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The arabinogalactan of dried mango exudate and its co-extraction during pectin recovery from mango peel

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ABSTRACT

Hot-acid extraction of the peel of fully ripe 'Nam Dokmai' (NDM) and 'Maha Chanok' (MHC) mangoes yielded pectins (P_{NDM}, P_{MHC}) being comparatively poor in uronic acids (38–47 g hg^{-1}) in favor of the neutral sugars (23 g hg^{-1}), chiefly galactose. Beyond the high-molecular-weight fraction, they displayed a characteristic, almost monodisperse fraction with a peak molecular weight of 18,200–19,100 relative to dextrans. In order to examine the hypothesized co-extraction of non-pectin polymers, the pectin P_{NDM} and the exudate of mature-green 'Nam Dokmai' fruit (SAP_{NDM}) were characterized regarding composition and molecular properties both before and after fractionation. By fractionation of P_{NDM} on a weak anion exchanger, the high-molecular-weight pectin was largely separated from this characteristic fraction, which was composed of arabinogalactans. Likewise, the almost monodisperse molecular weight distribution of the dried fruit exudate SAP_{NDM} with a peak molecular weight of 22,400 was shown to be caused by arabinogalactans being the main component (77.6 g hg^{-1}) with a molar ratio of 82/10/4/4 for the major carbohydrate residues galactose, arabinose, rhamnose, and glucuronic acid. Fractionation of SAP_{NDM} by size exclusion chromatography (SEC) and rechromatography (HPSEC) confirmed the high molecular uniformity of these arabinogalactans. The occurrence of hydroxyproline-rich proteins ($\leq$0.2 g hg^{-1}) in these exudate fractions may indicate small percentages of arabinogalactan-proteins. Co-extraction of residual fruit exudate may thus be considerable in pectin recovery from mango peel. Options for obtaining both pure pectins (galacturonic acid, 94 mol $hmol^{-1}$) and arabinogalactans from this source were discussed.

1. Introduction

Mango peel, being a by-product of the fruit-processing industry, has been deemed to be a promising source for the recovery of pectins (Kermani et al., 2014; Nagel, Neidhart, et al., 2014; Rehman, Salariya, Habib, & Shah, 2004; Sirisakulwat, Sruamsiri, Carle, & Neidhart, 2010). However, a high level of co-extracted impurities has repeatedly resulted in mango pectins, which hardly met the legal specification of commercial food-grade pectins concerning the minimum galacturonic acid content (Neidhart, Sirisakulwat, Nagel, Sruamsiri, & Carle, 2009; Sirisakulwat, Nagel, Sruamsiri, Carle, & Neidhart, 2008). Besides high contents of arabinose, galactose, and starch, a non-starchy fraction of comparatively low molecular weight (15,000–22,000), which was detected irrespective of cultivar and fruit ripeness, has been assumed to affect the functional properties of mango pectins crucially and to restrict their use as gelling agents (Neidhart et al., 2009; Sirisakulwat et al., 2008). Polysaccharide fractions being rich in arabinose and galactose had been found in aqueous extracts from mango peel (Kermani et al., 2014; Koubala et al., 2007) and pulp (Brinson, Dey, John, & Pridham, 1988; Iagher, Reicher, & Ganter, 2002; Ollé, Lozano, & Brillouet, 1996; Yashoda, Prabha, & Tharanathan, 2005). They had been ascribed to extensive arabinan, galactan, and arabinogalactan (AG) side chains of the pectic substances (Iagher et al., 2002; Kermani et al., 2014; Yashoda et al., 2005). Similarly, mango pectins that had been extracted using hot acid (Sirisakulwat et al., 2008) or chelating agents (Kermani et al., 2014; Prasanna, Prabha, & Taranathan, 2004; Prasanna, Yashoda, Prabha & Taranathan,

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2003) were shown to be particularly rich in arabinose and galactose. In general, arabinogalactans may be covalently linked to the rhamnose units of the rhamnogalacturonan-I (RG-I) parts of the pectins, but can also occur as free polysaccharides (Voragen, Beldman, & Schols, 2001).

According to the hypothesis treated in this study, the impurities of mango pectins include not only co-extracted structural and storage polysaccharides of the peel, but also carbohydrates of the fruit resin, because a network of resin ducts that pervades the fruit is particularly dense in the exocarp (Joel, 1981). Like other plants of the *Anacardiaceae* family, the mango fruit (*Mangifera indica* L.) secretes a transparent, reddish-brown, or black resin after wounding the bark or severing the fruit from the stalk (Nussinovitch, 2010). Its content of irritant alk(en)ylresorcinols (Hassan, Irving, Dann, Coates, & Hofman, 2009) has been deemed to define the levels of these phytoanticipins in the peel and mesocarp (Kienzle, Carle, Sruamsiri, Tosta, & Neidhart, 2014). Besides its lipophilic phase being rich in terpenes (Johnson & Hofman, 2009) and the alk(en)ylresorcinols, this exudate ('sap') comprises a hydrophilic phase containing enzymes (Saby John, Bhat, & Prasada Rao, 2011) and mucilaginous carbohydrates (Joel & Fahn, 1980; Saby John, Bhat, & Prasada Rao, 2003). The carbohydrate fraction has been reported to consist of a water-soluble, non-starchy, protein-containing polysaccharide of a molecular weight >2 kDa (Joel & Fahn, 1980; Saby John et al., 2003, 2011).

This study aimed at the characterization of the major water-soluble constituents in the exudate of 'Nam Dokmai' mango fruit. In regard to the above-mentioned hypothesis, the respective polysaccharides, presumably arabinogalactans, were to be characterized and compared to the polysaccharide fractions of the pectins, which are obtainable by hot-acid extraction of mango peel.

2. Material and methods

2.1. Sample material

For the recovery of pectins (P_{NDM}, P_{MHC}) from dried mango peel by hot-acid extraction (pH 1.5, 90 min) and alcohol precipitation on the laboratory scale (Sirisakulwat et al., 2008), peel waste from the industrial processing of fully ripe 'Nam Dokmai' (NDM) and 'Maha Chanok' (MHC) fruits (northern Thailand, season 2010) was used. For comparative analyses, the unstandardized pectins RP, AP, and CP from sugar beet, apple, and citrus pomace, respectively, were made available by Herbstreith & Fox (Neuenbürg, Germany). Gum arabic was obtained from J.T.Baker (Avantor Performance Materials, Griesheim, Germany).

Mango fruit exudate was collected from 30 mature-green 'Nam Dokmai' fruits, which had been harvested in northern Thailand within one day in May 2011 by cutting the stem 10 cm above the fruit base. In the laboratory, the stem was severed from the fruits in their abscission zones. The exuding resin was pooled (SAP_{fresh}) and dried at 25 °C for 48 h in order to transform it into a thin layer for the transport to Hohenheim University, Germany, where the dried sap was ground in a mortar and conditioned in a desiccator for 48 h (SAP_{NDM}).

2.2. Preparative methods

2.2.1. Pectin fractionation by preparative anion exchange chromatography

The mango pectin P_{NDM} (cf. 2.1) was fractionated by separation on a diethylaminoethanol (DEAE) Sepharose™ Fast Flow weak anion exchange resin (GE Healthcare, Uppsala, Sweden). The ion exchange chromatography (IEC) system comprised a C-695 borosilicate 3.3 plunger column (49 mm × 460 mm, 26 cm filling height), a C-660 fraction collector, a C-615 pump manager, a C-605 pump module, and the Sepacore Record 1.0 software (Büchi, Essen, Germany), besides a BT 8200 UV detector (Biotronik, Maintal, Germany). The eluents were adopted from Asgar, Yamauchi, and Kato (2003) with modifications. The column was equilibrated for 2 h at a flow rate of 15 mL min^{-1} with eluent A_{IEC} (0.05 M Sørensen buffer, pH 5.0) containing KH_2PO_4 (49.4 mmol L^{-1}) and $Na_2HPO_4 \cdot 2\ H_2O$ (0.6 mmol L^{-1}).

For the pectin solution (16.67 g L^{-1}), P_{NDM} was dissolved in eluent A_{IEC} and shaken overnight. This solution was centrifuged (8000 × *g*, 15 min). The resulting supernatant was filtered through an MN 615 ¼ folded filter in order to inject 20 mL of the filtrate into the IEC system via a sample loop. For gradient elution of the pectin fractions at a flow rate of 10 mL min^{-1} (3 bar max. pressure, ambient temperature), eluent B_{IEC} (eluent A_{IEC} plus 500 mM NaCl) was admixed with eluent A_{IEC} to increase the NaCl concentration stepwise as follows: (1) 0–40 min 0% B_{IEC} (0 mM NaCl), (2) 40–70 min linear increase to 12.5% B_{IEC}, (3) 70–100 min 12.5% B_{IEC} (62.5 mM NaCl), (4) 100–140 min linear increase to 25% B_{IEC}, (5) 140–170 min 25% B_{IEC} (125 mM NaCl), (6) 170–210 min linear increase to 100% B_{IEC}, and (7) 210–260 min 100% B_{IEC} (500 mM NaCl). The eluate was automatically collected in 20 mL-fractions covering elution intervals of 2 min. The fractions contributing to the same peak at 215 nm were pooled. The pooled fractions IEC-I to IEC-V were lyophilized to reduce their volumes. After they had been dissolved in adjusted volumes of ultra-pure water, they were dialyzed for 72 h against distilled water under stirring (1 kDa cut-off, Ø 29 mm, regenerated cellulose, Spectra/Por7®, Spectrum Laboratories, Compton, USA), and lyophilized again for further investigation.

For a representative IEC run, the characteristic composition of the 20 mL-fractions was monitored. For this purpose, their anhydro-uronicacid contents (AUA_{MHDP}) were quantified, using the *m*-hydoxydiphenyl (MHDP) assay (Sirisakulwat et al., 2008) without pre-hydrolysis. Their neutral-sugar contents ($ANS_{orcinol}$, expressed as anhydro-galactose) were determined by means of the orcinol-sulfuric acid assay (Miller, Slater, Birzgalis, & Blum, 1961) with subsequent correction for interfering uronic acids according to the respective results of the MHDP assay (Ralet & Thibault, 2002). In a test tube, 0.2 mL of the sample or calibration standard solution was mixed with 3 mL orcinol reagent (H_2SO_4, 70% v/v, plus 3,5-dihydroxytoluol monohydrate, 0.2% w/v), heated at 100 °C (20 min), and recooled in ice water (5 min) for the absorbance measurement at 515 nm with a Cary 100 UV/Vis spectrometer (Varian, Mulgrave, VIC, Australia) after 30 min.

2.2.2. Polysaccharide fractionation by semi-preparative size exclusion chromatography (SEC)

The system used for semi-preparative SEC comprised an HPLC compact pump type 1.709 and an LC-CaDI 22-14 control unit with the McDAcq32 software version 2.0 (Bischoff, Leonberg, Germany) plus a dynamic mixing chamber (Knauer, Berlin, Germany) and a 2410 RI-detector (Waters, Milford, MA, USA). A Superformance 26 plunger glass column (600 mm × 26 mm i.d., 336 mL volume; Merck, Darmstadt, Germany) was packed with Toyopearl HW-55F resin of 45 μm particle size (Tosoh Bioscience, Stuttgart, Germany) after covering the plungers with 20 μm-filter disks. For the eluent, 5 mM ammonium formate/formic acid buffer (pH 3.0) was filtered through a Whatman 0.2 μm nylon filter (Ø 47 mm).

Dried mango exudate (12 mg of SAP_{NDM}, cf. 2.1) was dissolved in 1.2 mL of this eluent by shaking for 10 min. After filtration (Chromafil, AO-45/15, 0.45 μm), 1 mL was injected onto the column for the separation at ambient temperature and a flow rate of 1 mL min^{-1} within a total run time of 260 min. While the effluent was monitored by RI-detection, two fractions (SF–I and SF-II; Fig. 1)