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Polysaccharide composition of raw and cooked chayote (*Sechium edule* Sw.) fruits and tuberous roots

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ABSTRACT

Chayote is a multipurpose table vegetable widely consumed in Latin America countries. Chayote fruits, leaves and tuberous roots contain complex carbohydrates as dietary fiber and starch, vitamins and minerals. The complex polysaccharides (cell walls and starch) were analyzed in the black and green varieties of chayote fruits as well as in green chayote tuberous root before and after a controlled cooking process to assess changes in their composition and structure. The monosaccharide composition and linkage analysis indicated pectins homogalacturonans and rhamnogalacturonan I backbones constitute about 15–20% of the wall mass, but are heavily substituted with, up to 60% neutral arabinans, galactans, arabinogalactans. The remainder is composed of xyloglucan, glucomannans and galactoglucomannans. Chayote cell-wall polysaccharides are highly stable under normal cooking conditions, as confirmed by the optical microscopy of wall structure. We found also that tuberous roots constitute a valuable additional source of quality starch and fiber.

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1. Introduction

Chayote is the common name of the fruit of *Sechium edule (Jacq.)* Swartz, a climbing plant belonging to the gourd family (Cucurbitaceae, subtribe Sicyinae). The name is the Spanish derivative of the Nahuatl word *chayojtli*. In Guatemala it is known as *güisquil*, in Louisiana as mirliton or merleton, and in Brazil as *chuchu* (Saade, 1996). The plant, characterized by its single pendulous ovule and single-seeded fruits (Morton, 1981; Saade, 1996), is considered a staple food in all Latin America countries. Almost all parts of the plant are edible and, since pre-Columbian times, their tuberous roots, shoots and fruits are widely consumed in what is now Mexico and Guatemala, while their leaves are used as fodder. They can be consumed raw in salad, pickled, boiled or as a jelly. In a raw state, the fruit pulp has a firm and crispy texture, but after cooking, the pulp has a delicate soft texture.

Poor eating habits associated with fiber-deficient diets contribute to increased occurrence of obesity and development of chronic degenerative diseases, such as heart disease, cancer, diabetes, and obesity. Populations that consume more fruits and vegetables, and consequently dietary fiber, have less incidence of these syndromes (Boeing et al., 2012). Fruits and vegetables are rich in soluble and insoluble dietary fiber, anti-oxidants, vitamins and minerals that contribute to the body health (Boeing et al., 2012). Chayote fruit and root are just such sources of beneficial dietary fiber, starch vitamins and minerals (Aung, Ball, & Kushadd, 1990; Mélo, Lima, Sucupira, Caetano, & Maciel, 2006). The fruit is also low in calories (19 kcal per 100 g) and in soluble sugars (1.6 g per 100 g), but is a rich source of minerals, such as potassium, calcium, phosphorus and magnesium, and vitamin C (11–20 mg per 100 g) (Mélo et al., 2006; USDA, 2014). In contrast to the fruit, chayote root is a rich source of starch. We show here that a single 2-years-old chayote plant can produce up to 5-kg of tubers and contain up to 65% starch (w/w; in dry basis). The root, known as *ichintal*, is widely consumed in Latin America. Plants produce the tuberized root after the first year of growth (Saade, 1996). They have a white color and tastes like potatoes after cooking, and are highly appreciated in almost all Latin America countries, but practically unknown in Brazil. One-hundred g of fresh root contains on average 19 mg of ascorbic acid, 34 mg of phosphorus and 0.4% (w/w)

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of dietary fiber (Saade, 1996). Compared to chayote fruits, there is scarce information about their nutritional composition and uses.

In Brazil, chayote fields are routinely cleared for replanting, resulting in a large production of root that is destroyed despite its potentially high commercial value. Also, fruits that are not in accordance with market classification standard are wasted. Better knowledge of fruits and root polysaccharide composition may help to promote the use of chayote root and fruit as an important source of starch and dietary fiber. Moreover, linear arabinans, such as those found in black and green chayote fruits, have high water holding capability when compared to branched arabinan; arabinan substituted with α-(1,2) and/or α-(1,3) linked L-arabinosyl residues (Ramaswamy, Kabel, Schols, & Gruppen, 2013), making chayote pectin suitable as thickener.

The chemical structure is responsible for the beneficial activities presented by the polysaccharides. Important properties as water holding capability of the dietary fiber, and the ease with they are metabolized by the colonic bacteria as well as the immunomodulatory activity are closely related to the chemical structure of the polysaccharides (Blackwood, Salter, Dettmar, & Chaplin, 2000; Paulsen & Barsett, 2005). This study aims to unravel the chemical structure of water-soluble and water-insoluble polysaccharides of two chayote cultivars and the changes produced in their structure by the cooking process.

We show chayote to be an excellent source of edible roots that vary in weight from 0.5 to 5 kg, with amounts of starch comparable to potatoes (67–75% of starch, in dry basis) (Brazinskiene et al., 2014). In contrast to root, chayote fruit provides arabinan- and galactan-rich pectic material that can be used as thickener by industry, as it retains favorable biophysical properties after cooking.

2. Materials and methods

2.1. Materials

Black and green chayote (*S. edule* Sw.) fruits and green chayote tubers were purchased from Batista Farm in Amparo County, São Paulo State, Brazil. Chayote fruit production begins approximately 80 to 110 days after seeding, and after 15 days after anthesis the immature fruits are ready to be harvested and classified. We selected fruits classified in the trade as "Extra", i.e. composed of fruits that weigh approximately 250–350 g and have no bruises or defects in the shape or skin. Three samplings were performed in the summer during the rainy season (February, March and April). Immature fruits of the black and green chayote cultivars were harvested approximately 15 days after anthesis. The tuberous roots were purchased from the same farm from 2-year-old green chayote plants. Chayote tuberous roots are produced in the second year after planting when plant vigor is reduced and production of fruits declines. The root samples were 500 g to 5 kg.

2.2. Sample preparation

The chayote fruits and tubers were peeled and halved along their axes. One half of the fruit and root sample was frozen in liquid N_2 and freeze dried. The other half of the fruit and root was cooked in boiling water (1:2; w/v) for 15 min and drained before freezing in liquid N_2 and freeze dried. The freeze-dried, raw and cooked materials were ground with a mortar and pestle, and then boiled in methanol: chloroform (1:1; v/v) for 30 min at 70 °C to inactivate enzymes and extract lipids. The suspensions were filtered using a sintered-glass funnel, and the residues were washed extensively with acetone to remove the remaining pigments. The residues were dried at ambient temperature using a SpeedVac® Plus System (Savant, New York, USA). The materials were named cellular material (CM) and used for non-starch polysaccharide extraction.

2.3. Non-starch polysaccharide extraction

About 100 mg of CM of chayote fruit pulp or tuber was soaked in 1.5 mL of sodium phosphate buffer (0.05 M; pH 6.0) and sequentially hydrolyzed with amylases and proteases. Heat-stable α-amylase from *B. licheniformis* (10 μL; Megazyme International Ireland; EC 3.2.1.1; 3000 U mL^{-1}) was added to the mixture, and the mixture was incubated at 90 °C for 1 h. The pH was adjusted to 4.5 with 0.4 M HCl, and 20 μL of amyloglucosidase (from *A. niger*; Megazyme International Ireland Limited; Ireland; EC 3.2.1.3; 3300 U mL^{-1} on soluble starch) was added. The mixture was then incubated at 60 °C for 1 h. At the end, the pH was adjusted to 7.5 with 0.4 M NaOH, and 10 μL of *B. licheniformis* proteases (Megazyme International Ireland Limited; Ireland; EC 3.4.21.14; 350 tyrosine U mL^{-1}) was added. The mixture was then incubated at 60 °C for an additional 30 min. The hydrolyzate was centrifuged at 9000 × *g* for 15 min, and the supernatant was pooled into a new flask and brought to 80% ethanol in water (v/v), heated to 70 °C for 15 min and ice-cooled to precipitate the water-soluble polysaccharides. The water-insoluble residue was washed five times with deionized water, frozen in liquid N_2, freeze-dried and weighed. This fraction was named the water-insoluble polysaccharide fraction. The ethanolic mixture was pelleted by centrifugation at 9000 × *g*. The ethanolic supernatant was dried, resuspended in water and analyzed by HPAEC-PAD to determine the glucose released from starch. The residue was washed extensively with ice-cold 80% ethanol in water (v/v), resuspended in water, frozen in liquid N_2, freeze-dried and weighed. This fraction was named the water-soluble polysaccharide fraction.

2.4. Soluble sugar determination

Soluble sugars of chayote pulps and roots were extracted three times with 80% ethanol in water (v/v) at 80 °C and analyzed by high pressure liquid chromatography with pulse amperometric detection (HPAEC-PAD; Dionex, Sunnyvale, CA, USA) using a PA1 column (Dionex, Sunnyvale, CA, USA) and an isocratic run of 18 mM NaOH during 25 min (Cordenunsi, Shiga, & Lajolo, 2008).

2.5. Cell-wall monosaccharide and linkage analysis

Duplicate samples of water-soluble and -insoluble cell wall materials from Chayote were carboxyl-reduced with $NaBD_4$ after activation with a water-soluble carbodiimide, as described by Kim and Carpita (1992) and modified by Carpita and McCann (1996). A colorimetric assay for uronic acids in the presence of neutral sugars (Filisetti-Cozzi & Carpita, 1991) was used to confirm reduction of the carboxyl groups was 95% or greater. For each set of materials, two samples of each were directed to monosaccharide and linkage analysis.

The sugar alditol acetates were prepared according to Gibeaut and Carpita (1991). Derivatives were separated by gas-liquid chromatography (GLC) on a 0.25-mm × 30-m column of SP-2330 (Supelco, Bellefonte, PA, U.S.A.). Temperature was held at 80 °C during injection, then ramped to 170 °C at 25 °C min^{-1}, and then to 240 °C at 5 °C min^{-1}, with a 10-min hold at the upper temperature. Helium flow was 1 mL min^{-1} with split-less injection. The electron impact mass spectrometry (EIMS) was performed with a Hewlett-Packard MSD at 70 eV and a source temperature of 250 °C. The proportion of 6,6-dideuteriogalactosyl was calculated using pairs of diagnostic fragments *m*/*z* 187/189, 217/219 and 289/291