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Isolation and structural characterisation of papaya peel pectin

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ABSTRACT

As pectins are structurally highly variable between plant species, waste streams of other sources than the commercially used citrus peel and apple pomace may contain industrially useful forms of pectin. The potential of papaya peel as a source for the extraction of pectin in countries of the South was investigated in this study. In particular, pectin extracted from the peel of a wild (Local) and an ameliorated (Solo) papaya variety was structurally characterised. Pectin was examined via physicochemical (sugar composition, solubility, linearity, branching, degree of methoxylation, molar mass) and immunological (binding of anti-pectin antibodies) analysis of fractionated cell walls and isolated polymers. Cell-wall material isolated from papaya peel appeared to be rich in pectin, indicating a potential (industrial) source of this polysaccharide. The peel of both papaya varieties predominantly consisted of low methyl-esterified, linear Ca^{2+}-cross-linked homogalacturonan with high molar mass, while only a limited amount of high methyl-esterified, branched water-soluble pectin could be retrieved from Solo and Local papaya peel. Differences between pectins obtained from the peel of the wild and ameliorated papaya varieties were rather limited. However, rhamnogalacturonan-I (a branched domain of pectin) retrieved from the peel of Solo had generally longer and/or more arabinan, galactan and/or arabinogalactan side chains than rhamnogalacturan-I from the peel of the Local papaya variety. In addition, pectin from Local papaya peel presented a lower degree of methyl-esterification than pectin from the Solo variety. The structural characteristics of papaya peel pectin revealed by this study can form a basis for understanding its functional properties as ingredient in food systems.

1. Introduction

Papaya (*Carica papaya L.*) or pawpaw belongs to the family of the Caricaceae. This fruit, native to the tropics of America is now disseminated throughout the world (Teixeira da Silva et al., 2007). Solo, Formose, Sunset, Golden and Sunrise are the most common varieties (Paull, Nishijima, Reyes, & Cavaletto, 1997). The fresh fruit is attractive to consumers due to its striking odours, high vitamin content (i.e. vitamin A and C) and high fibre content (Almora et al., 2004; Ittimongkol et al., 2002). In addition to the fruit's dietary value, papaya stems, leaves and fruits contain high levels of proteins and vitamins which are used in the elaboration of cosmetics and medications (Teixeira da Silva et al., 2007). Peels and pips which are the by-products of papaya processing and represent 20 to 25% of the fruit weight, can be used for animal feeding but are generally discarded into the environment causing organic pollution. However, it may be possible to valorise papaya peels due to the fact that proteases and pectins can be recovered from this by-product with acceptable yield (Boonrod, Reanma, & Niamsup, 2006; Chaiwut, Nitsawang, Shank, & Kanasawud, 2007).

Pectins are a family of complex polysaccharides present in the primary cell wall and middle lamella of dicotyledons (Voragen & Pilnik, 1995). They are generally described as an alternation of smooth (homogalacturonans, HGs) and hairy (type I and type II rhamnogalacturonans, RG-I and RG-II) regions (Ridley, O'Neill, & Mohnen, 2001; Schols & Voragen, 2002). According to Willats, McCartney, Mackie, and Knox (2001) HG is the most abundant pectic polysaccharide in plant cell walls. It is composed of a linear chain of (1,4)-linked α-D-galacturonic acid (Gal*p*A) residues in which some of the C-6 carboxyl groups are methyl-esterified. In this context, the degree of methoxylation (DM) is defined as the percentage of GalA units esterified by methanol. RG-I on the other hand contains a backbone which is composed of the repeating unit [4)-α-D-Gal*p*A-(1,2)-α-L-Rha*p*-(1] in which 20–80% of the rhamnosyl (Rha*p*) residues are substituted at O-4 with neutral sugar side chains, containing mainly arabinose and galactose (Ridley et al., 2001; Schols & Voragen, 2002). RG-II finally is a complex

Abbreviations: AIR, alcohol-insoluble residue; CSP, chelator-soluble pectin; DM, degree of methoxylation; HG, homogalacturonan; HM, high-methoxylated; LM, low-methoxylated; M_p, peak molecular weight; MPBS, phosphate-buffered saline containing milk powder; NSP, sodium-carbonate-soluble pectin; R, residue; RG-I, rhamnogalacturonan-I; RG-II, rhamnogalacturonan-II; WSP, water-soluble pectin.

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molecule whose backbone is composed of at least eight 1,4-linked α-D-Gal*p*A units to which four structurally conserved side chains, consisting of twelve different monosaccharides, are attached (Schols & Voragen, 2002).

Pectins are widely used as food additives for their thickening, gelling and emulsifying properties in jams, soft drinks and milk products (May, 1997). The functionalities of pectins depend largely on the structural characteristics of these polysaccharides, which in turn are determined by the plant source and the extraction conditions used. Pectins are highly heterogeneous with respect to their GalA and neutral sugar content, their degree of methoxylation, their degree of branching and their molar mass distribution (Kravtchenko, Voragen, & Pilnik, 1992; Ralet & Thibault, 2002). Commercially, pectins are mostly extracted by treating apple pomace or citrus peels with hot dilute mineral acids, mainly nitric acid. These pectins are generally highly methoxylated (HM, 55–80%). Low methoxylated (LM) pectins can be obtained by treating HM pectins with acid or strong alkali, while amidated pectins can be produced using ammonia, which converts ester groups into amide groups (Rolin, 2002). Besides apple pomace and citrus peels, there are some secondary sources of pectin but only sugar beet pulp and sunflower heads have been commercialised so far. As pectins are highly variable between plant species in structural terms, waste streams of other, not yet fully explored sources may also contain industrially useful forms of pectin. Sources from countries of the South, including residues of mango, guava, cocoa and papaya, are still being investigated (Berardini, Knodler, Schieber, & Carle, 2005; Boonrod et al., 2006; Voragen & Pilnik, 1995).

Many investigations have been done on the physicochemical properties of pectin fractions found in the cell wall of papaya flesh. Westerlund, Aman, Andersson, Andersson, and Rahman (1991) found that the major component of the water soluble pectin fraction of papaya fruit was a rhamnogalacturonan with a low content in arabinose and galactose residues and with a DM of 50%. Furthermore, Paull, Gross, and Qiu (1999) found that the pectin fractions of papaya fruit were mainly composed of rhamnose, glucose, xylose, galactose, mannose and arabinose in decreasing order of concentration. Cell-wall polysaccharide modifications during postharvest ripening of papaya fruit were also studied. However, regarding to papaya peels, till now, only few research works have been performed. In the few works that have been done so far, no fractionation of pectins based on solubility has been carried out after extraction of the pectins from the matrix (which was mostly done using one extracting solvent). Boonrod et al. (2006) did one of the few works on papaya peels in which pectin was extracted using HCl at different concentrations (0.02, 0.06 and 0.1 M). Analysis results showed a good extraction yield (2–6% fresh weight basis), the presence of low methoxylated pectins (46–51%) and a high galacturonic acid content (72%).

In countries of the South, food industries are faced with the problem of inadequate supply of additives. Hence, this work is a contribution to ensure the availability of pectins to be used in food industry. The main objective of the present study was to investigate the potential of papaya peels for the extraction of pectin. Specifically, pectin in the peel of a wild (Local) and an ameliorated (Solo) papaya variety was structurally characterised.

2. Material and methods

2.1. Sampling

Papaya fruits *(C. papaya L.)* from two varieties (Solo and Local) were obtained from Yaounde (Cameroon) market and used immediately. Solo, an ameliorated variety with a high production, is green when ripe and weighs around 600 g. Local papaya fruits on the other hand have an average weight of 1500 g and their colour changes from green to red–yellow upon ripening. Peels were removed from the ripe papaya fruit varieties and chopped into pieces of approximately 1 cm^2. These fresh peels were blanched at 95 °C for 5 min and subsequently dried in an air-convection oven at 50 °C for 2 days. The dried product was ground in a blender and passed through a sieve (Ø 0.5 mm) in order to obtain a homogeneous peel powder. This powder was used for the isolation of the cell-wall components as alcohol insoluble residue (AIR).

2.2. AIR isolation

In order to remove the majority of pigments, soluble sugars and lipids, the dried peel powder (0.5 g) was treated with ethanol and acetone at room temperature. First however, the peel powder was soaked in 200 mL of deionised water under stirring for 3 h at 4 °C. Afterwards, the suspension was filtered (Macherey-Nagel MN 615 Ø 90 mm) and the residue was homogenised in 64 mL of 95% (v/v) ethanol using a mixer (Buchi mixer B-400, Flawil, Switzerland). The suspension was filtered again and the residue was rehomogenised in 64 mL of 95% ethanol. This was repeated two or three times till the ethanol solution was colourless. Finally, the residue was homogenised in 96 mL acetone and filtrated before drying overnight at 40 °C to obtain the AIR (McFeeters & Armstrong, 1984).

2.3. Sequential fractionation of papaya peels pectin

Water-soluble pectin (WSP), chelator-soluble pectin (CSP) and sodium carbonate-soluble pectin (NSP) fractions were extracted from papaya AIR using different solvents whereby pectin losses were minimised. This extraction procedure was performed as described by Braga, Pessoni, and Dietrich (1998) for WSP and by Chin, Ali, and Lazan (1999) for CSP and NSP. WSP was extracted using deionised water. More specifically, 0.5 g of AIR was mixed with 90 mL of boiling water while stirring for 5 min. The suspension was then cooled under running tap water and filtered (Macherey-Nagel MN 615 Ø 90 mm). The filtrate (WSP) was collected and adjusted to 100 mL with water and the residue was resuspended in 90 mL of 0.05 M cyclohexanetrans-1,2-diamine tetra-acetic acid (CDTA) in 0.1 M potassium acetate (pH 6.5) for 6 h at 28 °C. The resulting suspension was filtered and the filtrate volume was adjusted to 100 mL with CDTA solution to obtain the CSP fraction. The residue was reincubated in 90 mL 0.05 M Na_2CO_3 containing 0.02 M $NaBH_4$ for 16 h at 4 °C, and subsequently for 6 h at 28 °C. After filtration, the filtrate volume was adjusted to 100 mL to obtain the NSP fraction. The final residue (R) was dried (40 °C) and weighed. The fractionation procedure was done in duplicate. WSP, CSP and NSP were frozen with liquid nitrogen and stored at −40 °C until further analysis. For neutral sugar analysis and molar-mass distribution analysis, pectin fractions were lyophilised using a freeze-dryer (Christ alpha 2-4, Osterode, Germany).

2.4. Pectin characterisation

2.4.1. GalA analysis

The GalA content of the AIR, pectin fractions (WSP, CSP, NSP) and fractionation residue (R) was determined colorimetrically by the m-phenylphenol method. Samples were first hydrolysed in concentrated sulphuric acid and afterwards, the GalA content was determined according to the spectrophotometric method described by Blumenkrantz and Asboe-Hansen (1973). The hydrolysis was performed in duplicate while three colorimetric analyses were carried out for each hydrolysate.

2.4.2. Neutral sugar analysis

The neutral monosaccharides (fucose, rhamnose, arabinose, galactose, glucose, xylose and mannose) of pectin fractions were determined using high-performance anion-exchange chromatography coupled with pulsed amperometric detection. To obtain the individual neutral-