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Process Development for Maltodextrins and Glucose Syrup from Cassava

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The objective of this study was to produce maltodextrins (MD) and glucose syrup (GS) throughout a small-scale process from the direct conversion of cassava roots collected in Burundi and previously detoxified.
The detoxified cassava slices were blended with water at ratios of 1:1.0; 1:1.3; 1:1.6. Then, the cassava mash was undergone previously to gelatinization and then to liquefaction experiments aimed at obtaining MD with a Dextrose Equivalent (DE) value < 20. The doses of 0.013, 0.016, 0.019, 0.025, and 0.075 % ($v_{enzyme}/w_{fresh\ mash}$) thermostable α-amylase (Liquezyme-X) were investigated to be added to cassava mash at pH 6.5 before and after 10 min - 90 °C step at atmospheric pressure (p_{atm}) or 143.27 kPa (110 °C) allowing the starch gelatinization. Then liquefaction times of 10, 15, 20, 30, 40, 45, 60, 90 and 120 min were tested. The saccharification step followed the liquefaction in order to obtain a GS with DE close to 99. The hydrolyzed cassava mash from liquefaction experiments was added at pH 5.4 and 60°C with 0.019 % ($v_{enzyme}/w_{fresh\ mash}$) glucoamylase (Dextrozyme GA) and pullulanase (Dextrozyme GX) testing 1, 2, 4, 6, 18, 24, and 48 h incubation times. All experiments were done in duplicate and analysis of variance (ANOVA) with Tukey's test at $p \leq 0.05$ was used to measure the effect of changing variables among treatments. Correlation Pearson's test were applied to measure the strength of the interactions between the variables.
Results showed that the 10 min-143.27 kPa (on lab-scale) and the 12 min-145÷152 kPa (on small-scale) burst of starch granules in 1:1.6 cassava: water mash with 0.013 % ($v_{enzyme}/w_{fresh\ mash}$) thermostable α-amylase at pH 6.5 followed by 15 min-90 °C liquefaction phase at p_{atm} allowed at obtaining MD with DE value < 20. In order to gain a GS having a DE value close to 99, a 4 h-60 °C saccharification phase at pH 5.4 with 0.019 % ($v_{enzyme}/w_{fresh\ mash}$) glucoamylase and pullulanase should be carried out. Finally, highly significant correlations were found out between the water amount in the cassava mash, the concentration of the α-amylase enzyme, and the liquefaction time.
This type of process had the advantage to be simple and practical, with reduced working times and enzyme doses, so to be popularizing especially in developing Countries. Further investigations are needed on some energy intensive operations, as like as pH adjustment, pressure increasing, and heating.

1. Introduction

Maltodextrins (MD) are enzymatic and/or acid hydrolysis products of starch, consisting of a-(1,4) linked D-glucose oligomers and/or polymers, which are normally defined as having a dextrose equivalent (DE) value < 20. They are commonly used as spray-drying aids for flavours and seasonings, carriers for synthetic sweeteners, texture providers, fat replacers, film formers, and bulking agents in the food industry (Chronakis, 1998). Glucose syrup (GS) is a food ingredient obtained from the hydrolysis of starch. High glucose syrup (HGS) is used mainly as a source of crystalline dextrose or as a substrate for high fructose syrup production (HFS). HGS finds application in beverage and confectionery industry while HFS is predominantly used in ice cream, yogurt, processed foods and as feed for honey bees (Johnson et al., 2009). Maize has been the main raw material for conversion of starch to sugars because of its high productivity. However, when compared with root starches, corn starch is not optimal for hydrolysis. The low content of lipids (0.1%) in cassava starch ensures that amylose–lipid complex formation is negligible; consequently, good liquefaction can be achieved at a lower temperature, and retrogradation problems are

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less severe. An additional advantage of cassava starch is its low protein content; consequently, less color is developed during hydrolysis, and refining requirements are reduced (Carpio et al., 2011). Moreover, according to the Food and Agricultural Organization of the United Nations, the properties of tropical starches must be studied in order to help the Country development for an efficient competition in the international trade of starch (Moore et al., 2005). Although, its primary use is as a food crop, cassava is widely used for the production of starch and its role has been increasingly recognized also in incoming Countries for the production of polylactic acid, bioethanol, GS, and HFS (Chinnawornrungsee et al., 2013; Lauven et al., 2013; Moore et al., 2005; Rickard et al., 1991).

The acid-catalyzed depolymerization of starch is still applied extensively in starch-processing factories, despite the full commercial availability, but at higher costs, of several enzymatic preparations for starch hydrolysis. The most intensively employed enzymes are the thermoresistant α-amylases (maltogenic for amylose, malto- and dextrinogenic for amylopectin; EC 3.2.1.1) and amyloglucosidases or glucoamylases (glucogenic for both starch fractions; EC 3.2.1.3). The combination of both types of enzyme involves two steps such as liquefaction where the enzyme α-amylase partially hydrolyzes starch to maltodextrins and saccharification where the low DE syrup is completely converted to glucose by glucoamylase (Fontana et al., 2001). In developing countries, cassava starch is hydrolyzed at artisanal and industrial scales (Ghildyal et al., 1989; Moore et al., 2005). The direct hydrolysis of cassava root slurry offered the advantage that part of the naturally occurring low molecular carbohydrates could be converted to reducing sugars (Berghofer and Sarhaddar, 1988). The possibility of using cassava chips for economic production of starch hydrolyzates was investigated by Ghildyal et al. (1989), but the gain in lower expenses on cassava chips as compared to cassava starch was overturned by the higher capital investment on plant and machineries and the requirement of different processing conditions. Given the great potential for cassava starch syrup production industries in tropical countries, there is a need for tools such as small-scale processes that permit optimization and scaling of the process (Morales et al., 2008).

This paper focused on a new methodology enabling a small-scale manufacturing process of cassava roots previously detoxified according an improved method (Lambri et al., 2013) into MD and GS reducing as much as possible both amounts of enzymatic preparations and times of treatments.

2. Materials and Methods

2.1 Raw materials

Cassava roots were collected directly from a rural market in Gitega, Burundi. The roots were peeled and cut with a knife. They were mixed together and grated into small sticks with Kenwood Chef Major Titanium KM020 and Vegetable Processor AT340 supplied by De Longhi Appliances s.r.l. (Treviso, Italy). Roots were detoxified according to the improved method reported by Lambri et al. (2013).

2.2 Enzyme sources

Thermostable α-amylase (from *Bacillus licheniformis*, 135 KNU/g, Liquezyme-X), glucoamylase (from *Aspergillus niger*, 400 AGU/g, Dextrozyme GA), and pullulanase (from *Bacillus acidopullulyticus*, 400 AGU/g, Dextrozyme GX) were purchased from M/s Novozymes A/S (Denmark). Pectolitic enzyme (with more than 200 units PL/g, Enartis Zym Quick) was provided by Enartis (Novara, Italy).

2.3. Preparation of wet root slurry

The detoxified cassava chips were blended in a laboratory electric blender (La Moulinette, Mulinex, Groupe SEB, France) using little water to a fine paste. The mash was made up testing cassava: water ratios of 1:1.0; 1:1.3; 1:1.6. (w:w). For lab-scale trials 30g of fine cassava paste was mixed with 30g, 40g, and 50g of water respectively. In small-scale process 800g of cassava paste was used.

2.4. Enzymatic hydrolysis to MD

The liquefaction phase of cassava mash aimed at obtaining a DE value < 20, was experimented under treatments 1 (T1), 2 (T2), and 3 (T3).

T1 – In order to search for the optimal dilution of cassava mash with water, 1:1.0; 1:1.3; 1:1.6 (w:w) cassava: water mixtures were adjusted to pH 6.5 and 10 min kept at 90 °C (p_{atm}) in a thermostatic ethylene glycol bath (F25 Julabo Labortechnik GMBH, Seelbach Germany). Then, 0.025 % ($v_{enzyme}/w_{fresh\ mash}$) thermostable α-amylase was added and the mixture was maintained at 90 °C for 1.0, 2.0, and 3.0 h.

T2 – In order to investigate for the lowest dose of thermostable α-amylase, 0.013%, 0.016%, 0.019%, 0.025%, and 0.075% ($v_{enzyme}/w_{fresh\ mash}$) was added to cassava mash prepared with the optimal cassava: water ratio (from T1) and adjusted to pH 6.5. The mixture was 10 min kept at 90 °C (p_{atm}) in the ethylene glycol bath and then incubated at 90 °C for 1.0, 2.0, and 3.0 h.

T3 – The mash with the optimal cassava: water ratio (from T1) was added of the lowest dose of thermostable α-amylase (from T2) before a 10 min-143.27 kPa step. Then, the mixture was brought to p_{atm} and maintained at 90 °C for 10, 15, 20, 30, 40, 45, 60, 90, and 120 min.

2.5. Enzymatic hydrolysis to GS

Treatment 4 (**T4**) was arranged to carry out the saccharification phase of MD in cassava mash to GS. Cassava mash at optimal ratio of cassava: water (from T1) was adjusted to pH 6.5 and 10 min treated under 143.27 kPa with the optimal dose of thermostable α-amylase (from T2). The mixture was then incubated at 90 °C (p_{atm}) for a time corresponding to that optimal from T3. Then, temperature and pH for adding the 0.019% ($v_{enzyme}/w_{fresh\ mash}$) of glucoamylase and pullulanase were investigated, and the shortest saccharification time allowing the greatest DE was chosen among 1, 2, 4, 6, 18, 24, and 48 h.

2.6. Small-scale process to MD and GS

From lab- to small-scale process treatment 5 (**T5**) was applied. Cassava mash produced with 800g of root slices at optimal ratio of cassava: water (from T1) was adjusted to pH 6.5 and added of thermostable α-amylase at the optimal dose (from T2). Then the mixture was submitted for 15 min at 145÷152 kPa and then incubated at 90 °C (p_{atm}) to shorten the time provided from T3. The temperature and the pH for the addition of 0.019% ($v_{enzyme}/w_{fresh\ mash}$) glucoamylase and pullulanase were arranged as provided in T4. Incubation continued with the attempt to shorten the time provided from T4.

2.7. Clarification and concentration of GS

GS obtained from small-scale process (T5) was 15 min-25 °C centrifuged at 5000g with Varifuge 20RS (Heraeus,Hanau, Germany) before and after a pectin elimination step (12 h-4 °C) with 110 µl/L of pectolitic enzyme. A protein elimination step (8 h-4 °C) with 1 g/L of bentonite (TopGran DC, Dal Cin, Concorezzo, Italy) was followed by centrifugation, and then a decolourization step (1 h-25 °C) with 0.2 g/L of charcoal (Carlo Erba, Milan, Italy) under slow agitation was done. The final filtration was performed with laboratory vacuum drum filter before submitting the limpid GS to a 40 %-60 % concentration step (60 °C; p=100mbar) with Büchi Rotavapor R-114 and Büchi Waterbath B-480 (Büchi, Swiss).

2.8. Analytical determination

Total sugar content of cassava

Twenty grams of detoxified sliced roots were extracted using 80 % ethanol (1:20 w/v). The alcoholic sugar filtrate and the residue containing starch added of 100 mL water were hydrolyzed at 100 °C with respectively 1 mL 18 N HCL for 30 min and 2 mL 18 N HCL for 5 h. Then, the samples from the acid hydrolyzed sugar and starch were clarified with the Carrez's reactive, and treated with charcoal before being filtered and titrated for detecting the total sugar content according to Lane and Eynon (1923).

Reducing sugars and DE of hydrolizates

Dextrose Equivalent (DE) determination was made on hydrolizates according to AOAC 935.62 and 923.09 methods (1990). The reducing sugars were determined with Lane and Eynon (1923) method and DE was computed using the formula: (%reducing sugars * 100) / (%dry matter). The glucose content of the hydrolizates was determined by the glucose oxidase–peroxidase method using the Megazyme D-Glucose (GODOP) assay kit (Megazyme International Ireland). HPLC of the clarified and concentrated GS with determinations of mono- and oligosaccharides was performed according to Hayes et al. (1995).

2.9. Statistical analysis

All experiments were done in duplicate and analysis of variance (ANOVA) with Tukey's test at $p \leq 0.05$ was used to measure the effect of changing variables among treatments. Correlation Pearson's test were applied to measure the strength of the interactions between the variables. The software IBM SPSS Statistics 19 (IBM Corporation, New York, USA) was used.

3. Results and discussion

Acid hydrolyzed cassava root slices used for the study showed a DE close to 99 and 43% glucose on dry weight (w/dw). Both values are greatest than those obtained by Johnson et al. (2009).

3.1 Enzymatic hydrolysis to MD

The first part of the process aimed at obtaining MD with DE < 20 was arranged under T1, T2, and T3 experiments. T1 was designed at optimizing the ratio between cassava and water of the mash which was adjusted to pH 6.5 and 10 min kept at 90 °C (p_{atm}) to attain starch gelatinization before the addition of 0.025 % ($v_{enzyme}/w_{fresh\ mash}$) thermostable α-amylase and the incubation at 90 °C (p_{atm}) for 1.0, 2.0, and 3.0 h. Results reported in Figure 1A showed that irrespective of the liquefaction time a mash prepared mixing cassava fine paste and water according to a ratio of 1:1.6 gave rise to a DE value > 15; conversely,

cassava mash prepared with a 1:1.0 ratio produced DE value < 15. The mash at 1:1.3 ratio gave a DE between 15 and 20, only when liquefaction lasted 3 h and highlighted a DE in the range 10 ÷ 15 after 1 and 2 h. This last result is similar to the Johnson and Padmaja (2013)'s data obtained under similar condition to T1 with the same thermostable α-amylase enzyme (Liquezyme-X) and 1 h liquefaction of a 20% cassava starch suspension. Under conditions far from T1 and from a suspension of 3% cassava starch, Moore et al. (2005) gained DE values of 31.4 and 53.2 after 1 and 2 h of liquefaction, respectively.
Results reported in Figure 1B are referred to T2 applied to reduce the dose of the thermostable α-amylase compared to that used by Johnson et al. (2009) in direct conversion of cassava roots into sugar syrups. Data demonstrated that 0.013% ($v_{enzyme}/w_{fresh\ mash}$) was broadly enough to obtain a DE slightly greater than 20 even after 1 h liquefaction. Moreover, under atmospheric pressure and 90°C, results on the 10 min gelatinization of 1:1.6 cassava: water mash without and with 0.025% ($v_{enzyme}/w_{fresh\ mash}$) thermostable α-amylase were provided from Figure 1A and Figure 1B, respectively. Data showed the same DE after 1, 2, or 3 h liquefaction time.
As a matter of fact, starch gelatinization is fundamental to increase the surface attack for the liquefying enzymes (Baskar et al., 2008). In the conventional liquefaction process, corn starch slurry is heated to 105 °C in a jet cooker for 5 min with α-amylase and then the mixture is cooled to 90-95 °C in a holding tank (Johnson and Padmaja, 2013). Even if Rickard et al. (1991) detected that cassava starch has the lowest gelatinization temperatures (66 ÷ 73 °C) among tuber starches, cassava starch susceptibility to enzyme attack is influenced by several factors, such as amylose and amylopectin content, crystalline structure, particle size and the presence of enzyme inhibitors. Among these factors, granular structure is believed to be the most important: cassava starch granules are dispersed or gelatinized in aqueous solution during liquefaction and mildly exo-corroded under thermostable α-amylase treatment (Adejumo et al., 2011).
From these inputs, the T3 was arranged by increasing the heat and the pressure during the starch gelatinization. This was attempted by applying a 10 min-143.27 kPa step before returning the mashes at patm and 90 °C for 10, 15, 20, 30, 40, 45, 60, 100, and 120 min to complete the liquefaction. The trend in Figure 2A evidenced the need to operate for times shorter than 40 min if MD with DE < 20 have to be delivered. This time is much shorter than that previously reported by Johnson et al. (2009). Moreover, under the conditions summarized in Figure 2B and by simply changing the liquefaction time it's open the possibility of obtaining MD with various DE with a wide spectrum of applications (Chronakis, 1998).
Finally, Person's test produced significant inverse correlations between cassava dilution amount and liquefaction time ($p \leq 0.05$), and between cassava dilution amount and enzyme dose ($p \leq 0.01$), highlighting the fact that together with starch gelatinization modality these are the key factors affecting the reducing sugar yield after liquefaction (Tatsumi and Katano, 2005).

3.2 Enzymatic hydrolysis to GS

Under the condition given in T4 and in Figure 2B, the saccharification phase of cassava mash to GS continued after liquefaction. Berghofer and Sarhaddar (1988) reported the need to use both liquefying and saccharifying enzymes to obtained sugar syrups with high maltose concentration and reduced amounts of higher sugars from cassava.
The temperature of the sample was fast brought down before adding glucoamylase and pullulanase enzymes, because of they are not so heat-stable as α-amylase is. Their optimum temperature is between 60-65°C, but not lower than 60°C (Hii et al., 2012). As a consequence, temperature was strictly monitored with Temp 3JKT Thermocouple Thermometer (Oakton Instruments, Vernon Hills, USA) having 0.1°C resolution. At 60°C the mash was acidified with HCl 2N up to optimal pH of 5.4 for glucoamylase and pullulanase activity (Hii et al., 2012) which were both added at 0.019% ($v_{enzyme}/w_{fresh\ mash}$). This dose corresponded to the 0.05% addition of enzyme volume on cassava weight of Johnson et al. (2009).
Table 1 reported the results of 1, 2, 4, 6, 18, 24, and 48 h saccharification times and highlighted the significantly greatest DE after 4 h. This was partially in agreement with other researchers (Johnson et al., 2009; Johnson and Padmaja, 2013) who hydrolyzed cassava mash under similar conditions, but without using pullulanase enzyme. Pullulanase has been widely utilised to hydrolyse the α-1,6 glucosidic linkages in starch, amylopectin, pullulan, and related oligosaccharides. As a debranching enzyme, it enables a complete and efficient conversion of the branched polysaccharides into small fermentable sugars. The use of glucoamylase together with pullulanase leads to the complete saccharification of the dextrins to glucose by hydrolyzing both α-1,4 and α-1,6 linkages at the non-reducing end (Hii et al., 2012). As demonstrated by our results, this use is particularly interesting on cassava starch which is composed of branched amylopectin (80±5%) and unbranched amylose (20±5%) (Rickard et al., 2001).

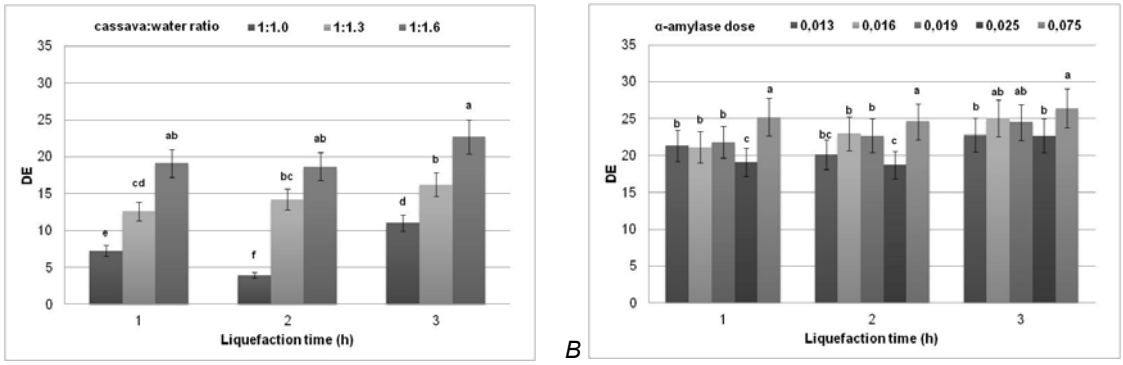

Figure 1: DE measured at the end of T1 and T2 treatments at varying the cassava: water ratio in the cassava mash (A), and the dose of the thermostable α-amylase added to the mash before starch gelatinization (B) Values are means ± SD (n=6). At each bar top, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

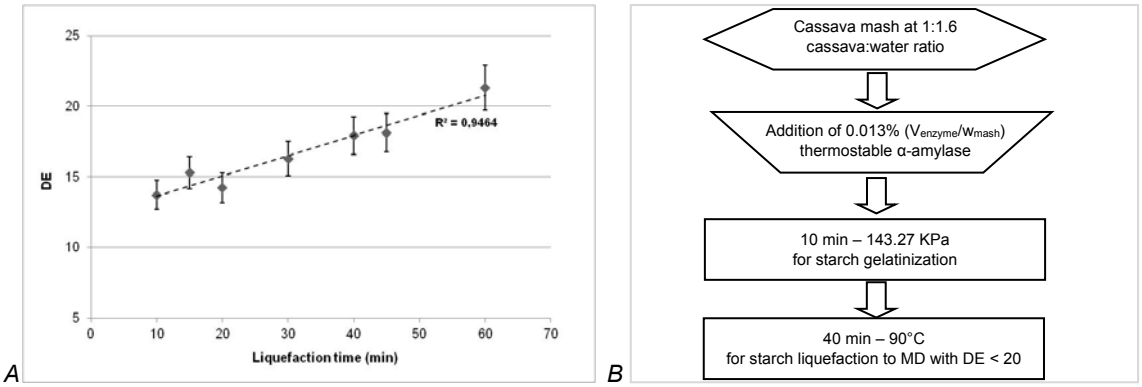

Figure 2: (A) DE mean values ± SD (n=6) measured at the end of liquefaction under T3 in function of liquefaction times from 10 to 60 min. (B) Process steps identified after T1, T2, and T3 for MD with DE < 20.

From lab- to small scale trials (T5) the process was arranged with the optimal variables stated at values detected in T1 and T2 for cassava mash and thermostable α-amylase dose, respectively. The use of an agitated reactor working for 15 min at 145÷152 kPa to attain the starch gelatinization, implied a further evaluation of the liquefaction time and, consequently, of the related temperature. Liquefaction time might be shortened to 12 min and temperature should be raised to 95°C (at p_{atm}), in order to gain a DE value of 16.4 at the end. Then, temperature has to be lowered to 65 °C, pH adjusted to 5.4 for allowing the saccharification with glucoamylase and pullulanase for 4h.

Working times were highly shortened if compared to the results of Johnson et al. (2009) who reported about 1 and 48 h for liquefaction and saccharification, respectively. In the final GS having a DE value of 98.1, 50.2% of reducing sugars, and 46.2% of glucose per dry weight (w/dw) appeared. The HPLC analysis of clarified and concentrated GS according to the procedure under the section 2.7, evidenced the following composition (w/dw): 2.0% oligosaccharides (> 3 units of simple sugars), 1.2% trisaccharides, 2.9% disaccharides, 1.4% fructose, and 92.5% glucose.

Table 1: DE measured after the saccharification phase under T4 conditions. Within each row, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

Saccharification time	1h	2 h	4 h	6 h	18 h	24h	48h
DE	44.3 e	81.4 ab	92.3 a	65.9 c	73.0 bc	60.9 d	69.5 c

4. Conclusion

Although being commonly used in the industrial manufacturing of glucose, the enzymatic lab-scale process developed from this study brought the advantage of the scalability of operations to small scale process, the shortening of working times, and the reduction of the enzyme doses required for both liquefaction and saccharification. Moreover, it allowed at obtaining the direct conversion of cassava mash into MD with various DE, and a GS with a high glucose purity. These advantages may enable the process to be popularized especially in developing Countries, but further investigations are needed to reduce energy intensive steps as pH adjustment, pressure increasing, and heating requests.

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