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Polysaccharides from raw and cooked chayote modulate macrophage function

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ABSTRACT

Chayote (*Sechium edule*) pulp is consumed raw or cooked and is source of polysaccharides with potential immunomodulatory properties in macrophages, which are cells that play important roles in homeostasis and innate immune responses to pathogens; however, these immunomodulatory effects of chayote polysaccharides remain unknown. Therefore, polysaccharides from raw (SeR) and cooked (SeC) chayote and from the hot water extract obtained after heating (SeH) were analyzed for their composition and effects on macrophages. Chayote has a high- (340 kDa) and a low- (46 kDa) molecular weight (MW) polysaccharide fraction containing high amounts of galactose, arabinose and galacturonic acid. After cooking, SeC was enriched in high-MW galactose-rich fractions, whereas SeH was enriched in low-MW arabinans and homogalaturonans-rich fractions. The polysaccharides induced tumor necrosis factor alpha (TNF-α) and nitric oxide (NO) secretion in macrophages; however, only SeR and SeH reduced TNF-α, NO and interleukin-6 secretion in activated macrophages. Furthermore, SeR and SeCW, but not SeC, affected phagocytic activity. In conclusion, bioactive polysaccharides in chayote modulate macrophage functions, and minor changes in composition resulting from the solubilization of a small proportion of low-MW arabinans and homogalacturonans during cooking accounted for the different effects of raw and cooked chayote.

1. Introduction

Sechium edule (*Jacq.*) Swartz is a plant of the Cucurbitaceae family, which has economic and nutritional relevance, mainly in Latin America. Almost all plant parts are edible, but the tuberous roots and fruits are most consumed (Aung, Ball, & Kushad, 1990). The root is a source of starch and is widely consumed cooked, whereas the fruit, known as chayote, is consumed raw or cooked (Shiga, Peroni-Okita, Carpita, Lajolo, & Cordenunsi, 2015).

Chayote is low in calories (19 kcal/100 g) and is a source of minerals (USDA, 2014). In addition, phenolic compounds from chayote show antioxidant and α-glucosidase inhibitory effects (Firdous, Neraja, Debnath, Singha, & Sravanthi, 2012; Sulaiman, Ooi, & Supriatno, 2013). Recently, it was shown that chayote is also a source of galactans and arabinan-rich polysaccharides (Shiga et al., 2015). In this regard, polysaccharides with a similar monosaccharide composition extracted from other botanical sources potentiate the innate immune response against tumors (Yang, Hsieh, Lu, & Lin, 2014), reduced the adverse effects of chemotherapy in colorectal cancer animal models, and showed modulatory effects in macrophages (Yang, Lu, & Lin, 2013). Despite this evidence, the anti-inflammatory, anticancer and immunomodulatory potential of chayote polysaccharides remains unknown.

Macrophages are cells located throughout body tissues, where they ingest and process foreign materials, cell debris and recruit other macrophages in response to inflammatory signals (Murray & Wynn, 2011). Under normal conditions, macrophages play important roles, ranging from development, homeostasis and repair, to innate immune responses to pathogens, and are crucial in the initiation, maintenance, and resolution of inflammation. Nevertheless, conditions of chronic low-grade inflammation can negatively influence the reparative and homeostatic functions of macrophages, resulting in a causal association of this cell with disease states (Wynn, Chawla, & Pollard, 2013). Thus, bioactive polysaccharides from foods that play a beneficial role in macrophage homeostasis, mainly through interaction with toll-like receptors (TLR 2 and TRL4), c-type lectin receptors (dectin-1 and mannose receptor), scavenger receptors (SR) and complement receptors (CR) in cell membranes (Schepetkin & Quinn, 2006), can enhance innate immune functions and augment host defense responsiveness (Yao, Xue, Zhu, Gao, & Ren, 2015).

In the present study, polysaccharides from raw chayote were analyzed for their composition and immunomodulatory effects in macrophages. To

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this end, both untreated and lipopolysaccharide (LPS)- or zymosan-pretreated macrophages were used to evaluate the immunomodulatory potential of polysaccharides in normal and pro-inflammatory conditions, respectively. Finally, as the fruit is usually consumed after cooking and heating, this processing might promote the solubilization or degradation of polysaccharides (Liao et al., 2015). Thus, the composition and effects of polysaccharides extracted from the cooked fruit and from the hot aqueous extract obtained after heating were also studied.

2. Material and methods

2.1. Materials and reagents

Mature chayote (*Sechium edule*) fruits (Green variety) used in the study were purchased in a local market (São Paulo, Brazil) ready to be consumed. The fruits weighing approximately 450–500 g were firm, fresh in appearance, free of foreign smell and taste and with no apparent defects in shape or skin, being classified as "extra" according to FAO guidelines (FAO/WHO, 1999). The RAW 264.7 murine macrophages and Caco-2 human colorectal cancer cell lines were from American Type Culture Collection (ATCC, Manassas, VA). Heat-inactivated fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) containing penicillin (100 UI/mL) and streptomycin (100 μg/mL) were from Cultilab (Campinas, Brazil). Neutral sugars, uronic acids, malto-oligosaccharides, amino acids and dextran standards, poly-D-galacturonic acid, 2-cyanoacetamide, Triton X-100, bovine serum albumin (BSA), Trypan blue, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT), crystal violet, zymosan A from *Saccharomyces cerevisiae*, LPS from *Escherichia coli* serotype 055:B5, sodium nitrite ($NaNO_2$), Griess reagent and May–Grünwald dye were from Sigma-Aldrich (St. Louis, MO). Heat-stable α-amylase from *Bacillus licheniformis*, amyloglucosidase from *Aspergillus niger* and endopolygalacturonase from *Aspergillus aculeatus* were from Megazyme International (Wicklow, Ireland). Water was from a Milli-Q purification system from EMD Millipore (Bedford, MA). Other chemicals were of analytical or HPLC grade.

2.2. Extraction and composition of chayote polysaccharides

2.2.1. Sample preparation

Fruits from two independent samplings were prepared similar as described by Shiga et al. (2015). In addition, the hot water obtained after heating was retained. Briefly, fruits were peeled, halved along their axes, and one-half of the fruit pulp was frozen in N_2 and freeze-dried. The other half was cut into cubes (2 cm^3) and was cooked in boiling water (1:2 w/v) until softening occurred (NEPA/UNICAMP, Center for Studies and Surveys on Food., 2011). The firmness of fresh and cooked fruit was analyzed at different cooking times (0–25 min). The softening was confirmed using a TA-TX2*i*/5 texture analyzer (Stable Micro Systems, Goldaming, England) equipped with a 3 mm diameter puncture probe at 2 mm/s for 5 mm after the probe contact. The firmness was determined as the maximum force (N) recorded after the contact between the probe and the fruit. After cooking and complete softening, the material was filtered (22–25 μm; Miracloth; Calbiochem, La Jolla, CA) and washed with water. Both the hot water obtained after heating and the drained water from the cooked material were pooled to constitute the hot water extract. The cooked fruit was frozen in N_2 and freeze-dried, whereas the hot water extract was concentrated under a vacuum at ambient temperature, frozen in N_2 and stored at −80 °C. After freeze-drying, the raw and cooked fruits were milled using a IKA A10 grinder (Staufen, Germany), passed thought a 60-mesh (260 μm) sieve and extracted with methanol:chloroform (1:1 v/v) for 30 min at 70 °C to extract lipids and inactivate enzymes. Extracts were filtered in a sintered-glass funnel and washed with acetone. Finally, the remaining solids from the raw and the cooked fruit were dried under a vacuum at ambient temperature and the non-starch water-soluble polysaccharides were extracted.

2.2.2. Extraction of non-starch water-soluble polysaccharides

Non-starch water-soluble polysaccharides were obtained after starch hydrolysis as described by Shiga et al. (2015). The remaining solids from the raw and the cooked fruit and the hot water extract were hydrolyzed in 50 mM sodium phosphate buffer with α-amylase (pH 6.0; 3000 U/mL) for 1 h at 90 °C and amyloglucosidase (pH 4.5; 3300 U/mL on soluble starch) for 1 h at 60 °C. Then, mixtures were incubated with stirring at 60 °C for 30 min, centrifuged (9000 *g*; 15 min; 4 °C) and the supernatants were collected. Ethanol was added to the supernatants to a concentration of 80% (v/v) ethanol. Extracts were heated at 70 °C for 15 min and ice-cooled overnight to precipitate water-soluble polysaccharides. After centrifugation (9000 *g*; 15 min; 4 °C), supernatants were collected, concentrated under a vacuum at ambient temperature and were separated for oligosaccharide analysis. Precipitates, which corresponded to the non-starch water-soluble polysaccharides, were extensively washed with ice-cold 80% (v/v) ethanol, solubilized in water, frozen in N_2 and freeze-dried. After freeze-drying, optical micrographs (light microscopy) of the extracts dispersed in Lugol's iodine staining were analyzed to confirm the complete removal of starch. The water-soluble polysaccharides from raw and cooked chayote and from the hot water extract were named SeR, SeC and SeH, respectively.

2.2.3. Total sugars, proteins and amino acids profile

Total sugars were determined by the phenol-sulfuric method (Masuko et al., 2005), using glucose as a standard. Total proteins were determined by the Bradford method (Bradford, 1976) adapted by Nagel, Sirisakulwat, Carle, and Neidhart (2014), using BSA as a standard. Amino acids profile was determined similar as described by White, Hart, and Fry (1986). Briefly, BSA (control) or polysaccharides (2 mg) were hydrolyzed with 6 N hydrochloric acid at 110 °C for 20 h and analyzed by high performance anion-exchange chromatography coupled to a pulsed amperometric detector (HPAEC-PAD). After hydrolysis, samples were diluted in water (1:10 v/v), filtered (0.45 μL; EMD Millipore) and analyzed in an ICS-5000 system (Dionex, Sunnyvalle, CA), equipped with an AminoPac PA10 (250 × 2 mm) column (Dionex). Analysis (0.25 mL/min; 40 min; 30 °C) was performed in 50 mM sodium hydroxide for 12 min, increasing to 80 mM until 16 min and decreasing to 60 mM until 24 min with a 0–400 mM sodium acetate gradient between 16 and 24 min. A mixture of 21 L-amino acids plus glycine was used as standard.

2.2.4. Oligosaccharide profile

Supernatants obtained during the precipitation of polysaccharides were analyzed for the presence of oligosaccharides by HPAEC-PAD (Morales, Corzo, & Sanz, 2008). Supernatants were filtered (0.45 μL; EMD Millipore) and analyzed in a DX-500 system (Dionex), equipped with a CarboPac PA100 (250 × 4 mm) column (Dionex). Analysis was performed in 100 mM sodium hydroxide (0.7 mL/min; 85 min; 30 °C) with a 30–100 mM sodium acetate gradient for 30 min, increasing to 200 mM until 70 min. Monosaccharide (glucose), disaccharide (maltose) and a mixture of malto-oligosaccharides containing maltoriose to maltoheptaose were used as standards.

2.2.5. Polysaccharide profile

Homogeneity and the average molecular weight of polysaccharides were analyzed by high-performance size-exclusion chromatography coupled to a refractive index and a multiple wavelength detector (HPSEC-RID/MWD). Polysaccharides were analyzed in a 1260 Infinity system (Agilent, Santa Clara, CA) equipped with PL-aquagel-OH columns 60, 50, 40 and 30 (300 × 7.5 mm; 8 μm; Agilent) connected in series using water eluent (1 mL/min; 50 min; 25 °C). The refractive index detector temperature was 30 °C and the multiple wavelength detector