h} \pm 0.0$	$44.1^{ab} \pm 0.1$	$191.5^{a} \pm 1.5$	$3.7^{e} \pm 0.1$	$181.3^{d} \pm 14.3$	$0.1^{f} \pm 0.0$	$73.2^{f} \pm 0.7$
9	4 (1)	30 (0)	50 (1)	0.3 ± 0.0^{i}	3.6 ± 0.4^{i}	$36.4^{i} \pm 0.1$	$3.5^{i} \pm 0.0$	$54.3^{e} \pm 0.0$	$186.7^{fg} \pm 1.5$	$39.8^{gh} \pm 0.2$	$43.9^{b} \pm 0.4$	$14.2^{g} \pm 0.0$	$37.0^{bg} \pm 0.7$
10	2 (−1)	30 (0)	50 (1)	0.2 ± 0.0^{j}	5.6 ± 0.2^{j}	$33.2^{j} \pm 0.2$	$6.4^{j} \pm 0.0$	$46.1^{h} \pm 0.1$	$208.3^{fe} \pm 1.2$	$16.4eh \pm 0.4$	$4.5^{b} \pm 0.3$	$7.6^{h} \pm 0.0$	$2.7^{g} \pm 0.1$
11	3 (0)	20 (−1)	50 (1)	0.2 ± 0.0^{k}	3.7 ± 0.1^{k}	$40.9^{k} \pm 0.4$	$4.6^{k} \pm 0.0$	$51.7^{e} \pm 0.1$	$199.7^{gc} \pm 0.0$	$33.6^{e} \pm 1.0$	$6.4^{b} \pm 0.2$	$13.8^{i} \pm 0.1$	$2.6^{bg} \pm 0.3$
12	3 (0)	40 (1)	50 (1)	0.5 ± 0.0^{l}	7.0 ± 0.4^{l}	$39.1^{l} \pm 0.2$	$4.9^{l} \pm 0.0$	$47.6^{i} \pm 0.1$	$199.0^{gc} \pm 1.5$	$7.5^{f} \pm 0.2$	$33.1^{a} \pm 0.6$	$1.5^{j} \pm 0.0$	$52.5^{h} \pm 0.3$
13	3 (0)	30 (0)	40 (0)	0.5 ± 0.0^{m}	4.7 ± 0.4^{m}	$36.1^{m} \pm 0.2$	$3.4^{m} \pm 0.0$	$38.3^{h} \pm 1.9$	$183.7^{f} \pm 1.5$	$17.8^{hg} \pm 1.9$	$4.2^{a} \pm 0.8$	$5.2^{k} \pm 0.2$	$3.5^{bg} \pm 0.3$
14	3 (0)	30 (0)	40 (0)	0.5 ± 0.0^{n}	4.1 ± 0.1^{n}	$36.2^{n} \pm 0.3$	$5.4^{n} \pm 0.0$	$37.9^{h} \pm 1.6$	$185.3^{cd} \pm 0.6$	$13.7^{i} \pm 1.1$	$4.2^{b} \pm 0.0$	$4.4^{l} \pm 0.0$	$4.5^{bg} \pm 0.6$
15	3 (0)	30 (0)	40 (0)	0.5 ± 0.0^{p}	$4.2^{p} \pm 0.1$	$35.7^{p} \pm 0.2$	$5.4^{p} \pm 0.0$	$39.0^{fi} \pm 0.6$	$186.0^{fe} \pm 1.0$	$12.6^{d} \pm 0.7$	$14.4^{a} \pm 3.7$	$7.1^{m} \pm 0.0$	$3.3^{e} \pm 0.1$

Marked differences (superscript letters) are significant at $p < 0.05$

Moisture Content

Film moisture determination was performed in two stages: (i) initial weight loss (WL) and (ii) moisture content (MC). The initial WL allowed films to lose free moisture and transformed them into films which could be subsequently applied. Herewith, 15 films (8.4 cm diameter each), formulated according to the experimental design (Table 1), were dried at three different temperatures (30, 40, 50 °C) and their WL monitored every 30 min until constant readings were obtained. These films were designated WLF. To evaluate the effect of processing conditions on the MC, triplicate samples from each batch of freshly dried WLF were determined gravimetrically. WLF was dried in a hot air circulation oven at 105 °C for 9 h until when the WLFs had constant weights. MC was calculated as the ratio of the mass of water lost to the total WLF weight and expressed in percentage, wet basis. Three replicates of each WLF were tested.

Optical Properties

Film optical property (transparency level) was determined as described (Muo et al. 2012) with slight changes. Film strip of each formulation was carefully inserted into cuvettes and placed inside a spectrophotometer cell. Spectrum intensity of an empty cuvette (I_0) (as a baseline) was run concurrently with the sample film. Transmission was measured using a spectrophotometer (Biochrom Libra S22 UV/vis, Cambridge CB4 0FJ, UK) at wavelength 700 nm. Transparency ($T\%$) was calculated using Eq. 1. For each of the values, the higher T implies that less light passed through a film, thus described as opaque. Three replicates of each film were tested.

$$\% T = \log I_0/(I\delta)100 \quad (1)$$

where I, the light intensity through the film inserted into the cuvette and δ, film thickness (mm).

Solubility

Film solubility (FS) in water was measured as described in Belibi et al. (2014) with minimal modifications. Previously oven-dried film strips (3 × 2 cm) were weighed on an aluminium foil, submerged in a beaker with 50 mL of distilled water and tightly covered with parafilm to minimise water loss and airborne contaminants. The contents were kept at 23 °C for 30 days, intermittently agitated every 24 h to allow dissolution and monitored for change in weight. The films were partially dehydrated (where necessary filtered) on filter paper and dried in an air-circulating oven at 70 °C until constant weight. 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.

Water Vapour Permeability

Film water vapour permeability (WVP) was determined gravimetrically at 38 °C, 95 % RH according to ASTM E96-05 (2005) method. Films for WVP were formulated based on experimental design (Table 1), cast on 8.4-cm diameter dishes to maximise uniformity and permeation cell fitting specificity. Each previously conditioned (54 % RH, 23 ± 2 °C, at least 48 h) film was carefully positioned between acrylic permeation cell containing $CaCl_2$ (0 % RH) and enclosed in a humidity-controlled plastic container partially filled with 1000 mL of KNO_3 salt solution, corresponding to a relative humidity of 95 %. The container was stored in temperature-controlled incubator at 38 °C, and cell weight gain was recorded every 2 h for 10 h and used for WVP calculations. WVP was calculated using Eq. 2.

$$\mathrm{WVP} = \left(\acute{m}\,\delta\right)/[AP(r_{95}-r_0)] \quad (2)$$

where $\acute{m}$, mass flow rate (g/day); δ thickness (mm); A, cross-sectional area (m^2); P, saturation partial pressure at 38 °C (kPa) and $r_{95}-r_0$, relative humidity of outside environment (95 %) and cell (0 %). All tests were conducted in triplicate and mean values were used for calculating WVP.

Thermal Characterisation

Thermal characteristics, namely glass transition (T_g) and melting temperatures (T_m) of bitter cassava films were evaluated using a differential scanning calorimeter (DSC 200 F3, Germany) equipped with a thermal analysis data station.

Films were prepared based on experimental design (Table 1) and each film (10 mg) was placed into a pre-weighed DSC pan. The pan was hermetically sealed, heated from 20 to 220 °C at a rate of 10 °C/min, cooled back rapidly in liquid nitrogen at a rate of 20 °C/s and reheated at 5 °C/min to 220 °C. The purpose of rapid cooling and second heating was to give film samples thermal history, a key in understanding the effect of previous processing on thermal characteristics of films. The T_g and T_m were calculated using the built in software (NETZSCH Proteus® 6.0, Germany) and determined by considering the midpoint of the heat capacity change observed on the second heating. All samples were evaluated in triplicate and mean measurements reported. An empty pan was used as a reference.

Mechanical Analysis

Mechanical properties, tensile strength (TS), elongation at break (E), elastic modulus (EM) and puncture resistance (PR) were evaluated by a TA.HD Plus Texture Analyser (Stable Microsystems, UK) equipped with a 50 kg load cell, according to ASTM882-09 method (2009).

For TS, E and EM measurements, an initial grip separation (50 mm) and cross head speed (1.0 mm/s) were used. Measurements were taken for at least five close values to obtain cross-sectional area (thickness × initial grip distance). Ten film strips (25 × 100 mm) were cut from each formulation according to the experimental design (Table 1). 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.

For PR, a circular opening (10 mm), probe diameter (3 mm) and a speed (1.0 mm/s) were used, and 7-mm diameter film discs from each formulation according to the experimental design (Table 1). PR was calculated as a maximum penetration force at the tear.

Model Development and Film Optimization

The response polynomial models were developed using factorial and Box-Behnken response surface design by varying parameters namely cassava derivatives (2, 3, 4 % *w*/*v*), glycerol (2, 3, 4 % *w*/*w*) and drying temperature (30, 40, 50 min) based on experimental design (Table 1) described in "Development of Intact Bitter Cassava Films" section. Appropriately, four models (linear, combined two factor interaction, quadratic) were fitted to the data in order to obtain the second order polynomial equations, their regression coefficients and R^2 values. The aliased cubic model was not considered for analysis.

Fig. 1 Example of films produced from intact bitter cassava as illustrated by their visual image when formulated with cassava, 4 % *w*/*v*; glycerol, 30 % *w*/*w*; drying temperature, 30 °C

Analysis of variance (ANOVA) was used for regression coefficient determination and significance of examination. The model adequacy was determined by coefficient of determination (R^2) and illustrated by the mean square pure error (MSPE). Processing conditions were matched with properties to determine significant effects for optimisation purposes.

The optimisation of conditions (parameter balance) and film properties (desired functional combination) were achieved by a desirability methodology after fitting polynomial models to the data as suggested by Derringer and Suich (1980) (Eq. 3) and reported widely.

$$D = \left[d_y\,(Y)^{1/n} \right] \tag{3}$$

where D, over all desirability; Y, responses; d_y (Y), response desirability function and n, number of responses. $(n=1)$; d_y $(Y)=0$, perfectly undesirable; d_y $(Y)=1$, perfectly desirable.

Validation of optimisation was accomplished by comparing experimental results and predicted values obtained from fitted model equations.

The Statistica 7.1 software (StatSoft Inc., Tulsa, USA) was used to perform the above tasks.

Results and Discussion

Example of Transparent and Homogeneous Biobased Films

An example of biobased films produced using intact bitter cassava is shown in Fig. 1. Notwithstanding film individual unique properties, all formulations produced homogeneous, flexible, transparent films, demonstrating the potential of this novel sustainable material to reduce cassava-borne environmental waste and develop biodegradable materials for food packaging applications.

Characterisation of Intact Bitter Cassava Biobased Films

Both formulation and optimisation experiments demonstrated that variations in processing conditions strongly associate with intact bitter cassava film properties. Moreover, processing conditions showed highly significant ($p<0.05$) difference in film pattern properties (Tables 1 and 2), suggesting that individual and compounded effects are important in matching parameters with ultimate properties.

Table 2 Regression coefficients and analysis of variance for film properties. Mean (β_0), cassava content (β_1), glycerol content (β_2), drying temperature (β_3), their corresponding interactions (β_{12}, β_{13}, β_{23}) and mean square residual error (MSPE)

Coefficients	Moisture content (%)	Optical (%)	Solubility (%)	Water vapour permeability (gmm/(m^2.day.kPa))	Glass transition (°C)	Melting temperature (°C)	Tensile strength (MPa)	Elongation at break (%)	Elastic modulus (MPa)	Puncture resistance (J)
β_0	1.713*	90.548*	−203.520*	−19.467***	274.725*	440.500*	92.103**	−1402.20*	13.314	623.252*
Linear										
β_1	−1.344*	−54.204*	66.155*	5.599	−83.098*	−155.833*	−25.362	1007.93*	31.785*	−252.896*
β_2	−0.177*	1.001*	8.665*	1.315*	−8.301*	3.800**	5.245*	1.58	−0.114	−29.588*
β_3	0.045*	−2.000*	0.822*	0.021*	−2.840*	−6.792*	−6.171*	30.24*	−1.902*	−4549*
Quadratic										
β_{11}	0.139*	12.840*	−8.524*	0.778	9.214*	31.875*	5.743	−195.016*	−7.309*	35.027*
β_{22}	0.002*	−0.061*	−0.112*	−0.021	0.134*	−0.041**	−0.060*	1.09*	0.027*	0.502*
β_{33}	−0.000*	0.019*	−0.009*	0.008*	0.030*	0.028*	0.066*	−0.24*	0.032*	0.062*
Interactions										
β_{12}	0.107*	0.572*	−2.258*	−0.061	2.070*	−2.688*	−0.832	−27.16*	−0.163	8.358*
β_{122}	0.001*	0.024*	0.021*	0.006**	−0.031*	0.031*	0.005	−0.21*	−0.016*	−0.099*
B_{112}	−0.009*	−0.344*	0.191*	−0.055**	−0.050***	0.096***	−0.011	6.61*	0.173*	−0.427*
β_{13}	−0.010*	0.261*	0.878*	−0.214***	0.051**	3.563*	0.800***	−5.76*	−0.315**	0.637*
B_{113}	0.001*	−0.045*	−0.144*	0.018	−0.062**	−0.629*	−0.048	0.95*	0.065**	−0.189*
β_{23}	−0.001*	−0.0014	−0.017*	−0.009*	−0.002	0.006	−0.024*	−0.11*	−0.006*	−0.029*
R^2	0.999	0.987	0.999	0.877	0.987	0.984	0.986	0.993	0.987	1.000
MSPE	0.000	0.108	0.079	0.244	0.485	1.646	2.714	37.338	0.355	0.470

*Significant at 1 % level

**Significant at 5 % level

***Significant at 10 % level

Thickness

The potential influence of thickness on film properties has been widely reported with examples from Maran et al. (2013b) and de Moraes et al. (2013). In this study, extreme care was taken to minimise variations in film thickness to an average of 0.025 ± 0.005 mm for all experiments. Therefore, there were no significant differences ($p > 0.05$) due to influence of processing conditions on the thickness. Nevertheless, insignificant deviation was expected to be caused by combined differences in processing parameters, with formulations falling below centre points (such as cassava:glycerol, 2:20 %) producing films close to 0.02 mm due to high loss of water during heating and drying stages. Maran et al. (2013b) and de Moraes et al. (2013) reported film thickness of 0.027–0.046 and 0.070–0.299 mm when using cassava starch, 1–3 % *w/v*; glycerol, 0.5–1.0 mL; agar, 0.5–1.0 g and Span 80, 0.1–0.5 L and 85–99 % with starch, 3–5 % *w/v*; glycerol, 20 % *w/w* and cellulose fibre, 0.3 g, respectively.

Moisture Content

Film moisture content (MC) was highly influenced by the processing conditions (Table 1 and 2). Glycerol and cassava had a significantly high effect on MC than temperature in the linear and quadratic ranges, respectively. Film MC determined following constant weight drying was in the range of 0.22–0.71 % (*w/w*, wet basis), which was very low suggesting that, in general, drying had an impact on MC. It can be thought that increasing the content of cassava biopolymer derivatives in the matrix would reduce the amount of water needed. However, this was not observed in the present work, since derivatives worked associatively with glycerol to increase the moisture content of the films. This could be explained by the following: (i) the high hygroscopic nature of glycerol which held water molecules into the film matrix by creating more hydrophilic hydroxyl groups as active sites with high affinity for water molecules, (ii) characteristics of the derivatives of intact bitter cassava such as bigger granule size that absorbed more water in order to swell and (iii) pre-formed gel or post thermal gelation during drying such that these strong gels were able to hold water firmly within the matrices.

Optical Properties

Intact bitter cassava film optical properties demonstrated highly significant associations with processing conditions. Drying temperature showed a highly significant ($p < 0.05$) impact on film optical properties (low transparency values), with films becoming more transparent as temperature increases linearly and non-linearly, as shown in Tables 1 and 2 and Fig. 2a_1, a_2. Cassava and glycerol had a significant negative effect on film transparency, with films becoming more opaque when the cassava quadratic and glycerol linear combination effects became more apparent. However, films demonstrated more transparency when the former and latter combined effects were transposed. Also, notable is the individual processing conditions contribution on film transparency, with glycerol quadratic and linear, and cassava quadratic effects showing positive influences while cassava linear effect exhibited a negative impact. Results showed that increasing cassava content improves film transparency. This is inconsistent with what is known (de Moraes et al. 2013), whereby an increase in cassava starch caused translucency or opaqueness in films, thus suggesting that intact root processing might be associated with modification of starch and general increase in film transparency.

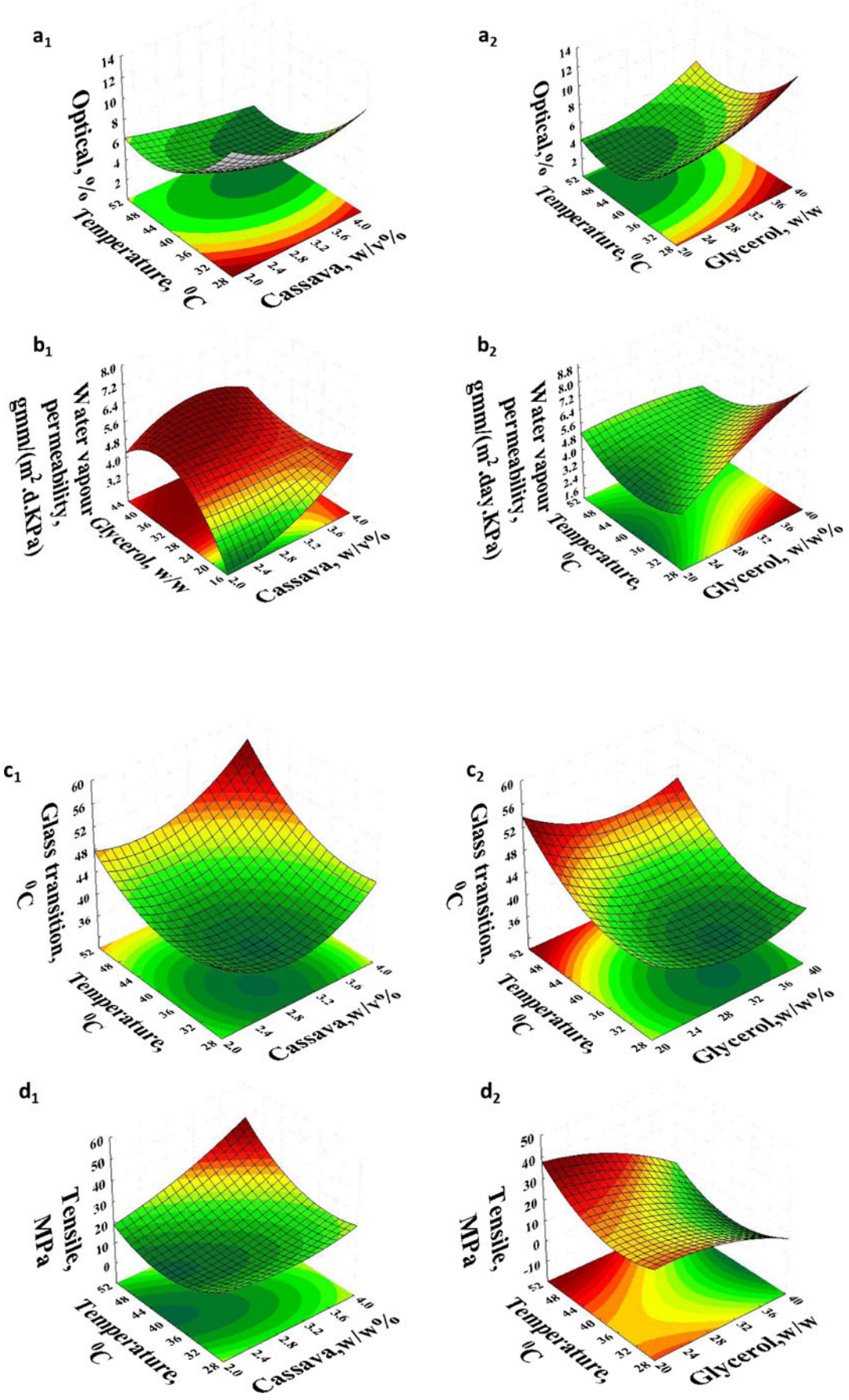
Fig. 2 Fitted response surfaces for **a** optical properties (MPa), **b** water vapour permeability (gmm/m^2/day/kPa), **c** tensile strength (MPa) and **d** glass transition (°C) as a function of different levels of cassava (% *w/v*), glycerol (% *w/w*) and drying temperature (°C)

Solubility

The film solubility as a function of processing conditions is presented in Tables 1 and 2. Similar to optical properties, drying temperature caused the highest film solubility in water, increasing linearly when drying temperature increased. Additionally, the main (cassava and glycerol) effects, interaction between cassava and glycerol, and between cassava and drying, linearly and quadratic, were significant ($p < 0.05$). Regardless of the high solubility-enhancing drying temperature, causing film structural disruptions and high water mobility, low solubility of 16–40 % was obtained after 30 days. Film solubility (16–40 %) was considered to be low compared to 11–41 % obtained when solubility was measured within 24 h (Belibi et al. 2014). Low values might be explained by relatively stable network components in the film structure imparted by intact root and other processing properties. The stability of solubility values after 30 days could also be explained by decreased swelling ability in glycerol plasticized films and their ability to resist degradation.

Water Vapour Permeability

Water vapour permeability (WVP) significantly ($p < 0.05$) decreased, linearly and quadratically, with an increase in drying temperature and linearly with combined interaction effects of cassava-drying temperature (DT) and glycerol-DT (Tables 1 and 2 and Fig. 2b_1, b_2). Conversely, glycerol alone, and when combined with cassava, caused a positive impact on WVP, increasing linearly and quadratically as the concentrations increased. The patterns observed can be explained by the effectiveness of glycerol in lowering intermolecular forces between polymer chains leading raised WVP and perhaps the


a1
Optical, %
Temperature, 0C
Cassava, w/v%
a2
Optical,%
Temperature, 0C
Glycerol, w/w
b1
Water vapour permeability, gmm/(m2.d.KPa)
Glycerol, w/w
Cassava, w/v%
b2
Water vapour permeability, gmm/(m2.day.KPa)
Temperature, 0C
Glycerol, w/w%
c1
Glass transition, 0C
Temperature, 0C
Cassava,w/v%
c2
Glass transition, 0C
Temperature, 0C
Glycerol,w/w%
d1
Tensile, MPa
Temperature, 0C
Cassava,w/w%
d2
Tensile, MPa
Temperature, 0C
Glycerol,w/w

disruption of these forces at high temperatures leading to their strength.

Thermal Properties

The impact of processing conditions on thermal properties of intact bitter cassava, associated with glass transition (T_g) and melting (T_m) temperatures, are shown in Tables 1 and 2. The cassava content had the most significant ($p < 0.05$) effect on T_g followed by the glycerol content, both having the expected impact. However, the drying temperature showed a more pronounced effect, which increased with the proportional increase in the concentration of cassava (Fig. $2c_1$).

This is likely due to the glass transition temperature of the dried film being in the range of temperatures of the drying process itself. Therefore, the extent of vitrification of the structure during drying depends on this processing variable. In the case of T_m, the effect of drying temperature is even more important than that of glycerol content (Table 2). Altogether, the observed extensive variations in effects of processing conditions on T_g and T_m point to the need to find a balance so as to produce films with desired T_g and T_m.

Mechanical Properties

Film formulations produced wide variations in mechanical behaviour (Tables 1 and 2), with statistical significance ($p < 0.05$) among tensile strength (TS) (Fig. $2d_1$, d_2), elongation at break (E), elastic modulus (EM) and puncture resistance (PR). Glycerol presented the highest negative linear impact on TS and EM and highest linear positive effects on E and PR, statistically shown in Table 2. Additionally, an interaction between cassava and glycerol and cassava and drying temperature was significant, imparting a negative linear effect on all mechanical properties. Altogether, these results showed the extent of plasticising and effects of glycerol on mechanical properties.

Modelling of Film Characteristics

Tables 2 shows the regression coefficients and highlights significant terms for response surface quadratic model fittings and choice. Thus, the quadratic models were highly significant for all responses ($p < 0.05$), whereas there was an aliased condition for cubic models. Accordingly, the quadratic model and corresponding linear and combined two factor interactions was used in determining the association between processing conditions and film properties. Consequently, the model equations (3 to12) which adequately predicted the association between conditions and properties, as best-fitting responses were used, showing that processing conditions had positive and negative influence on properties. Generally, all formulations, except for WVP, for the quadratic model used presented a significant influence ($p < 0.05$), with $R^2 > 0.90$ and their differences <0.2, respectively, alluding that the model amply projected the tangible association between the processing conditions and film properties. Moreover, obtained R^2 suggested that over 90 % of conditions and response data explained the adequacy and significance of the models.

Desirability Optimisation of Packaging Films

The optimal individual values for film properties as a function of optimal processing conditions using a desirability function (DF) is shown in Table 3. DF falls between 0 and 1, with 0 as minimum and 1 maximum. As values tend to be 1, the more optimised the process is achieved, the more desirable properties are provided by optimal processing conditions. With the exception of optical properties, the higher individual desirability values show that most optimised parameters were highly desired.

In order to elucidate a universal optimal formulation of processing conditions that would concurrently deliver the most desirable film properties, a global desirability (GD) of 0.7 was determined for all parameters (Fig. 3). The overall desirability considers the combined magnitudes of individual desirability (Table 3), expressed as a mean and achieved using the Statistica software. The multiple criteria optimisation was validated by comparing values relative to an absolute optimal process (Fig. 4) in order to check the relative deviation from the optimal and determine desired properties. Except for moisture content (53.2 %), solubility (16.7 %) and optical properties (12.4 %), parameters had a relatively low deviation (<10 %), indicating that the optimal values of processing parameters and conditions produced optimal properties with less effect on each other. Thus, to achieve the maximum possible match properties with low cost and maximum film functional performance, optimal parameters, within the experimental scope, were found to be the following: cassava powder, 2.0 % *w*/*v*; glycerol, 40.0 % *w*/*w*; drying temperature, 50.0 °C; moisture content, 0.3 %; transparency, 3.4 %; solubility, 21.8 %; water vapour permeability, 4.2 gmm/m^2/day/kPa; glass transition, 56 °C; melting temperature, 212.6 °C; tensile strength, 16.3 MPa; elongation at break, 133.3 %; elastic modulus, 5.1 MPa and puncture resistance, 57.9 J. Suffice to mention that individual optimal properties (Table 3) are slightly different from those obtained with a global optimal formulation, possibly due to the need to match all properties to a single formulation of processing conditions. Therefore, it can be possible to use individual or a combination of optimal parameters to produce films. Nonetheless, with demand of robust design procedures at low-cost production, the global procedure would suffice.

To validate the simultaneous optimisation design, a comparison between the experimental and predicted responses was determined after a confirmatory controlled

Table 3 Optimal values of intact bitter cassava films as determined by individual response desirability function

Parameter		Objective		Experimental		Optimal	Desirability
Property	Condition	PC	FP	Lower limit	Upper limit		
Moisture content			Minimise	0.22	0.71	0.19	1.00
	c	Minimise		2	4	4.00	
	g	Minimise		20	40	20.00	
	dt	Minimise		30	50	50.00	
Optical properties			Minimise	3.06	11.07	3.43	0.85
	c			2	4	2.00	
	g			20	40	40.00	
	dt			30	50	40.00	
Solubility			Minimise	16.58	41.34	15.52	1.00
	c			2	4	2.00	
	g			20	40	20.00	
	dt			30	50	35.00	
Water vapour permeability			Minimise	3.28	7.72	3.19	1.00
	c			2	4	2.00	
	g			20	40	20.00	
	dt			30	50	35.00	
Glass transition			Maximise	36.10	54.30	56.25	1.00
	c			2	4	4.00	
	g			20	40	25.00	
	dt			30	50	50.00	
Melting temperature			Maximise	182.00	220.00	213.63	1.00
	c			2	4	2.00	
	g			20	40	40.00	
	dt			30	50	50.00	
Tensile strength			Maximise	1.80	41.05	48.44	1.00
	c			2	4	4.00	
	g			20	40	25.00	
	dt			30	50	50.00	
Elongation at break			Maximise	2.38	200	187.27	0.95
	c			2	4	2.00	
	g			20	40	40.00	
	dt			30	50	40.000	
Elastic modulus			Maximise	0.10	14.20	15.95	1.00
	c			2	4	4.00	
	g			20	40	25.00	
	dt			30	50	50.00	
Puncture resistance			Maximise	1.54	74.07	81.02	1.00
	c			2	4	4.00	
	g			20	40	40.00	
	dt			30	50	35.00	

c cassava derivative (% *w*/*v*), *g* glycerol (% *w*/*w*), *dt* drying temperature (°C), *PC* processing condition, *FP* film property

step was performed with all parameters at optimal conditions. Table 4 presents analogous divergence ( ), delineated as the percentage of the differences between experimental and predicted values for individual properties. With >0 and GD of 0.7, it implies that the optimisation process was ideal and represented the best

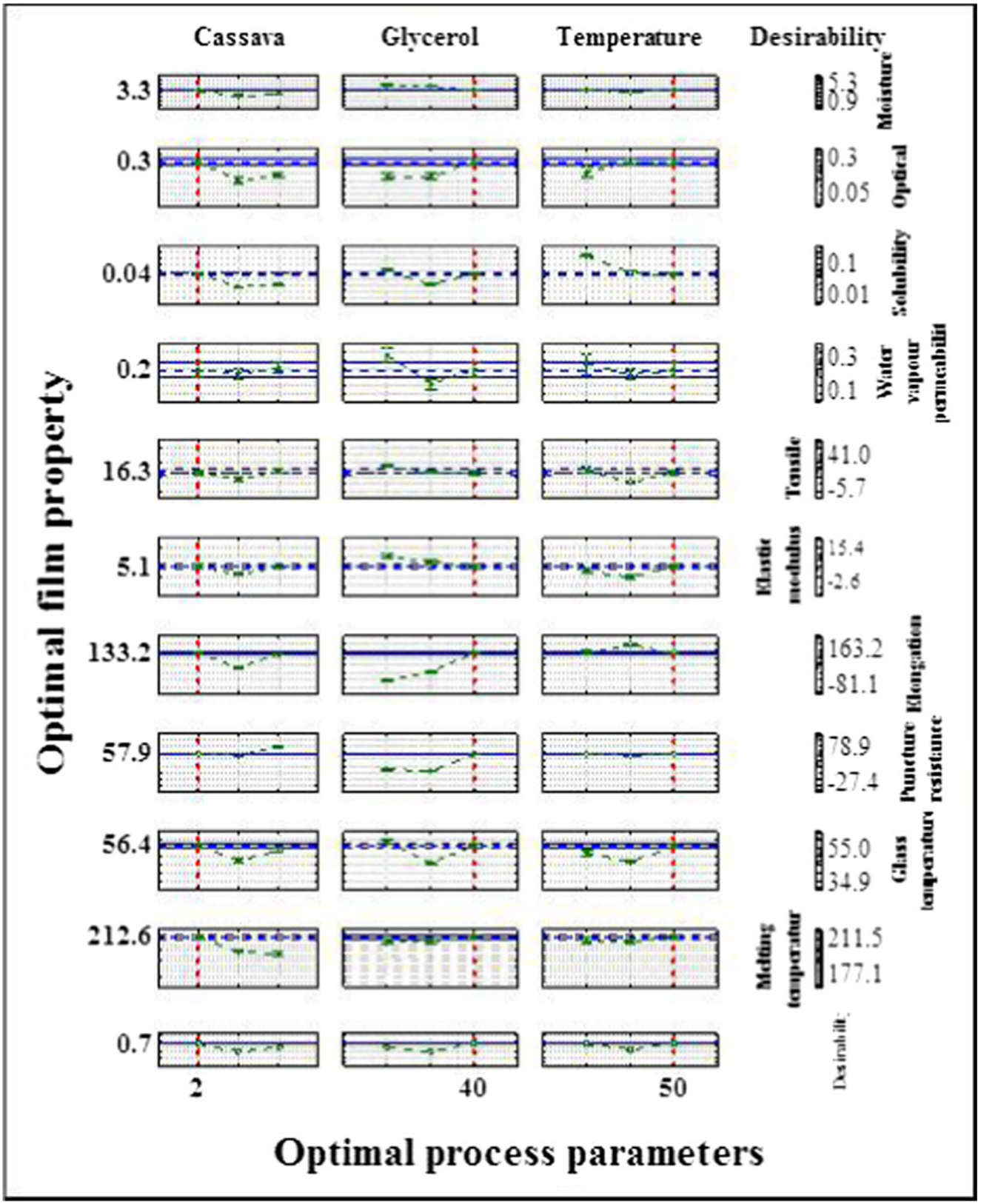


Fig. 3 Predicted response variables and desirability values used in multiple response optimisation of process conditions and properties during development of intact bitter cassava films

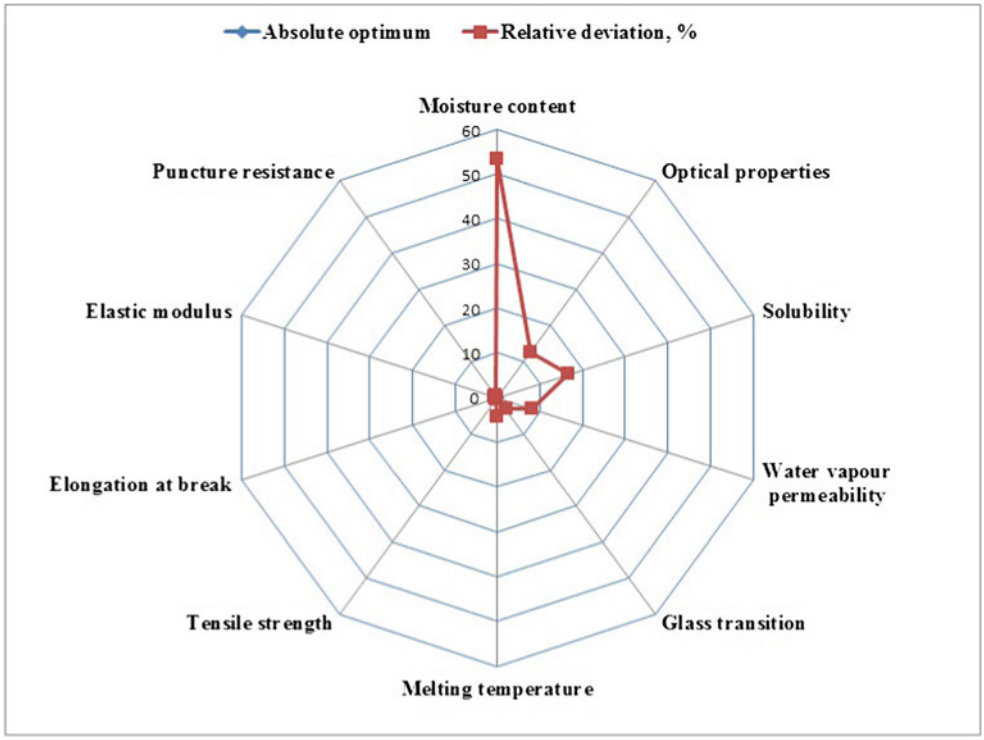


Fig. 4 Multiple criteria optimization technique for the validation of optimal values deviating from the set absolute optimal point (—◆—)

association between the processing conditions and film properties over the scope of the parameters studied. In addition, calculated coefficient of determination (R^2), 0.996 and mean relative percent deviation modulus ($\dot{P}$) > 50 % further confirm the adequacy of optimisation. Since, in practice, fitted properties' values were used instead of observed values in desirability optimisation, a $\dot{P}$ of 56 % implied that better (above/set criteria) values were achieved experimentally which correspond to better-quality films.

Conclusion

Packaging films with desirable properties were produced from intact bitter cassava, demonstrating the potential of this novel sustainable material to reduce cassava borne environmental waste and develop biodegradable materials for food packaging applications.

Desirable film properties were produced using optimised processing conditions, i.e. 2 % *w/v* cassava derivative, 40.0 % *w/w* glycerol and 50 °C drying temperature. With these

Table 4 Validation of the effectiveness and adequacy of the optimization process for development of intact cassava films

Response property	Observed (optimal) value[a]	Predicted value	Difference ( ), %
Moisture content, %	0.19	0.45	58 (↓)
Optical, %	3.43	5.29	34 (↓)
Solubility, %	15.52	30.54	50 (↓)
Water vapour permeability, gmm/(m^2.day.kPa)	3.19	4.50	29 (↓)
Glass transition temp, °C	56.23	44.05	22
Melting temp, °C	213.63	193.57	9
Tensile strength, MPa	48.44	3.71	92
Elongation at break, %	187.03	181.33	3
Elastic modulus, MPa	15.95	0.11	99
Puncture resistance, J	81.02	25.57	68

↓, relative deviations tending to minimum

[a] Values achieved with optimal processing conditions: cassava derivative, 2 % *w/v*; glycerol, 40 % *w/w*; drying temperature, 30 °C

processing conditions, the film properties obtained were a follows: 0.3 %; transparency, 3.4 %; solubility, 21.8 %; water-vapour-permeability, 4.2 gmm/m^2/day/kPa; glass transition, 56 °C; melting temperature, 212.6 °C; tensile strength, 16.3 MPa; elongation, 133.3 %; elastic modulus, 5.1 MPa and puncture resistance, 57.9 J. The use of drying temperature in the range of values of the glass transition temperatures of the dried film provided significant effects to modulate the properties of the films with the processing variables. A set of empirical equations was developed relating the properties to the processing conditions for tailoring to specific packaging applications.

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