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Complementary effects of cell wall degrading enzymes together with lactic acid fermentation on cassava tuber cell wall breakdown

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ABSTRACT

Entrapment of starch granules within cassava parenchyma cells constitutes a major challenge in starch extraction from cassava tuber. This was addressed by applying cellulolytic and hemicellulolytic enzymes coupled with spontaneous lactic acid bacteria fermentation in the pre-treatment of wet milled cassava. The hydrolytic activities of the enzymes and pH lowering by the fermentation resulted in fragmentation of the cassava cell walls, with an improved release of free starch granules. The residual cell wall material after the treatments was characterized by microscopy and gas chromatography. Lignified material was resistant to hydrolysis. Material that was not hydrolysed by the enzymes consisted of arabinose, galactose, rhamnose, xylose and glucose, with the latter two the most abundant. It appears that the gums and hemicelluloses were hydrolysed first, enabling subsequent hydrolysis of the cellulosic materials. The complementary effects of these treatments could improve wet milling extraction of cassava starch.

1. Introduction

Cassava (*Manihot esculenta* Crantz L.) is grown mainly for its large starch-rich storage roots (Blagbrough et al., 2010). Cassava serves as an important food crop for millions of people in tropical and sub-tropical Africa, Asia and Latin America. After rice and maize, cassava is the third most important source of calories in the world (FAO, 2015). The agronomic characteristics of the cassava crop such as its tolerance to low-fertility soil and drought stress contribute to its global economic importance (Poonsrisawat et al., 2014). Total cassava world production in 2013 was approx. 277 million tonnes, of which Africa represented about 57% (FAO, 2015).

Proximate analysis of five different cassava genotypes indicated that starch and crude fibre composition ranges between 80 and 86% and 1.5–3.5% dry weight basis, respectively (Charles et al., 2005). Ccassava root tuber is processed commercially mostly for its starch (Teixeira et al., 2009). The process of extracting cassava starch is mostly by wet milling, due to the root's high moisture content (between 60 and 70%) (reviewed by Breuninger et al., 2009). In the cassava tuber, the starch granules are located within the parenchyma cells (Sriroth et al., 2000b). The process of extracting the starch involves pre-cutting the tuber into small pieces before rasping to open up the cells to release the starch granules. However, the efficiency of cassava starch extraction and yield are low due to a substantial quantity of starch not being recovered from the pulp (cell wall materials) (Dzogbefia et al., 2008). This is attributable to the starch granules being trapped within the parenchyma cells. In fact, the residual starch content of the pulp can range between 50 and 60%, dry weight basis (Sriroth et al., 2000a).

Improvement in starch extraction and starch yield through the use of hydrolytic enzymes have been investigated to treat the cassava mash and the waste materials (Sriroth et al., 2000a; Dzogbefia et al., 2008; Teixeira et al., 2009). Enzymatic hydrolysis opens up the compact cell wall structure leading to the release of the trapped starch granules (Kordylas, 1990). However, the right combination of suitable enzymes has been noted to be fundamental in the pre-treatment of the cassava mash in order to achieve an increase in starch extraction (Dzogbefia et al., 2008). Traditionally, cassava root tubers are commonly processed by spontaneous lactic acid bacteria (LAB) fermentation in producing food products such as gari (fried cassava flakes) (Kostinek et al., 2005). As reported by Kostinek et al. (2007), a wide range of endogenous LAB have been characterized in cassava root tuber.

Hence, application of suitable exogenous enzyme treatments with multiple cell wall degrading activities to the cassava cake (wet milled cassava tuber) coupled with LAB fermentation could more effectively modify the cassava cell wall structure. The objective of

Abbreviations: CWM, cell wall material; LAB, blactic acid bacteria; TA, titratable acidity.

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this study was to examine treatment of cassava tuber after wet milling using cellulolytic and hemicellulolytic enzymes in combination with LAB fermentation.

2. Materials and methods

2.1. Materials

Fresh cassava root tubers grown in Mozambique were obtained from a retail outlet in Pretoria. The commercial enzyme preparations used were Viscozyme L (a cocktail of cellulase and multi-hemicellulase enzymes) and Ultraflo Max (a cocktail of multi-hemicellulase enzymes), and Termamyl SC (a thermal-stable α-amylase enzyme) kindly provided by Novozymes (Benmore, South Africa). The Ultraflo preparation has β-glucanase, xylanase and other pentosanase activities, while the Viscozyme enzyme preparation has β-glucanase, cellulase, xylanase, pentosanase (presumably more general pentosanase) and arabanase activities (Novozymes, 2008).

2.2. Methods

Pre-weighed fresh cassava root tubers were washed, peeled, chopped into chips and re-weighed. The cassava chips were milled using a Waring blender for 2 min at low speed and 1 min at high speed, with a small quantity of water added to aid the wet milling process. After milling, the cassava cake (wet milled cassava tuber) was weighed again.

2.2.1. Effects of the enzyme preparations on cassava cell wall degradation

Cassava cake (450 g) was weighed into beakers. Nine ml Viscozyme and Ultraflo enzyme solution, individually and in 50:50 combination, were diluted with distilled water and added to give 250 ppm of the enzyme preparations (relative to cassava cake solids) and mixed thoroughly. The cassava cake samples were covered with Parafilm to obtain an anaerobic condition to aid spontaneous LAB fermentation. A control treatment was obtained by mixing the cassava cake with 9 mL distilled water. The cassava cake samples were incubated at 24 °C for one week and two weeks. The treatments were performed twice. Enzyme activity was stopped by freezing the cassava cake samples at −20 °C.

Spontaneous fermentation of the cassava cake by LAB was monitored over the period of incubation by determining the pH and titratable acidity (TA). TA was expressed as % lactic acid equivalents.

2.2.2. Starch removal

Starch was removed to enable characterization of the remaining cassava cell wall materials after incubation. It was carried out using a BRF mashing bath (Brewing Research Foundation, Nutfield, UK). The pH of the cassava cake samples was adjusted to pH 5.0 with 0.1 M NaOH solution, the optimum pH for the α-amylase used. Six ml diluted Termamyl SC was added to give 100 ppm of the enzyme preparation in the slurry. It was then cooked for 1 h at 96 °C. After cooking for 1 h, 1 mL full strength Termamyl SC was added to the mash and the cooking continued until the starch was negative by iodine. After starch removal, the samples were centrifuged at 470*g* for 2 min and the clear supernatant carefully removed. The insoluble solids residue was frozen for later analysis.

2.2.3. Purification of the treated cassava cake and mashed solid materials

After incubation for one week and two weeks, 25 g cassava cake samples before and after were weighed and diluted to 50 g with distilled water in 100 mL glass centrifuge tubes. They were centrifuged at 470*g* for 2 min and the clear supernatant carefully removed. The samples were then re-suspended in distilled water and re-centrifuged in order to completely wash out the soluble solids and this was repeated four times in total. The purified residual solid materials of the treated cassava cake samples both before and after starch removal were analysed for total solids, starch, soluble and insoluble fibre contents, particle size (sieve analysis) and by light microscopy.

2.3. Analyses

2.3.1. Total solids

Total solids content of the washed enzyme treated and untreated cassava cake samples was determined based on dry matter remaining, by drying at 103 °C for 3 h.

2.3.2. Starch content

Starch content of the cellulolytic and hemicellulolytic enzyme treated and untreated cassava cake samples before starch removal was determined using the Megazyme Total Starch Assay Procedure (Amyloglucosidase/α-Amylase Method) (Megazyme International, 2011).

2.3.3. Insoluble fibre content

After starch removal and washing of the remaining solid material to remove the soluble solids, total insoluble fibre content was estimated based on the dry matter remaining, by drying at 103 °C for 3 h.

2.3.4. Particle size

Particle size of the cassava cake samples before and after starch removal was determined by weighing 10 g samples of the cassava cake samples and then sieving through 500 and 250 μm opening sieves with a small amount of distilled water. The solid materials retained by the 500 and 250 μm sieves and the materials that passed through the 250 μm sieve were dried and then weighed.

2.3.5. Light microscopy

Microscopic examination of the cassava cake samples was carried out before and after starch removal, as well as on the particle size fractions. The cake samples before starch removal were stained with iodine solution to identify the starch granules.

2.3.6. Gas chromatography characterization of hydrolyzable residual cell wall materials

Purified remaining insoluble cell wall materials were freeze dried. They were characterized for compositional and structural properties, as described by Ciucanu and Caprita (2007) and Laine et al. (2002), respectively, but with modifications as outlined in Du Clou and Walford (2010). Hydrolysis of the cell wall materials into their monosaccharide components was carried out with acidified methanol. Samples for structural analysis were first per-methylated using Hakamori's reagent in dimethyl sulphoxide (Hakamori 1965). This was followed by hydrolysis with acidified methanol. Following derivatization, the cell wall monosaccharide structural linkages were determined using a GC fitted with a VF-5 ms column (Agilent Technologies, Santa Clara, CA) and flame ionization detector. For composition analysis, 1 μL of sample was injected at a 1:100 split with the injector temperature set at 280 °C. The carrier gas was nitrogen, set at a flow of 1.3 mL/min. The initial oven temperature of 140 °C was held for 1 min before rising to 158 °C (at 2 °C/min), then 186 °C (at 1 °C/min), then 280 °C (at 10 °C/min) and holding for 2 min. For structural linkage analysis, the injector temperature was set at 260 °C and the gas flow rate at 1 mL/min. The oven programme was altered so that the initial