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Pectin isolation and characterization from six okra genotypes

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ABSTRACT

Pectin was isolated by aqueous extraction at pH 6.0 from the pods of six different okra genotypes (*Abelmoschus esculentus* L.). Genetic diversity was determined using fragment length analysis (FLA) of ten simple sequence repeat (SSR) markers. Physical and chemical evaluation of pectin was performed by means of FT-IR and NMR spectroscopy, sugar composition analysis (GC-MS), size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLS), dilute solution viscometry and steady shear rheology assisted by principal component analysis (PCA). Each of the SSR markers detected on average 4.1 alleles and revealed unique genotypes for each sample. Extraction yield was between 11 and 14% resulting in pectin with galacturonic acid content between 43 and 63%, low degree of methyl-esterification (17–25%) and high degree of acetylation (20–40%). All samples were of high weight-average molar mass (M_w) (700–1700 × 10^3 g mol^{-1}) and sugar composition analysis revealed the structural diversity of samples with HG/RG-I ratios ranging between 1.3 and 3.1. The present work shows that individual okra genotypes provide pectin with different structural properties that could potentially provide a new source of functional pectin for the food or pharmaceutical industries.

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1. Introduction

Okra (*Abelmoschus esculentus* L.) is cultivated throughout the tropical, sub-tropical and temperate regions of the world including the shores of the Mediterranean Sea owing to its high economic and nutritional value. Pectin has been identified as the responsible hydrocolloid for the viscous texture of okra extracts and is of major technological interest for food and pharmaceutical applications (Alba, Laws, & Kontogiorgos, 2015; Georgiadis et al., 2011; Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014; Ghori et al., 2017). The functional properties of pectins are typically influenced by their chemical and macromolecular properties such as molecular weight, charge and charge-density, polymer conformation, and flexibility of polymer chains (Kontogiorgos, Margelou, Georgiadis, & Ritzoulis, 2012; Ndjouenkeu, Goycoolea, Morris, & Akingbala, 1996). Depending on the plant source, genotype, stage of ripening and extraction method, pectins can exhibit heterogeneity in macromolecular characteristics, which subsequently affect their functional properties (Alba & Kontogiorgos, 2017).

Okra pectins have been previously isolated by following various extraction strategies using aqueous buffers (Alamri, Mohamed, & Hussain, 2012; Alba et al., 2015; Archana et al., 2013; Georgiadis et al., 2011; Samavati, 2013; Sengkhamparn, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009; Woolfe, Chaplin, & Otchere, 1977; Zheng et al., 2014). Isolated okra pectins are rich in rhamnogalacturonan-I (RG-I) segments with varying composition of side chains and molecular weights ranging from 10 – 767 × 10^3 g mol^{-1}. Although the effect of extraction conditions on structural and macromolecular characteristics of okra polysaccharides is well investigated and understood, the impact of different okra genotypes on those features has not yet been evaluated. The variability of chemical structures of cell wall polysaccharides (*e.g.*, pectin and hemicellulose) is related to both genetic and developmental factors and has been the subject of several studies in dicotyledonous plants focusing on investigation of the compositional changes of cell-wall polysaccharide structural domains (Gálvez-López, Laurens, Devaux, & Lahaye, 2012; Lahaye, Devaux, Poole, Seymour, & Causse, 2013; Lahaye, Falourd,

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Quemener, Devaux, & Audergon, 2014; Lahaye et al., 2012).

The understanding of the impact of each structural parameter (*e.g.*, neutral sugar composition, degrees of methylation and acetylation) of cell-wall polysaccharides may serve as the basis for plant design with remodelled functionality. Isolation of pectin from different okra genotypes may also result in structural dissimilarities and consequently may impact functional properties of okra pectin isolates. The aim of the present work, therefore, was to investigate the physicochemical properties of extracted pectins of six different okra genotypes in order to evaluate their potential as novel functional ingredients for the food and pharmaceutical industries.

2. Material and methods

2.1. Cultivation of okra genotypes and pectin isolation

Okra genotypes (Asha, Agbagoma, Asontem, Balabi, Sengavi and Penkrumah) were cultivated in Ghana (Akrofu, Volta Region) from October 2015 to January 2016 and all agricultural practices including thinning, weed control and watering were carried out under controlled environmental conditions. The soil at the experimental site was sandy-loam with the rainfall pattern remaining very low (<20 mm) whereas temperature ranged between 22 and 31 °C. A standardized crop descriptor for okra (Resources, 1991) was used to measure the various phenotypic characteristics of the different genotypes. Pectin was extracted from dried okra pods using phosphate buffer at pH 6.0. Following extraction, the polysaccharides were precipitated using alcohol, dialysed and finally freeze-dried. The details of the isolation protocol are described elsewhere in detail (Alba et al., 2015).

2.2. Genetic diversity analysis of okra samples

Fragment length analysis (FLA) was performed by Ecogenics GmbH (Switzerland) using singleplex PCR and FAM-labelled oligonucleotides. Forward and reverse primer sequences (Table 1) targeting 10 simple sequence repeats (SSRs) were based on known loci with high information content (Schafleitner, Kumar, Lin, Hegde, & Ebert, 2013). Analysis of fragment length polymorphism data was conducted using Peak Scanner™ software (Applied Biosystems, US) to determine allele sizes in base pairs for each primer pair. Alleles were scored in a dominant manner as absent (0)/present (1), and dissimilarity was calculated in the Darwin package (Perrier & Jacquemoud-Collet, 2006) using Jaccard's coefficient $d_{ij} = b + c/(a + b + c)$, where a equals to the number of alleles that are common to both genotypes i and j. b equals to the number of alleles that are unique to genotype i (absent in j). c equals to the number of alleles that are unique to genotype j (absent in i). A phylogenetic tree was generated using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering in Darwin (Pereira et al., 2006).

2.3. Yield and chemical characterization of okra pectin

The yield was calculated based on dry weight basis using the following equation:

$$Yield\ (\%) = \frac{mass\ of\ freeze\ dried\ pectin}{mass\ of\ dried\ okra\ powder} \times 100 \quad (1)$$

Protein quantification was performed using Bradford assay (Bradford, 1976) and total carbohydrate content of okra pectin powder was determined by phenol-sulphuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Galacturonic acid content of pectins was determined using *m*-hydroxydiphenyl method (Filisetti-Cozzi & Carpita, 1991). All measurements were performed in triplicate. Methoxyl content was determined by titration (Schultz, 1965) and acetyl content was established with the hydroxamic acid method (McComb & McCready, 1957). Degrees of methylation and acetylation were calculated using the equations, as described previously (Alba et al., 2015). Neutral sugars were determined using methanolysis conducted with 1 M methanolic HCl at 85 °C for 24 h, as described previously (Bleton, Mejanelle, Sansoulet, Goursaud, & Tchapla, 1996). Sugar derivatives were analysed using an Agilent 7890A GC system (Santa Clara, CA, USA) coupled to an Agilent 5675C quadrupole MS. The samples were eluted from an HP-5 column (30 m × 0.25 mm, 0.25 μm film) using helium as carrier at a flow rate of 1 mL min^{-1} by applying the following temperature settings: start temperature 140 °C hold time 1 min and final column temperature 220 °C with 25 °C min^{-1} gradient. Principal component analysis (PCA) of normalized data (yield, protein content, carbohydrate content, D-GalA, DM, DA, Mw, polydispersity, D-Gal, L-Ara, D-Glc, HG/RG-I, intrinsic viscosity, Huggins constant, c* and c**) were used to perform a PCA (correlation matrix and minimum of five components) using Minitab 17 (Minitab Inc., Philadelphia, U.S.A.).

2.4. Spectroscopic analysis

FT-IR spectra were obtained between 500 and 4000 cm^{-1} for all okra samples in attenuated total reflection (ATR) mode at a resolution of 4 cm^{-1} using 128 scans (Nicolet 380, Thermo Scientific, UK). Spectral smoothing was applied using instrument software (OMNIC 3.1). NMR analysis was conducted using a Bruker AV 500 spectrometer (Bruker Co., Switzerland) at 500 MHz ^{1}H and 125.76 MHz ^{13}C. Prior to analysis, samples (5% w/v) were dispersed overnight in D_2O (99.9% D, Goss Scientific Instruments Ltd., Essex). Proton decoupled spectra were recorded at 70 °C using 12,800 scans with a relaxation delay of 2 s and a 30° pulse angle. ^{1}H–NMR spectra were recorded with 64 scans at the same temperature.

Table 1
Forward and reverse oligonucleotide sequences used for FLA. Sequences based on markers identified by Schafleitner (Schafleitner et al., 2013).

Marker Name	Forward Primer	Reverse Primer	Number of alleles detected in the six genotypes
AVRDC-Okra1	ATGGAGTGATTTTTGTGGAG	GACCCGAACTCACGTTACTA	3
AVRDC-Okra9	ACCTTGAACACCAGGTACAG	TTGCTCTTATGAAGCAGTGA	5
AVRDC-Okra17	ACGAGAGTGAAGTGGAACTG	CTCCTCTTTCCTTTTTCCAT	3
AVRDC-Okra28	CCTCTTCATCCATCTTTTCA	GGAAGATGCTGTGAAGGTAG	3
AVRDC-Okra39	TGAGGTGATGATGTGAGAGA	TTGTAGATGAGGTTTGAACG	4
AVRDC-Okra52	AACACATCCTCATCCTCATC	ACCGGAAGCTATTTACATGA	4
AVRDC-Okra54	CGAAAAGGAAACTCAACAAC	TGAACCTTATTTTCCTCGTG	2
AVRDC-Okra56	GGCAACTTCGTAATTTCCTA	TGAGTAAAAGTGGGGTCTGT	7
AVRDC-Okra64	AAGGAGGAGAAAGAGAAGGA	ATTTACTTGAGCAGCAGCAG	7
AVRDC-Okra89	TTTGAGTTCTTTCGTCCACT	GTATTTGGACATGGCGTTAT	3

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