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Mechanism of cassava tuber cell wall weakening by dilute sodium hydroxide steeping

Martin Odoch, Elna M. Buys, John R.N. Taylor *

Institute for Food, Nutrition and Well-being and Department of Food Science, University of Pretoria, Private Bag X 20, Hatfield 0028, South Africa

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ABSTRACT

Steeping of cassava root pieces in 0.75% NaOH in combination with wet milling was investigated to determine whether and how dilute NaOH modifies cassava cell walls. Gas chromatography data of cell wall constituent sugar composition and Fourier transform infrared (FTIR) data showed that NaOH steeping reduced the level of pectin in cassava cell walls. FTIR and wide-angle X-ray scattering spectroscopy also indicated that NaOH steeping combined with fine milling slightly reduced cellulose crystallinity. Scanning electron microscopy showed that NaOH steeping produced micropores in the cell walls and light microscopy revealed that NaOH steeping increased disaggregation of parenchyma cells. Steeping of ground cassava in NaOH resulted in a 12% decrease in large residue particles and approx. 4% greater starch yield with wet milling. Therefore dilute NaOH steeping can improve the effectiveness of wet milling in disintegrating cell walls through solubilisation of pectin, thereby reduced cell wall strength.

1. Introduction

The cassava (*Manihot esculenta* Crantz L.) plant is mainly cultivated for its tuberous, starch-containing roots (Alves, 2002). It is widely grown in tropical regions of Africa, Asia, and Latin America, and is the third most important source of calories worldwide after rice and maize (FAOSTAT, 2015). Harvested cassava roots have the following approximate composition: moisture 60 g/100 g, total carbohydrate 38 g/100 g, dietary fibre 1.8 g/100 g, protein 1.4 g/100 g, potassium 271 mg/100 g, magnesium 21 mg/100 g, calcium 16 mg/100 g, sodium 14 mg/100 g, and ascorbic acid 21 mg/100 g (USDA, 2016). The high starch content of cassava roots, approx. 26–52 g/100 g (as is basis), and the plant's low fertiliser requirement and drought tolerance also makes it attractive for starch production for industrial use (Jansson, Westerbergh, Zhang, Hu, & Sun, 2009).

The cassava root is generally processed fresh by mechanical attrition (wet milling). However, a major impediment in the root for food and for starch extraction is disintegration of the recalcitrant cell walls (Sriroth, Piyachomkwan, Wanlapatit, & Oates, 2000). Disintegration of cassava cell walls is also important in detoxification of processed starch (Sornyotha, Kyu, & Ratanakhanokchai, 2010), thermal softening of cooked roots (Favaro, Beléia, Junior, & Waldron, 2008) and reduction in viscosity of milled cassava (Huang et al., 2016).

Disintegration of cassava root cell walls is a multifactorial problem as plant cell wall strength depends on the properties of its components, their composition, intra-structural interactions and the molecular mechanism of cell adhesion (Favaro et al., 2008). Specifically, in cassava cell wall, pectin content has been found to be inversely related to root friability (Franck et al., 2011). It is recognised that, generally, pectin interactions within the middle lamella, are the principal factor responsible for cell adhesion (Parker, Parker, Smith, & Waldron, 2001). Research indicates that cassava root cell wall adhesive strength is influenced by the proportions of alkali-soluble and chelator-insoluble pectins plus the presence of divalent cations, which are thought to strengthen pectin cross-linking (Favaro et al., 2008). Cassava cell walls also contain soluble hemicellulosic mucopolysaccharides, referred to as cassava tuber mucilage (Charles, Huang, & Chang, 2008), which probably act as an adhesive component within the intercellular spaces.

Weakening of cassava cell walls by various treatments such as ammonia (Moorthy, 1991), enzymatic (Adetunji, du Clou, Walford, & Taylor, 2016; Eyini & Rajapandy, 2007) and microbial

Abbreviations: ATR-FTIR, attenuated total reflectance Fourier transform infrared; CWM, cell wall material; GC, gas chromatography; LOI, lateral order index; NSP, non-starch polysaccharides; SEM, scanning electron microscopy; TCI, total crystallinity index; WAXS, wide-angle X-ray scattering.

* Corresponding author.
E-mail address: john.taylor@up.ac.za (J.R.N. Taylor).

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(Nanda & George, 1996) have been investigated to improve cellular disintegration by wet milling.

Recently, the mechanisms of cassava cell wall weakening by enzymatic (Adetunji et al., 2016; Huang et al., 2016) and retting (spontaneous fermentation) involving endogenous and exogenous enzymes (Ngea et al., 2016) have been described. Adetunji et al. (2016) and Ngea et al. (2016) found that cassava root tissue weakening was related to opening up of the cell wall structure, partly through pectin degradation and associated the degradation with specifically modifications to galactan, homogalacturonan and arabinan pectic polymers. Weakening of cassava cell walls during retting is thought to primarily involve activities of pectin methylesterase and polygalacturonate lyase (Brauman, Keleke, Malonga, Miambi, & Ampe, 1996).

It has been found that pectins and xyloglucans in cassava root cell walls can be solubilised by sequential extraction using chelating agents imidazole and cyclohexane-*trans*-1, 2-diamine-N, N, N′, N′-tetraacetate and then under alkaline conditions with Na_2CO_3 followed by KOH (Favaro et al., 2008). Furthermore, sodium hydroxide (NaOH) treatment has been shown to weaken grass/cereal cell walls by saponification of the intermolecular ester bonds cross-linking xylan hemicellulose and lignin, depolymerisation of lignin by cleavage of inner-molecular α- and β-aryl ether linkages, and removal of hemicellulose acetyl groups and uronic acid substitutions (reviewed by Zhao, Zhang, & Liu, 2012). NaOH treatment also causes cellulose from plant primary cell walls to swell, with a concomitant decrease in chain crystallinity (Dinand, Vignon, Chanzy, & Heux, 2002).

However, the effect of alkaline steeping on cassava cell wall structure and disintegration has not been investigated. Hence, on the basis of the above research, it was hypothesised that steeping of cassava roots under alkaline conditions with dilute NaOH could be an effective way of chemically modifying and weakening the parenchyma cell walls to aid their disintegration during wet milling. The aim of this was to test the hypothesis that dilute alkali steeping would chemically modify cassava parenchyma cell structure and as a consequence result in greater disintegration of the cells during wet milling. The specific objective of the study was therefore to characterize dilute NaOH induced weakening of cassava root cell walls and to identify any existent effects in relationship to starch extraction.

2. Materials and methods

2.1. Materials

Cassava roots (sweet variety '*South Africa*') were harvested from eleven month old plants grown in a single field at Tonga, Mpumalanga Province, South Africa.

2.2. Transport and storage

Directly after harvesting, the roots were placed into polystyrene insulated boxes with closed lids and transported in a covered vehicle with an internal temperature of 20–23 °C to the University of Pretoria. Directly on arrival, the roots were washed, peeled to remove the periderm and phelloderm layers, and then chopped into chips (approx. 9 cm^3). The chips were placed in high density polypropylene bins with closed fitting lids and stored at −20 °C for up to 4 months until utilized. The entire process from harvest to storage was completed within 18 h. The pH and titratable acidity of ground freshly harvested cassava roots and cassava chips after storage at −20 °C were determined. They both had a pH of 6.3 ± 0.1 and a titratable acidity of 0.1 ± 0.0%. This showed that there was no spontaneous fermentation during storage.

2.3. Cassava chip size reduction

Prior to utilization, the frozen chips were thawed at 4 °C for 24 h, then ground into smaller pieces using an electric rotary meat mincer fitted with an 8 mm opening plate.

2.4. NaOH steeping and wet milling

Cassava pieces (400 g) were mixed with 400 ml 0.75% (w/w) NaOH solution, final pH 11.5. Control samples were mixed with distilled water. All samples were steeped for 2 h at 25 °C. The cassava pieces were pulverised in a Waring blender for 1 min using low and high speed for 30 s each. The pulp was suspended in 5x its volume distilled water and wet milled at 12,000 rpm using a Retsch EZ200 wet mill (Haan, Germany). Three screen opening sizes were investigated: 500 μm, 1000 μm and 2000 μm.

2.4.1. Preparation of cassava starch and residue fractions

The wet milled pulp was separated into residue (remaining on the sieve) and filtrate (passing through the sieve) using a 106 μm opening sieve. The residue was re-suspended in 200 ml distilled water and the sieve separation process was repeated five times in total. The collected filtrate was allowed to stand for 12 h at 25 °C to facilitate starch sedimentation and the clear supernatant was discarded. The wet cassava starch (the sediment) and the sieve-retained cassava residue, were dried in a forced draught oven at 50 °C for 72 h. The dried starch and residue fractions were milled using a Janke and Kunkel micro hammer mill (Staufen, Germany) fitted with a 500 μm opening screen. The milled samples were stored in sealed polyethylene bags at 4 °C until assayed.

2.4.2. Starch removal from the cassava residue fraction

The starch in the residue fraction was removed by enzymic hydrolysis to enable characterization of the insoluble cell wall material. Residue fraction, 6 g, was suspended in 100 ml distilled water and the slurry pH adjusted to pH 5.0 using 1 M HCl. The slurry was centrifuged at 3100*g* for 15 min, 4 °C and the clear supernatant carefully decanted. Pure thermostable α-amylase from *Bacillus licheniformis* (Megazyme, Bray, Ireland) was used to hydrolyse the starch under conditions in accordance with the supplier's Total Starch Assay. The enzyme was diluted (1:30) using sodium acetate buffer (0.1 M, pH 5.0) containing calcium chloride (0.05 M). The diluted enzyme, 185 ml, was added and the slurry incubated at 100 °C for 20 min in a shaking water bath at 80 rpm. The starch enzymic hydrolysis step was repeated, until starch was absent in the residue fraction as assessed by the iodine test. The residue was re-suspended in 30 ml distilled water, and washed twice. The residue material after starch enzymic hydrolysis, referred to as cell wall material (CWM), was freeze dried, milled using an air-cooled, knife-type laboratory mill (IKA, Staufen, Germany) and stored at 4 °C in zip-lock type polyethylene bags until required. Fig. 1 summarises the stages followed during cassava starch extraction and isolation of the cassava cell wall material.

2.5. Analyses

2.5.1. Residue fraction particle size

Residue fraction particle size, prior to starch enzymic removal, was determined by sieving 10 g wet residue through a 250 μm opening sieve followed by 25 μm opening sieve using 1 L distilled water. Retained particles on both sieves were dried separately by AACC Method 44-15A (AACC International, 2000).