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Enzymatic hydrolysis of cassava peels as potential pre-treatment for peeling of cassava tubers

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ABSTRACT

This study focused on the optimization of enzymatic treatments of cassava tuber peels to enhance hydrolysis and therefore improve the peeling process. A commercial enzyme was applied on the cassava peels. The parameters of the enzymatic treatments comprising temperature, pH, incubation time, and dose of enzyme were varied using the response surface methodology by the central composite design to optimize the enzymatic treatment of cassava peels. The glucose content and total soluble solids were determined as response factors for screening the enzymatic treatment efficacy. After 30 runs of enzyme treatments in the central composite design, multivariate correlation of the glucose content was established with R^2 at 0.956 and *MAPE* at 1.14 %. Results show that glucose content and total soluble solids were significantly ($p < 0.05$) affected by the enzyme dose and incubation time. Under optimal conditions the maximum glucose content, the maximum soluble solids and the loss in peel thickness were 21.2 %, 2.5 °Bx and 1.74 ± 0.2 mm, respectively, at a pH of 4.5, a temperature of 49.8 °C, an incubation time of 3.9 h and the addition of 1.25 mL enzyme per 1 g of cassava peels. Further investigation of enzyme treatment efficiency on peeling of cassava tuber was conducted under optimum conditions. Results showed that enzyme treatment can improve the peeling process of cassava tuber, with a reduction of peeling time to 75.0 ± 21.5 s and an increase of peeling yield to 82.0 ± 1.9 %.

1. Introduction

Cassava (*Manihot esculenta* Crantz), commonly known as manioc, tapioca or yucca, is an important crop in tropical and sub-tropical lands of Africa, Asia and Latin America (Burrell, 2003). Cassava has a higher carbohydrate production than other starchy staples such as rice and maize (Tonukari, 2004). Around one billion people consume cassava as a staple food (Latif and Müller, 2015).

Cassava roots can be consumed after cooking like other starchy tubers (Siritunga et al., 2004; Jimoh and Olukunle, 2012). Cassava tubers are also widely used as a raw material in different industries such as food, feed, biofuels, pharmaceutical and garment industries (Bokanga et al., 1994; IITA, 2011; Fakir et al., 2012). Fresh cassava does not have a long shelf life during storage due to its high moisture content (Chandrasekara & Kumar, 2016). Cassava is usually processed to obtain various relatively shelf-stable products (Reilly et al., 2003).

Peeling is one of the essential operations of cassava processing. Currently, it is performed by mechanical, thermal and chemical methods (Egbeocha et al., 2016). Practically, each method of peeling has its own advantages and disadvantages. Hand peeling is the traditional peeling method, which is still mainly done by women and teenage girls (Kehinde et al., 2007). Based on other studies, the average rate of manual peeling was 21.8 kg h^{-1} (Odigboh, 1985) but it could be as high as 43.7 kg h^{-1} (Igbeka et al., 1992). However, manually peeling is time consuming and labor intensive (Jimoh and Olukunle, 2012).

Another method is mechanical peeling, which is applied in small and large-scale industries. The mechanical peeling causes high losses due to the different shapes and sizes of cassava tubers, which is one of the main difficulties to mechanize the cassava peeling process (Ezekwe, 1976). Several prototypes have been constructed to develop efficient cassava peeling machines in Africa, Brazil and China (Odigboh, 1985; Adetan et al., 2005; Olukunle, 2007; Oluwole and Adio, 2013). Previous studies have reported different rates and efficiencies for peeling cassava tubers. Olukunle and Akinnuli (2013) reported that 88.7 % cassava peeling efficiency can be achieved with a mechanical peeling machine. A peeling efficiency of 85.0–94.6 % was reported in a recent study of Jimoh et al. (2016). Oluwole and Adio (2013) employed the abrasive method for peeling cassava tubers and the average peeling efficiency was 70.3 %.

Furthermore, Ebegbulem and Ngoddy (2013) found that immersing

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Table 1
Comparison of peeling methods for cassava tubers.

Method	Peeling rate (kg/h)	Peeling efficiency (%)	Losses (%)	Remarks	References
Manual	21.8	-	-		Odigboh (1985)
	37.5	99	4		IITA (1990)
	43.7	-	-		Igbeka et al. (1992)
Mechanical	44.8	83	5.4		Akintunde et al. (2005)
	10.4	75	7		Agbetoye et al. (2006)
	410	> 80	8		Olunkunle et al. (2010)
	725	72.2	17.37		Kamal and Oyelade (2010)
	442	75	41		Jimoh and Olukunle (2012)
	583	88.7	14		Olukunle and Akinnuli (2013)
	-	70.3	5.1		Oluwole and Adio (2013)
	96	85	5		Ukenna and Okechukwu (2014)
	520	72	33		Ugwu and Ozioko (2015)
	-	63.7	36.3		Daniyan et al. (2016)
	1046.5	94.6	11.2		Jimoh et al. (2016)
Chemical	-	-	-	quality not acceptable	Igbeka (1984)
Thermal					Sajeev et al. (2009)
	-	-	-	quality not acceptable	Abdullahi et al. (2010)
					Oluwole and Adio (2013)

cassava tubers in 15 % sodium hydroxide solution for 20 min at 60 °C would loosen and soften the cassava peels. In comparison with potato peeling using hot sodium hydroxide solution, cassava tubers require a higher concentration of sodium hydroxide, higher temperature and longer incubation time, which is not suited for the peeling process due to the extra costs and the possibility of food poisoning (Adetan et al., 2006; Jimoh and Olukunle, 2012).

The comparison of different peeling methods for cassava tubers in terms of peeling rate, peeling efficiency and losses is shown in Table 1.

Despite all the efforts undertaken to improve the cassava tuber peeling process, this step is still a drawback in cassava processing. Application of enzymes for cassava peeling is a novel concept and particular enzymes can hydrolyze the cell wall component of the peel and soften it enough for peeling. In addition, the enzyme technology as an environment-friendly, safe and effective substitution to the existing food processing, has a relevant effect on the sustainable technologies development in the food industry (Andreaus et al., 2016; Zhang et al., 2018). Furthermore, cassava peels as a main waste of cassava processing contain high amount of toxic compound such as cyanogenic glucosides which has a negative impact on environment (Sudharmono et al., 2016). Application of cell wall degrading enzymes can also reduce the toxicity by enhancing the release of linamarin and linamarase (Sornyotha et al., 2010). Several studies have been conducted to apply enzymes in the peeling process of fruits and vegetables such as oranges, peaches, apricots, nectarines and grapefruits (Pretel et al., 1997; Suutarinen et al., 2003; Toker and Bayindirli, 2003; Pretel et al., 2007a, b; Pagan et al., 2010; Noguchi et al., 2015). Betiku et al. (2013) and Bishai et al. (2015) also employed enzymes for hydrolyzing the peels of sweet potato and for peeling potato, respectively.

Depending on the compound of the peel, specific enzymes were chosen for the peeling process. For hydrolysis of sweet potato and potato peel, glucoamylase, cellulase and xylanase were employed (Betiku et al., 2013; Bishai et al., 2015).

According to our knowledge, there are no studies on the application of enzymes for cassava peeling. Therefore, the main objective of this study was to optimize the enzymatic treatment by varying operational conditions including pH, temperature, incubation time and enzyme dosage for the cassava peel hydrolysis for later application in cassava peeling process.

2. Materials and methods

2.1. Samples

Cassava tubers were bought from the local market in Stuttgart and peeled manually by removing the corky periderm (outer layer) and cortex (inner layer) separately. The peels were stored at −20 °C for further analysis.

2.2. Characterisation of cassava peels

Proximate composition of the corky periderm and cortex of cassava peel samples were analyzed by standard method of AOAC (1990). Dry matter content was determined using a cabinet dryer, (UM 700, Memmert GmbH & Co.KG, Schwabach, Germany) with the DIN CEN/TS 14774-3 (2004) method. The ash content was analyzed according to DIN EN 14775 (2012). The measurements for cellulose, hemicellulose and lignin were carried out according to the methods of Van Soest et al. (1991). The thickness of cassava peels was measured using a Vernier caliper with an accuracy of 0.1 mm at three different points.

2.3. Enzymes

Cellulose and hemicellulose are the responsible polysaccharides for the adhesion of peels to the flesh of tubers and the peel's hardness (Pagan et al., 2010). Therefore, hydrolyzing cellulose and hemicellulose can improve the peeling process of cassava tubers. Based on the composition of the cassava peels, Viscozyme® L with hemicellulase and cellulase activities were chosen for the enzymatic treatments in this study.

Viscozyme® L was purchased from Novozymes GmbH, Germany. This enzyme is a beta-glucanase with xylanase, cellulase and hemicellulase activities by declared activity of 100 beta-glucanase unit (FBGU/g) from supplier, produced from a selected strain of *Aspergillus aculeatus* that hydrolyzes (1, 3) - or (1, 4)-linkages in beta-D-glucans.

The manufacturer of this enzyme recommends following reaction conditions: temperature range of 25–55 °C, pH range of 3.5–5.5 and a minimum holding time of 1 h (Combo et al., 2011).

2.4. Enzymatic hydrolysis

About 3 g (3 ± 0.2) of cassava peels (both corky periderm and cortex) with thickness of 2.1 ± 0.3 mm and size of 40 × 30 mm were immersed in 100 mL of distilled water in a 250 mL Erlenmeyer flask (Duran group, Wertheim am Main, Germany). The Erlenmeyer flask with the solution was placed on a heating plate with adjustable temperature settings and stirring function with a speed of 100 rpm (RT 15, IKA, Staufen im Breisgau, Germany). The pH was adjusted by 0.01 M HCl to the required pH. A different dose of enzyme was added to the solution (0, 1.25, 2.5, 3.75 and 5 mL in 100 mL distilled water). The

mixture was incubated at a temperature of 40, 45, 50, 55 and 60 °C, at pH of 3.5, 4, 4.5, 5 and 5.5 and at a time of 1, 2, 3, 4 and 5 h. After the incubation time, the mixture was heated to its boiling point for 2–3 min to deactivate the enzyme. Then, the solution was passed through filter paper (4–12 μm). The filtrate was kept at −20 °C for further analyses.

2.5. Measurement of glucose content

To quantify the hydrolytic effect of the enzyme treatments, glucose as one the metabolites was analyzed. The glucose content was determined using high-pressure liquid chromatography (HPLC) with a refractive index detector (RID-20A, Shimadzu, Kyoto, Japan). All 20 μL samples were injected into a Repro-Gel Pb column (9 μm, 300 × 8 mm; Dr. Maisch GmbH, Ammerbuch, Germany). Water was used as the mobile phase, and elution was followed at 80 °C with a flow rate of 0.3 mL min^{-1} for 60 min. The concentration was estimated according to the retention times and peak area equivalent standards. A mixture of various sugars according to Picha (1985), 1 % of saccharose, glucose, galactose, arabinose, xylose and mannose was used as standard.

2.6. Measurement of TSS content

As a less sophisticated alternative to the glucose analysis, the total soluble solids (TSS) was also used as an indicator for the hydrolytic effect of the enzyme treatments. During the enzymatic hydrolysis, insoluble hemicellulose and cellulose is hydrolyzed to different soluble, low molecular weight sugars, not only glucose. The reducing sugars including all monosaccharides such as glucose and some disaccharides, such as cellobiose, are the main components of TSS (Grohmann and Baldwin, 1992). Therefore, the TSS was expected to increase after enzymatic hydrolysis. TSS measurement was carried out with a handheld digital refractometer (PR-201, Atago Co, Tokyo, Japan). After each run of the enzymatic treatments, one to two drops of the solution were placed onto the prism glass of the refractometer and the percentage of °Bx was determined.

2.7. Experimental design for the statistical optimization of enzymatic treatments of cassava peels

Based on previous studies, temperature, incubation time, pH and enzyme dose were considered as the main factors effecting the enzymatic hydrolysis (Bruemmer, 1981; Pretel et al., 2007a,b). To study the effect of these key factors, response surface methodology (RSM) was applied using the central composite design.

The complete central composite design (CCD) comprised 30 combinations (6 central points), with a four-level full factorial design using coded factors −2, −1, 0, +1 and +2. The selected independent variables were incubation time (1–5 h), temperature (40–60 °C), pH (3.5–5.5) and enzyme dose (0–5 mL in 100 mL distilled water). Glucose content and TSS were determined as response factors for screening the enzymatic treatment efficacy assuming that the breakdown of cellulose and hemicellulose will result in an increase of the glucose content and total soluble solids. At the optimum conditions, the thickness of enzyme treated cassava peels were measured with an accuracy of 0.1 mm.

2.8. Scanning electron microscopy of enzyme-treated cassava peels

To investigate surface characteristics of the cassava peels, scanning electron microscopy study (SEM) were obtained from the cassava peels without enzyme treatment (control) and with enzyme treatment at optimum conditions. The dried samples were located on a graphite layer and covered with 80 % palladium and 20 % gold for few minutes. Afterward, the samples were observed at magnifications of × 200–500 using a scanning electron microscope (DSM-940, ZEISS, Jena, Germany) under high-vacuum conditions with an accelerating voltage of 5.0 kV. Several images were obtained from different areas of the samples (at least 5 images per sample). Microscopy observations were made at the Institute of Botany (210) of the University of Hohenheim, Germany.

2.9. Manual peeling test

After optimizing the enzyme treatment for hydrolysis of cassava peels, the effect of enzyme treatment on the peeling process of cassava tubers was investigated. Cassava tubers weighing 352.90 ± 79.77 g with mass proportion of peels of 18.6 ± 0.5% were washed and scored with a sharp knife by that the enzyme solution can penetrate through the cortex of cassava peels. Cuts were made following meridian lines with a distance of 2 cm on cassava tubers and reached the cortex of cassava peels, but not the flesh. The scored cassava tubers were treated by enzyme solution at the optimum conditions found in this study. After enzyme treatment, cassava tubers were peeled manually. The peeling of cassava tubers treated with enzyme was compared to scored cassava tubers treated by the same procedure (pH, temperature and incubation time) as enzyme treatment but without adding enzyme to the solution (blank solution) and by scored cassava tubers without any treatment (control).

Five test persons, who have not been informed about the different treatments applied on cassava tubers, were called in to conducted the single-blind peeling experiment. Each test person was assigned to peel the cassava tubers treated with enzyme solution, blank solution and control. The peeling of cassava tubers was evaluated in terms of peeling yield, peeling time and easiness of peeling according to Pretel et al. (1998), Rock et al. (2012) and Mohamad et al. (2017).

The peeling yield was calculated as:

$$peeling\ yield\ (\%) = \frac{m_2}{m_1} \cdot 100 \quad (1)$$

where m_1 (g) is the initial mass of cassava tuber before peeling and m_2 (g) is the mass of cassava tuber after manual peeling.

The peeling time (s) of each cassava tuber was measured by a stopwatch. The easiness of peeling was determined based on a subjective scale reaching from very difficult (1 point) to very easy (5 points). For each treatment, 15 cassava tubers of similar size, about 6 kg in total, were peeled. The results of peeling yield, peeling time and easiness of peeling were reported in details and as mean ± SD.

2.10. Penetration force of cassava tubers

To investigate the texture quality of cassava tubers, the force to penetrate the cassava tuber flesh after each treatment was measured using a penetrometer (PCE-FM200, PCE Deutschland GmbH, Meschede; Germany).

2.11. Statistical analysis

For designing the experiments and analyzing the obtained data, a student version of Design Expert 10 (STATCON GmbH, Witzenhausen, Germany) was used. With respect to the statistical analysis, the correlation coefficient (R^2) and the mean absolute percentage error (*MAPE*) were determined to estimate the accuracy of the mathematical model. To validate the optimization of the enzyme treatment, three replicates were conducted at optimum conditions. The peeling experiments were also conducted in three replicates and the mean values were reported as mean ± SD. The significant difference among the mean values was analyzed by ANOVA at a level of $\alpha = 5$ %.

3. Results and discussion

3.1. Characteristics of cassava peels

The results of the proximate analysis of cassava peels comprising

Table 2
Characteristics of the cassava peels.

	Portion of mass %	Moisture content % w.b.	Ash g/100 g d.b.	Cellulose g/100 g d.b.	Hemicellulose g/100 g d.b.	Lignin g/100 g d.b.
Cortex	74	80.01 ± 0.85	4.23 ± 0.20	10.14 ± 0.32	30.40 ± 0.13	5.01 ± 0.56
Corky periderm	26	22.47 ± 0.40	4.38 ± 0.10	12.49 ± 0.67	6.54 ± 0.20	48.91 ± 0.37

Note: Data are presented as mean ± SD. Cellulose and hemicellulose are calculated by ADF-ADL and NDF-ADF, respectively.

corky periderm and cortex are presented in Table 2. The results show that the corky periderm had a lower water content than the cortex. It was determined that the corky periderm had a lower hemicellulose content and a higher lignin content compared to the cortex. Furthermore, there was no obvious difference in the ash content of corky periderm and cortex. Similar to the cellulose content of the cortex and the corky periderm of 10.14–12.49 % in this study, Pooja and Padmaja (2014), Lounglawan et al. (2011) and Babayemi et al. (2010) found that the cellulose content of cassava peels was 14 %, 11.58 %, and 14 %, respectively. In addition, the hemicellulose content of the cortex is in line with the findings of Adekunle et al. (2016) (32.3 %) and Kouteu Nanssou et al. (2016) (27 %). However, the hemicellulose content was different in other studies (Aderemi and Nworgu, 2007; Lounglawan et al., 2011; Pooja and Padmaja, 2014). The differences among the results can be explained by climatic conditions, soil fertility, harvesting time, soil types or cultivar used (Dien et al., 2006).

3.2. Effect of enzyme treatment on cassava peels

The RSM design matrix for enzyme treatment is presented in Table 3 together with the glucose content and TSS of cassava peels. The glucose content increased from 1.7 % to 22.5 % (wet mass basis) by varying the condition parameters (pH, temperature, incubation time, enzyme dose). The TSS increased from 0.3 to 2.9 °Bx. The highest glucose content (22.5 %) with the highest TSS (2.9°Bx) was observed at a pH value of 4.5 and a temperature of 50 °C, with 3 h incubation time and an enzyme dose of 1.67 mL per 1 g of cassava peels.

The quadratic equation obtained from RSM for the effect of enzyme treatment on glucose content was:

$$Glc = 17.70 - 0.22{\cdot}pH - 0.39{\cdot}T + 1.04{\cdot}t + 4.64{\cdot}E - 0.56{\cdot}pH{\cdot}T + 0.22{\cdot}pH{\cdot}t - 0.12{\cdot}pH{\cdot}E - 0.65{\cdot}T{\cdot}t + 0.73{\cdot}T{\cdot}E + 0.10{\cdot}t{\cdot}E - 1.96{\cdot}pH^2 - 1.93{\cdot}T^2 - 0.64{\cdot}t^2 - 1.57{\cdot}E^2 \quad (2)$$

where *Glc* is the glucose content after enzymatic hydrolysis (%, wet mass basis), *T* is the temperature (°C), *t* is the incubation time (h) and *E* is the enzyme dose (mL).

The coefficients in Eq. (2) indicate that an increase in incubation time and enzyme dose had a positive effect on the glucose content. However, the increase in pH and temperature had a negative impact. Regarding the square terms, all variables had a negative impact on the glucose content.

The effects of the individual variables and their interaction on the glucose content was examined in Table S1. The accuracy of the model was indicated by R^2 and an adjusted R^2 of 0.956 and 0.915, respectively. The *MAPE* was 1.14 %. Incubation time and enzyme dose influenced the glucose content of cassava peels significantly ($p < 0.05$).

Table 3
RSM design matrix and experiment results for enzyme treatment.

Run	Condition parameters				Coded factors				Glucose content (%)		TSS (°Bx)	
	pH X_1	Temperature(°C) X_2	Incubation time(h) X_3	Dose of enzyme (mL) X_4	X_1	X_2	X_3	X_4	Observed	Predicted	Observed	Predicted
1	4	55	4	1.25	−1	1	1	−1	6.3	6.6	0.9	1.0
2	4.5	50	5	2.5	0	0	2	0	19.4	17.2	2.0	1.8
3	4.5	50	3	2.5	0	0	0	0	19.1	17.7	1.8	1.9
4	3.5	50	3	2.5	−2	0	0	0	10.5	10.3	1.7	1.5
5	5	55	4	1.25	1	1	1	−1	5.8	5.7	0.8	1.0
6	4	55	2	1.25	−1	1	−1	−1	5.8	6.4	0.9	0.8
7	5	45	2	1.25	1	−1	−1	−1	7.6	6.7	0.9	0.9
8	5.5	50	3	2.5	2	0	0	0	10.5	9.4	1.6	1.6
9	4	45	4	3.75	−1	−1	1	1	16.3	17.3	2.2	2.3
10	4	45	4	1.25	−1	−1	1	−1	9.8	9	0.9	1.1
11	4.5	50	3	2.5	0	0	0	0	17	17.7	1.9	1.9
12	4.5	60	3	2.5	0	2	0	0	9.8	9.2	1.6	1.4
13	4	55	4	3.75	−1	1	1	1	15.8	17.8	2.1	2.1
14	4.5	50	3	2.5	0	0	0	0	17.8	17.7	2.0	1.9
15	4.5	50	3	2.5	0	0	0	0	17.3	17.7	1.8	1.9
16	4	45	2	3.75	−1	−1	−1	1	13	14.1	2.2	2.0
17	5	45	4	1.25	1	−1	1	−1	8	10.3	1.1	0.9
18	5	55	2	1.25	1	1	−1	−1	5.3	4.7	0.8	0.9
19	4.5	40	3	2.5	0	−2	0	0	11.5	10.8	1.6	1.6
20	4.5	50	3	0	0	0	0	−2	1.7	2.1	0.3	0.3
21	4.5	50	3	2.5	0	0	0	0	16.8	17.7	1.8	1.9
22	5	45	2	3.75	1	−1	−1	1	14.1	14.1	2.2	2.3
23	4	45	2	1.25	−1	−1	−1	−1	6.6	6.3	0.9	1.0
24	4.5	50	3	2.5	0	0	0	0	18.2	17.7	1.9	1.9
25	5	45	4	3.75	1	−1	1	1	17.7	18.1	2.4	2.5
26	4.5	50	1	2.5	0	0	−2	0	12.2	13.1	1.5	1.5
27	5	55	2	3.75	1	1	−1	1	13.2	15	2.2	2.0
28	5	55	4	3.75	1	1	1	1	15.7	16.4	2.2	2.3
29	4.5	50	3	5	0	0	0	2	22.5	20.7	2.9	2.7
30	4	55	2	3.75	−1	1	−1	1	19.2	17.2	1.2	1.7

The *MAPE* of glucose content and TSS was 1.14 % and 8.56 %, respectively.

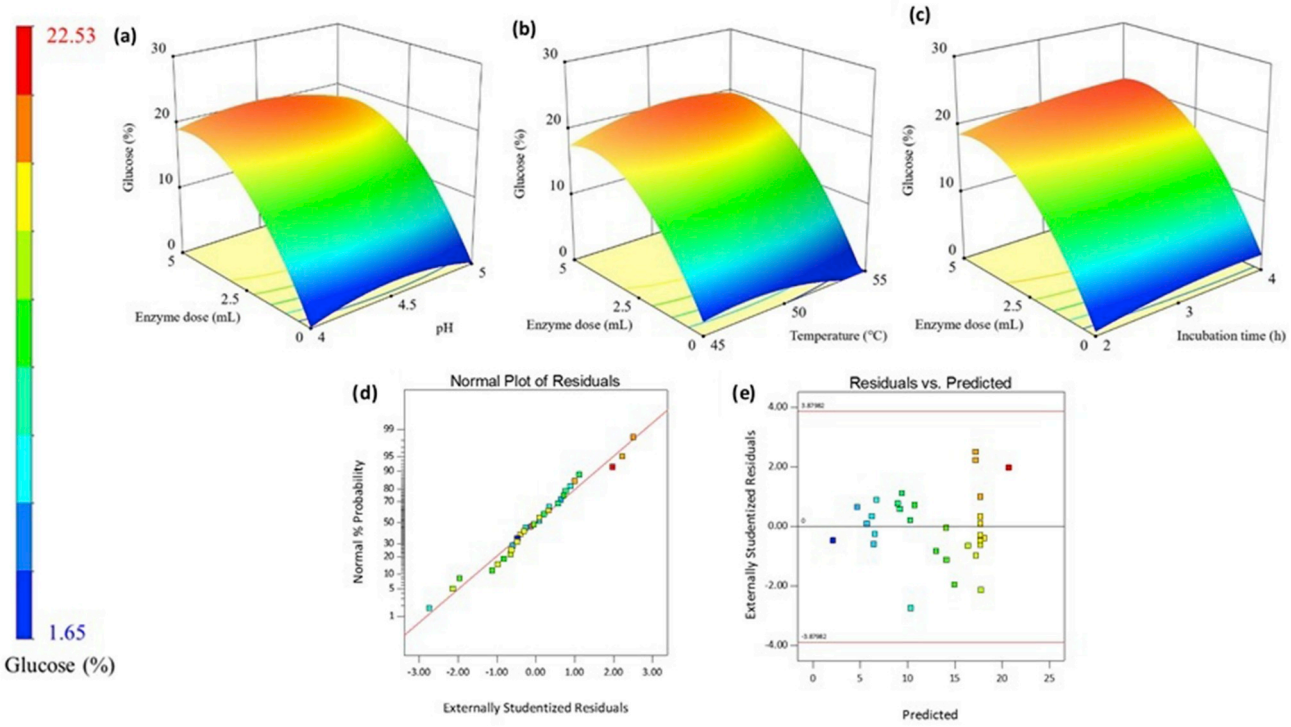

Fig. 1. Glucose content (%) of cassava peels after enzyme treatment, (a, b, c) surface plots indicating the effect of pH, temperature (°C), incubation time (h), enzyme dose (mL), (d) normal probability plot of the residuals and (e) plot of residuals versus predicted values.

Higher p-values for pH, temperature and interaction terms suggested little impact on the glucose content.

The surface plots for glucose content as a function of pH, temperature, incubation time and enzyme dose are shown in Fig. 1a, b and c. The model was further analyzed based on the normal probability plot for the externally studentized residuals. It was found that most of the residuals fall on the straight line (Fig. 1d). This indicates a normal distribution of data. Furthermore, the plot of residuals versus predicted values in Fig. 1e shows no clear pattern among data, which implies that there were no biases (Wilk and Gnandesikan, 1968; Johnson and Bhattacharyya, 2010).

The quadratic equation obtained from RSM for the effect of enzyme treatment on the TSS content was:

$$TSS = 1.87 + 0.046 \cdot pH - 0.071 \cdot T + 0.096 \cdot t + 0.61 \cdot E + 0.031 \cdot pH \cdot T - 0.031 \cdot pH \cdot t + 0.081 \cdot pH \cdot E + 0.031 \cdot T \cdot t - 0.056 \cdot T \cdot E + 0.056 \cdot t \cdot E - 0.080 \cdot pH^2 - 0.093 \cdot T^2 - 0.055 \cdot t^2 - 0.093 \cdot E^2 \quad (3)$$

where *TSS* is the total soluble solids after enzymatic hydrolysis (° Bx), *T* is the temperature (°C), *t* is the incubation time (h) and *E* is the enzyme dose (mL).

The coefficients in Eq. (3) indicate that an increase in pH, incubation time and enzyme dose had a positive effect on the TSS. However, the increase in temperature had a negative impact. Regarding the square terms, all variables had a negative impact on TSS.

The effects of the individual variables and their interaction on the TSS are demonstrated in Table S2. The accuracy of the model was indicated by R^2 and the adjusted R^2 at 0.938 and 0.880, respectively. The *MAPE* was 8.56 %. Incubation time and enzyme dose influenced the TSS of cassava peels significantly ($p < 0.05$). Higher p-values for pH, temperature and interaction terms suggested little impact on the TSS.

Figure S1, a, b and c illustrate the surface plots for TSS as a function of pH, temperature, incubation time and enzyme dose. Normal distribution of the data and no distortion of the model was shown in Figure S1 d and 2e, respectively.

Based on the model, a maximum glucose content of 21.2 % with a maximum TSS of 2.5 °Bx was predicted for a pH value of 4.5, a temperature of 48.9 °C, an incubation time of 3.9 h, and an enzyme dose of 1.25 mL per 1 g of cassava peels. Three experiments were conducted at optimal conditions to validate the prediction of the model. The maximum glucose content was 20.6 ± 0.24 % (mean ± SD), which was close to the predicted maximum glucose content of the model. In addition, the loss in peel thickness was 1.74 ± 0.2 mm at the optimum conditions.

3.2.1. TSS vs. glucose measurement

Other metabolites measured by HPLC under optimum conditions after the enzyme treatment and without enzyme treatment in addition to the glucose content and TSS are presented in Fig. 2.

The analysis of various sugars indicates that glucose content alone was not an adequate indicator for beta-glucanase hydrolysis because

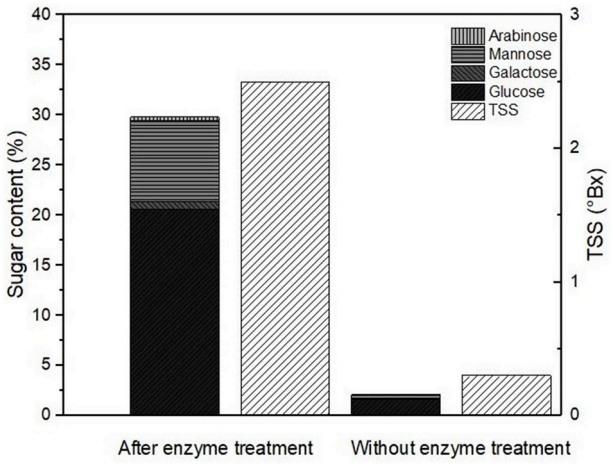

Fig. 2. The sugar content (%) and TSS (°Bx) of cassava peels after enzyme treatment at the optimum conditions and without enzyme treatment.

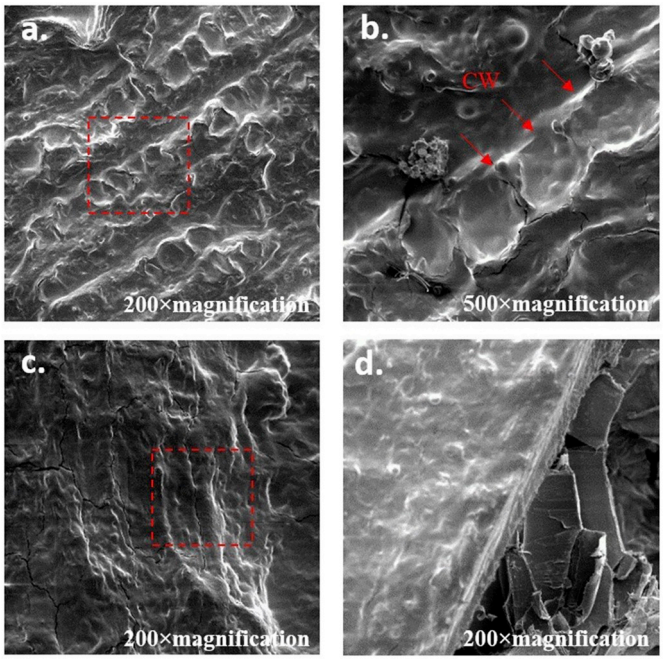

Fig. 3. Scanned electron micrograph of cassava peels without enzyme treatment (a,b), and after enzyme treatment (c,d) at optimum conditions (CW: cell wall).

other sugars increased as well after the enzyme treatment (e.g. cellobiose and maltose). The cellobiose, as one of the metabolites after enzyme treatment, which was not determined by HPLC in this study may have increased after enzyme treatment as well. For further studies, also the cellobiose content should be investigated. Determining reducing sugar would be a better indicator for exhibiting the efficiency of enzymatic hydrolysis. Based on previous studies, the dominant components of the solubilized solids are reducing sugars (Grohmann and Baldwin, 1992; Waes et al., 1998). Consequently, higher TSS indicates higher reducing sugars after enzyme treatment. Therefore, TSS is more adequate to indicate hydrolysis efficiency of cassava peels in this study. Moreover, measuring TSS is less expensive and quicker than measuring glucose by HPLC. Therefore, TSS measurements can be considered as a fast method for screening the efficiency of enzymatic hydrolysis, even though, TSS is not as accurate as a HPLC sugar analysis.

3.3. Scanning electron microscopy of cassava peels

The micrographs of the cassava peels are presented in Fig. 3. The scanned electron micrographs show a regular alignment of cells enclosed with cell walls in the cassava peels without enzyme treatment (3 a, b). Through the hydrolytic effect of the enzyme treatment, the peel surface became scattered, loosened and rugged, which might be explained by the breakage of cell walls (3 c, d). Furthermore, the cell structure of cassava peels was more porous, collapsed and damaged in enzyme treatments compared to the cassava peels without enzyme treatment. These results are in accordance with Agarwal and Bosco (2014) in which SEM images revealed the change in microstructure of coconut kernel treated with enzyme. It was observed that the cell-wall degrading enzyme can disorganize the cell structure because of the breakdown of polysaccharides in the cell wall. Similarly, Bishai et al. (2015) reported that treatment with enzymes could result in the rupture of the cell structure and breakage and collapse of the cell wall.

3.4. Influence of enzyme treatment on manual peeling performance and penetration force of cassava tubers

Table 4 shows the peeling yield, peeling time, easiness of peeling for cassava tubers treated with enzyme solution, blank solution and control. It was observed that enzyme-treated cassava tubers had the lowest peeling time (75.0 ± 21.5 s), highest peeling yield (82.0 ± 1.9 %) and easiest peeling (4.7 ± 0.4 points) followed by blank solution and control. The statistical analysis of measured traits for cassava tubers treated with enzyme solution, blank solution and control are shown in Table S4. There were significant differences among peeling yield

Table 4
Peeling yield (%), peeling time (min), easiness of peeling of cassava tubers treated with enzyme solution, blank solution and without treatment (control).

		Peeling yield (%)		Peeling time (s)		Easiness of peeling*	
		Mean	SD	Mean	SD	Mean	SD
Enzyme solution	Test person 1	80.1	2.0	56.4	8.3	5.0	0.0
	Test person 2	81.6	1.8	101.3	22.2	4.0	0.0
	Test person 3	82.4	0.6	79.4	10.3	5.0	0.0
	Test person 4	80.8	1.1	87.7	7.9	4.5	0.6
	Test person 5	85.0	1.0	50.0	3.0	5.0	0.0
	Mean ± SD	82.0 ^{bc} ± 1.9		75.0 ^{a} ± 21.5		4.7 ^{bc} ± 0.4	
Blank solution	Test person 1	78.5	0.7	89.8	14.3	3.7	0.6
	Test person 2	80.6	1.6	123.0	33.8	3.3	0.5
	Test person 3	77.5	1.6	202.7	22.8	2.8	1.0
	Test person 4	79.0	1.7	139.7	1.7	2.5	0.7
	Test person 5	77.3	1.7	70.1	7.7	3.3	0.6
	Mean ± SD	78.6 ^{ab} ± 1.3		125.1 ^{ab} ± 51.2		3.1 ^{ab} ± 0.5	
Control	Test person 1	74.6	1.1	109.9	12.5	3.3	0.6
	Test person 2	80.1	0.5	191.8	30.6	3.0	0.0
	Test person 3	72.5	2.3	228.9	77.9	2.3	1.0
	Test person 4	79.5	1.6	175.8	86.0	2.3	1.1
	Test person 5	78.2	0.3	102.0	10.0	2.0	0.5
	Mean ± SD	77.0 ^{a} ± 3.3		161.7^{bc} ± 54.5		2.6 ^{a} ± 0.6	

Reported values of each test person are presented as mean values ± SD (n = 3).
Mean values with the same letter in a column for each measured trait are not significantly different as indicated by Tukey's test ($p < 0.05$).
* very difficult (1 point) - very easy (5 points).

($p = 0.015$), peeling time ($p = 0.031$) and easiness of peeling ($p < 0.0001$). As those differences have been significant, it can be stated that the enzyme treatment by hydrolyzing the composition of cassava peels improved the peeling efficiency of cassava tubers. Similar to these results, Pretel et al. (2007a) and Mohamad et al. (2017) stated that by enzymatic peeling of oranges and mangoes, the peeling yield increased, the peeling time decreased and the peeling process became easier.

The peeling performance showed quite large variations between the test persons. This can be explained by the difference in peeling experience of the test person. In accordance to this study, a wide range of peeling rate (21.8–43.7 kg/h) for manual peeling of cassava tubers was also found in literature (Odigboh, 1985; Igbeka et al., 1992).

Regarding the texture of the tubers, there was no significant difference in penetration force of tubers treated with enzyme solution, blank solution and control (Tables S3 and S4). This indicates that the enzyme treatment did not soften the cassava flesh, as observed in other studies where thermal and chemical treatments negatively affected the quality of the tubers and led to rejection of these methods (Table 1).

Based on previous studies regarding enzymatic peeling of fruits and vegetables, cultivar, size, shape, thickness of peel and adhesion of the peels to the flesh would affected the peeling time and peeling yield (Pretel et al., 1997; Toker and Bayindirli, 2003; Pretel et al., 2007a,b). Therefore, those influencing parameters also have to be investigated in further studies on the peeling of cassava tubers.

4. Conclusion

The results indicate that enzymes with cellulose and hemicellulase activity can be effective to improve the cassava peeling process by hydrolyzing the cassava peels. The highest hydrolytic effect was achieved at a pH of 4.5, a temperature of 49.8 °C, an incubation time of 3.9 h and an enzyme dose of 1.25 mL per 1 g of cassava peels. The results of enzyme treatment on peeling of cassava tubers show that enzyme treatment positively affected the peeling process by increasing the peeling yield and decreasing peeling time.

A SEM study illustrated cell wall degradation of cassava peels after enzyme treatments. Further studies are planned to integrate enzymatic treatment in the mechanical peeling process of cassava tubers for later industrial-scale use.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101247.

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