

1 **Pectin isolation and characterization from six okra genotypes**

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20 **Abstract**

21 Pectin was isolated by aqueous extraction at pH 6.0 from the pods of six different okra
22 genotypes (*Abelmoschus esculentus L.*). Genetic diversity was determined using fragment length
23 analysis (FLA) of ten simple sequence repeat (SSR) markers. Physical and chemical evaluation
24 of pectin was performed by means of FT-IR and NMR spectroscopy, sugar composition analysis
25 (GC-MS), size exclusion chromatography coupled to multi-angle laser light scattering (SEC-
26 MALLS), dilute solution viscometry and steady shear rheology assisted by principal component
27 analysis (PCA). Each of the SSR markers detected on average 4.1 alleles and revealed unique
28 genotypes for each sample. Extraction yield was between 11–14 % resulting in pectin with
29 galacturonic acid content between 43–63 %, low degree of methyl-esterification (17–25 %) and
30 high degree of acetylation (20–40 %). All samples were of high weight-average molar mass (M_w)
31 ($700-1700 \times 10^3 \text{ gmol}^{-1}$) and sugar composition analysis revealed the structural diversity of
32 samples with HG/RG-I ratios ranging between 1.3 and 3.1. The present work shows that
33 individual okra genotypes provide pectin with different structural properties that could
34 potentially provide a new source of functional pectin for the food or pharmaceutical industries.

35

36 **Keywords:** okra; isolation; characterization; genotypes; pectin

37 **1. Introduction**

38 Okra (*Abelmoschus esculentus L.*) is cultivated throughout the tropical, sub-tropical and
39 temperate regions of the world including the shores of the Mediterranean Sea owing to its high
40 economic and nutritional value. Pectin has been identified as the responsible hydrocolloid for the
41 viscous texture of okra extracts and is of major technological interest for food and
42 pharmaceutical applications (Alba, Laws, & Kontogiorgos, 2015; Georgiadis, Ritzoulis, Sioura,
43 Kornezou, Vasiliadou, & Tsiptsias, 2011; Ghorji, Alba, Smith, Conway, & Kontogiorgos, 2014;
44 Ghorji, Mohammad, Rudrangi, Fleming, Merchant, Smith, & Conway, 2017). The functional
45 properties of pectins are typically influenced by their chemical and macromolecular properties
46 such as molecular weight, charge and charge-density, polymer conformation, and flexibility of
47 polymer chains (Kontogiorgos, Margelou, Georgiadis, & Ritzoulis, 2012; Ndjouenkeu,
48 Goycoolea, Morris, & Akingbala, 1996). Depending on the plant source, genotype, stage of
49 ripening and extraction method, pectins can exhibit heterogeneity in macromolecular
50 characteristics, which subsequently affect their functional properties (Alba & Kontogiorgos,
51 2017).

52 Okra pectins have been previously isolated by following various extraction strategies
53 using aqueous buffers (Alamri, Mohamed, & Hussain, 2012; Alba, et al., 2015; Archana, Sabina,
54 Babuskin, Radhakrishnan, Fayidh, Babu, Sivarajan, & Sukumar, 2013; Georgiadis, et al., 2011;
55 Samavati, 2013; Sengkhampan, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009; Woolfe,
56 Chaplin, & Otchere, 1977; Zheng, Zhao, Feng, Wang, Zou, Zheng, Takase, Li, Wu, Yang, &
57 Wu, 2014). Isolated okra pectins are rich in rhamnogalacturonan-I (RG-I) segments with varying
58 composition of side chains and molecular weights ranging from $10 - 767 \times 10^3 \text{ g mol}^{-1}$. Although
59 the effect of extraction conditions on structural and macromolecular characteristics of okra

60 polysaccharides is well investigated and understood, the impact of different okra genotypes on
61 those features has not yet been evaluated. The variability of chemical structures of cell wall
62 polysaccharides (*e.g.*, pectin and hemicellulose) is related to both genetic and developmental
63 factors and has been the subject of several studies in dicotyledonous plants focusing on
64 investigation of the compositional changes of cell-wall polysaccharide structural domains
65 (Gálvez-López, Laurens, Devaux, & Lahaye, 2012; Lahaye, Devaux, Poole, Seymour, & Causse,
66 2013; Lahaye, Falourd, Quemener, Devaux, & Audergon, 2014; Lahaye, Falourd, Quemener,
67 Ralet, Howad, Dirlewanger, & Arús, 2012).

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

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77 **2. Material and methods**

78 *2.1. Cultivation of okra genotypes and pectin isolation*

79 Okra genotypes (Asha, Agbagoma, Asontem, Balabi, Sengavi and Penkrumah) were
80 cultivated in Ghana (Akrofu, Volta Region) from October 2015 to January 2016 and all
81 agricultural practices including thinning, weed control and watering were carried out under
82 controlled environmental conditions. The soil at the experimental site was sandy-loam with the

83 rainfall pattern remaining very low (< 20 mm) whereas temperature ranged between 22 and 31
84 °C. A standardized crop descriptor for okra (Resources, 1991) was used to measure the various
85 phenotypic characteristics of the different genotypes. Pectin was extracted from dried okra pods
86 using phosphate buffer at pH 6.0. Following extraction, the polysaccharides were precipitated
87 using alcohol, dialysed and finally freeze-dried. The details of the isolation protocol are
88 described elsewhere in detail (Alba, et al., 2015).

89 *2.2. Genetic diversity analysis of okra samples*

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

102 *2.3 Yield and chemical characterization of okra pectin*

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

$$104 \quad \text{Yield (\%)} = \frac{\text{mass of freeze dried pectin}}{\text{mass of dried okra powder}} \times 100 \quad (1)$$

105 Protein quantification was performed using Bradford assay (Bradford, 1976) and total
106 carbohydrate content of okra pectin powder was determined by phenol-sulphuric acid assay
107 (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Galacturonic acid content of pectins was
108 determined using *m*-hydroxydiphenyl method (Filisetti-Cozzi & Carpita, 1991). All
109 measurements were performed in triplicate. Methoxyl content was determined by titration
110 (Schultz, 1965) and acetyl content was established with the hydroxamic acid method (McComb
111 & McCready, 1957). Degrees of methylation and acetylation were calculated using the
112 equations, as described previously (Alba et al., 2015). Neutral sugars were determined using
113 methanolysis conducted with 1 M methanolic HCl at 85 °C for 24 h, as described previously
114 (Bleton, Mejanelle, Sansoulet, Goursaud, & Tchaplal, 1996). Sugar derivatives were analysed
115 using an Agilent 7890A GC system (Santa Clara, CA, USA) coupled to an Agilent 5675C
116 quadrupole MS. The samples were eluted from an HP-5 column (30 m x 0.25 mm, 0.25 µm film)
117 using helium as carrier at a flow rate of 1 mL min⁻¹ by applying the following temperature
118 settings: start temperature 140 °C hold time 1 min and final column temperature 220 °C with 25
119 °C min⁻¹ gradient. Principal component analysis (PCA) of normalized data (yield, protein
120 content, carbohydrate content, D-GalA, DM, DA, Mw, polydispersity, D-Gal, L-Ara, D-Glc,
121 HG/RG-I, intrinsic viscosity, Huggins constant, *c** and *c****) were used to perform a PCA
122 (correlation matrix and minimum of five components) using Minitab 17 (Minitab Inc.,
123 Philadelphia, U.S.A.).

124 *2.4. Spectroscopic analysis*

125 FT-IR spectra were obtained between 500 and 4000 cm⁻¹ for all okra samples in
126 attenuated total reflection (ATR) mode at a resolution of 4 cm⁻¹ using 128 scans (Nicolet 380,
127 Thermo Scientific, UK). Spectral smoothing was applied using instrument software (OMNIC

128 3.1). NMR analysis was conducted using a Bruker AV 500 spectrometer (Bruker Co.,
129 Switzerland) at 500 MHz ^1H and 125.76 MHz ^{13}C . Prior to analysis, samples (5 % w/v) were
130 dispersed overnight in D_2O (99.9 % D, Goss Scientific Instruments Ltd., Essex). Proton
131 decoupled spectra were recorded at 70 °C using 12800 scans with a relaxation delay of 2 s and a
132 30 ° pulse angle. ^1H -NMR spectra were recorded with 64 scans at the same temperature.

133 2.5. *Molecular weight determination*

134 The weight-average molecular weights (Mw) of the okra polysaccharides from different
135 genotypes were estimated using size exclusion chromatography coupled to multi-angle laser
136 light scattering (SEC-MALLS) at 25 °C. Pectins were solubilised in 0.1 M NaNO_3 solution (3
137 mg mL^{-1}) at ambient room temperature with stirring overnight. Samples were subsequently
138 injected onto a SEC system (15 μm particle size, 25 cm \times 4 mm, Agilent, Oxford, UK) which
139 consisted of a PL Aquagel guard column linked in series with PL Aquagel-OH 60, PL Aquagel-
140 OH 50 and PL Aquagel-OH 40. Pectins were eluted with 0.1 M NaNO_3 solution at a flow rate of
141 0.7 mL min^{-1} . The eluent was then detected online firstly by a DAWN EOS light scattering
142 detector (Wyatt Technology, Santa Barbara, U.S.A.) and finally by a rEX differential
143 refractometer (Wyatt Technology, Santa Barbara, U.S.A.). The refractive index increment, dn/dc
144 was taken to be 0.146 mL g^{-1} (Chapman, Morris, Selvendran, & O'Neill, 1987; Morris, de al
145 Torre, Ortega, Castile, Smith, & Harding, 2008; Morris, Foster, & Harding, 2000).

146 2.6. *Dilute solution viscometry and steady shear rheology*

147 Okra pectins were dispersed at 0.01 – 0.5 % g dL^{-1} in 0.1 M NaCl at pH 7.0 in Sorensen's
148 phosphate buffer with 0.02 g dL^{-1} NaN_3 as a preservative. Pectins were stirred overnight to
149 ensure complete solubilization. Intrinsic viscosities $[\eta]$ of okra pectins were measured using an

150 Ubbelohde capillary viscometer (PSL Rheotek OB. C 80705) at 20 ± 0.1 °C. Determination of
151 the intrinsic viscosities for each of the different okra pectin extracts were obtained by
152 extrapolation to infinite dilution (Huggins, 1942):

$$153 \quad \frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \quad (2)$$

154 where $\eta_{sp} = \eta_{rel} - 1$ and $\eta_{rel} = t/t_o$, where t is the average flow time of the solutions at each
155 concentration, t_o is the flow time for water, k_H , is the Huggins constant, and c the biopolymer
156 concentration (g dL^{-1}). Zero shear viscosity measurements were carried out at 20 °C using a
157 Bohlin Gemini 200HR nano rotational rheometer equipped with a double gap geometry or cone-
158 and-plate geometry (55 mm diameter, cone angle 2°). All measurements were completed in a
159 steady shear mode in the range of $0.01\text{-}1000 \text{ s}^{-1}$ at 20 °C.

160 **3. Results and discussion**

161 *3.1. Genetic diversity of okra samples*

162 SSR (microsatellite) markers were used to determine the genetic diversity and
163 relationships between the six samples of okra. The SSR profile of a diploid or polyploid
164 individual may not always express the genotype, as the observed banding pattern cannot make
165 the distinction between homology of fragments of the same size and the possibility of loss of
166 PCR products (Kosman & Leonard, 2005). Therefore, SSRs were considered as dominant
167 markers, with no assumptions made on the genetic nature of the alleles. Each allele was scored
168 for simple presence/absence and dissimilarity was calculated using the Jaccard index to
169 determine genetic diversity. The advantage of the Jaccard index being that the shared absence of
170 SSR markers in any pairwise comparison does not contribute to the dissimilarity score, therefore
171 reducing the likelihood of over-estimating genetic distance. The SSR profiles over 10 loci
172 revealed a large amount of polymorphism with a mean of 4.1 alleles per locus and unique allele
173 combinations for each sample. All SSR markers showed polymorphism, and the number of
174 alleles varied from 2 (Okra54) to 7 (Okra56 and Okra64) (**Table 1**). This high polymorphism
175 rate, mostly consisting of variable-length triplet repeats, is consistent with previous studies
176 (Schafleitner et al, 2013). Without implying the evolutionary history, it is informative to
177 investigate the genetic diversity and relationships between the okra genotypes and correlate this
178 with phenotypic variation. Phylogenetic analysis revealed clustering of Asha-Asontem-
179 Penkruma as a group, Agbagoma-Balabi as a second group and Sengavi as an outlier (**Figure 1**).
180 The horizontal length between branches indicates relative genetic distance, and demonstrates that
181 the SSR-polymorphisms were sufficient to separate the samples into six distinct genotypes.

182 Having established the genetic diversity of the samples we proceeded with extraction and
183 characterisation of pectin from the samples, as described in the following sections.

184 3.2. Chemical characterisation of okra pectins

185 Pectins extracted from different okra genotypes using hot buffer extraction at pH 6.0
186 varied in yield and purity levels (**Table 2**). The pectin yields ranged between 11.3 and 14.6 %
187 w/w in the order of Asha > Penkruma > Agbagoma > Asontem > Balabi > Sengavi and were
188 comparable to yields previously reported in the literature (12.0 - 15.7 % w/w) (Alba, et al., 2015;
189 Samavati, 2013). Total carbohydrate values ranged between 66 and 87 % w/w and protein
190 content between 3.3 and 7.1 % w/w. Since the same extraction protocol was followed for all
191 samples, differences in yield and purity of the samples (*i.e.*, total carbohydrates and protein) is an
192 indication of structural variations of pectin backbone from the different genotypes. The D-GalA
193 content of samples ranged from 42.8 to 63.4 % w/w and presented low degree of methyl-
194 esterification ranging between 17.0 and 25.5 % w/w. In addition, the acetyl content of okra
195 pectin was between 3.0 and 6.1 % w/w and degree of acetylation varied from 19.9 to 40.1 %
196 w/w. Pectin with low degree of methyl esterification and high degree of acetylation is commonly
197 extracted from okra pods (Alba, et al., 2015; Sengkhamparn, et al., 2009). This data confirms
198 that irrespective of the source, cultivation techniques or genotypes of the raw material, pectin
199 extracted from okra always has a low degree of methylation (*i.e.*, LM-pectin) and high degree of
200 acetylation. Although okra pectin has not shown potential as a gelling agent (Alba, 2015) it has
201 demonstrated excellent emulsifying capacity for emulsions at low pH environments (Alba, Sagis,
202 & Kontogiorgos, 2016). In that work, the stability of emulsions and performance was concluded
203 to be linked to the neutral sugar composition and the side chains of the backbone. The neutral
204 sugar composition of okra pectin showed that the main neutral sugar was galactose followed by

205 rhamnose and arabinose (**Table 3**). Low glucose content indicates that the present isolation
206 protocol results in pectin isolates with low amounts of co-extracted hemicelluloses, cellulosic
207 oligomers or starch. The sugar molar ratios for the different pectins were calculated based on the
208 sugar content determined experimentally. There are a number of different molar ratios ($R_1 - R_4$),
209 which can be calculated from the monosaccharide composition (Denman & Morris, 2015;
210 Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011) to reveal important structural information
211 of pectins (**Table 3**). R_1 is the ratio of D-GalA to the neutral sugars that are present on the side
212 chains and gives an estimate of the linearity of pectin (**Table 3**). Penkruma genotype was
213 distinctively more linear compared to the rest of the samples. This is also confirmed by ratios R_2
214 and R_3 that represent the contribution of RG-I segments to the structure. In particular, greater R_2
215 values reveal greater proportion of RG-I chains whereas high R_3 values indicate greater
216 branching of the RG-I segments. Asontem, Agbagoma and Sengavi show extensive branching
217 compared to the other three samples. In addition to this, high R_4 values indicates long branches
218 something that is particularly evident for samples Asontem and Agbagoma. The HG molar ratio
219 of the okra pectins studied ranged from 53.7 to 72.1 % whereas the RG-I varied between 23.6 to
220 42.7 %. Penkruma is essentially a “smooth” pectin due to the predominance of HG regions in the
221 structure. On the contrary, in genotypes Asha and Balabi the presence of RG-I units is
222 particularly high revealing the abundance of “hairy” regions in these samples. These results show
223 that it is possible to tailor the structure of the extracted pectin by selecting the appropriate
224 genotype and create LM-pectins with an extensive spectrum of functionalities. As mentioned
225 earlier, okra pectins have not shown potential as gelling agents due to extensive branching and
226 high DA that interfere with chain association. However, Asha and Balabi pectins are good

227 candidates for emulsion stabilisation applications due to their extensive branching (Alba, et al.,
228 2016).

229 3.2. FT-IR and NMR spectroscopy

230 The FT-IR spectra of pectins from different okra genotypes cultivated under controlled
231 environmental condition are shown in **Figure 2**. The region in the range of 3200 – 3600 cm^{-1}
232 corresponds to the O–H stretching absorption due to inter- and intramolecular hydrogen bonding
233 of the GalA backbone. Bands in the region of 3000 – 2800 cm^{-1} are assigned to the C-H
234 absorption that includes CH, CH₂ and CH₃ stretching vibrations (Monsoor, Kalapathy, &
235 Proctor, 2001). Absorption bands between 1730–1720 cm^{-1} correspond to C=O stretching
236 vibration of methyl esterified groups and COOH groups whereas bands in the region 1630 and
237 1600 cm^{-1} are related to stretching vibration of carboxylate anion (COO⁻) (Manrique & Lajolo,
238 2002). The bands at 1416, 1380 and 1230 cm^{-1} arise from bending of CH₂, OH and –CH₃CO
239 stretching (Pereira, Oliveira, Rosa, Cavalcante, Moates, Wellner, Waldron, & Azeredo, 2016;
240 Sun, Xu, Sun, Geng, Fowler, & Baird, 2005; Zhang, Dong, Ma, Zhang, Wang, & Hu, 2015). In
241 the finger print region (1200-900 cm^{-1}) of pectin spectra, intense bands at 1140 and 1040 cm^{-1}
242 were assigned to stretching vibrations of glycosidic bonds (C–O) and pyranoid rings (C–C)
243 (Pereira, et al., 2016). Less intense spectral bands in the region 1140–1040 cm^{-1} are typically
244 attributed to the vibrations of monosaccharide components and indicate similarities in the neutral
245 sugars composition between okra pectins. Proteins that typically occur at around 1651 cm^{-1}
246 (amide I) and 1555 cm^{-1} (amide II) were not detected with FT-IR analysis due to the possible
247 wavelength overlap between protein and pectin vibrations and also due to the relatively low
248 amount of protein in okra pectins.

249 A comparison of ^1H -NMR spectra of okra isolated (**Figure 3a**) showed structural
250 similarity between pectins from the different okra genotypes. Okra pectins from all genotypes
251 showed a broad peak at around 3.75-3.84 ppm that signifies the presence of methyl groups
252 connecting to carboxyl groups of D-GalA (Wang, Ma, Jiang, Hu, Zhi, Chen, Ding, Ye, & Liu,
253 2016). The intensity of the aforementioned signal varied among pectins from different okra
254 genotypes. The signal in the range of 2.05-2.13 ppm is indicative of the presence of *O*-acetyl
255 substituent that is similar to that reported in previous study for okra pods (2.10 ppm). The signals
256 from methyl groups of unbranched α -(1 \rightarrow 2)-linked and branched α -(1 \rightarrow 2) and α -(1 \rightarrow 4)-linked
257 rhamnose were detected in all okra pectins and varied from 1.23 to 1.27 ppm and 1.19 to 1.35
258 ppm depending on the okra genotype used for pectin isolation. The low-field region at around
259 3.70-5.28 ppm contains typical signals of protons originating from D-GalA.

260 The ^{13}C NMR spectra of pectin from the different samples (**Figure 3b**) showed peaks
261 around 173 to 173.64 ppm indicative of the carbonyl group of galacturonic acid. Signals
262 occurring in the range of 50 to 53.04 ppm in the ^{13}C NMR spectra of the different okra pectins
263 confirmed the presence of a methyl group esterified to the carboxyl group of the galacturonic
264 acid. The $-\text{OCH}_3$ signal is similar to those observed for pectin from cacao pod husk (Vriesmann,
265 de Mello Castanho Amboni, & de Oliveira Petkowicz, 2011), cupuassu (Vriesmann & de
266 Oliveira Petkowicz, 2009) and white cabbage (Westereng, Michaelsen, Samuelsen, & Knutsen,
267 2008) and okra (Alba et al., 2015). Peaks in the range of 20.63 to 20.83 ppm can be assigned to
268 acetyl groups and the signals ranging from 16.91 to 18.39 ppm corresponds to the methyl groups
269 of rhamnose residue.

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272 3.5. Macromolecular characteristics

273 Molecular size of the isolated pectins were evaluated by size exclusion chromatography
274 (SEC) and weight average (M_w), number average (M_n) molecular weights, and polydispersity
275 index (M_w/M_n) were determined. Elution profiles of all samples revealed bimodal M_w
276 distributions representing populations of polymers of high and low molecular weights (elution
277 profiles not shown). The weight-average molecular weight (M_w) ranged from $791 \times 10^3 \text{ g mol}^{-1}$
278 (Balabi) to $1693 \times 10^3 \text{ g mol}^{-1}$ (Sengavi) (**Table 4**). The polydispersity indices (M_w/M_n) of the
279 macromolecular components of pectin ranged from 1.2 to 1.5 which was also reflected in the
280 distribution of the elution curves. The molecular weight was generally higher than previously
281 reported for pectin extracted by sequential extraction (Kontogiorgos, et al., 2012; Sengkhampan,
282 Sagis, de Vries, Schols, Sajjaanantakul, & Voragen, 2010; Sengkhampan, et al., 2009) but
283 comparable to extracts obtained with the same protocol (Alba, et al., 2015).

284 Intrinsic viscosity of the purified okra pectins from the different genotypes ranged from
285 2.91 to 5.10 dLg^{-1} (**Table 4**). The genotype Sengavi had the highest whereas Balabi had the
286 lowest intrinsic viscosity value in accordance with the M_w values. Intrinsic viscosity values of
287 all isolates are either consistent (Alba, et al., 2015; Ndjouenkeu, et al., 1996) or higher than those
288 obtained using sequential extraction methods (Kontogiorgos, et al., 2012) exemplifying the
289 influence of isolation protocol on the molecular structure of the samples. Huggins constant (K_H)
290 can be used to obtain information about the extent of coil expansion (Hesarinejad, Razavi, &
291 Koocheki, 2015). Values between $0.3 - 0.5$ reflect chains in good solvents, $0.5 - 0.8$ polymers in
292 theta solvents and values greater than 1 polymers in poor solvents with possible formation of
293 aggregates (Curvale, Masuelli, & Padilla, 2008; Irani, Razavi, Abdel-Aal, Hucl, & Patterson,
294 2016; Ma & Pawlik, 2007). K_H values calculated for okra pectins reveal that pH 7.0 with

295 addition of 0.1 M NaCl is a theta solvent (apart from Balabi) *i.e.*, pectin chains behave as ideal
296 adopting random coil conformations. A double logarithmic plot of η_{sp} versus $c[\eta]$ was
297 constructed in order to determine the critical coil overlap concentrations (c^* , c^{**}) *i.e.* the
298 transition from dilute to semi-dilute (c^*) and from semi-dilute to concentrated regime (c^{**}) of
299 the isolates. Double logarithmic plots of the polysaccharide extracts from the different okra
300 genotypes superimposed closely (**Figure 4**). Pectins in this study demonstrated three different
301 concentration regimes (dilute, semi-dilute and concentrated) and correspondingly showed three
302 different slopes (**Table 4**). Similar observations have been done in a rheological study of okra
303 gum, which demonstrated three regimes of concentration-dependence with slope 1 (1.17), slope
304 2 (2.1) and slope 3 (4.0) (Ndjouenkeu, et al., 1996). The transition at c^* can be associated with
305 the initial interaction between the individual coils of each polymer and has been noted to usually
306 occur when $c^*[\eta] \approx 1$ whereas $c^{**}[\eta]$ varies between 2 and 10 (Morris, Cutler, Ross-Murphy,
307 Rees, & Price, 1981). In the present investigation, results obtained are in agreement with the
308 above generalization although Asontem and Agbagoma exhibited somewhat lower $c^*[\eta]$ values.
309 Shear rate sweeps (0.01 to 1000 s^{-1}) reveal the viscosity dependence of okra pectin on shear rate
310 at different concentrations for all samples (**Figure 5a**). Almost all pectins exhibited a transition
311 from Newtonian to pseudoplastic flow behaviour at polymer concentration of about 1 % w/v,
312 except Balabi that becomes pseudoplastic at about 2 % w/v. Samples exhibited decreasing
313 viscosity in the order of Agbagoma > Sengavi > Asha > Penkruma > Asontem > Balabi (**Figure**
314 **5b**). Closer examination of this order reveals that viscosity is not a simple function of Mw. It is
315 difficult to draw a clear relationship between structure and viscosity but it appears that
316 galacturonic acid content and RG-I regions play central role in the solution behaviour in the
317 concentrated regimes of the biopolymers. To address the complexities of the present systems,

318 principal component analysis (PCA) was used that is able to reduce the data of multi-
319 dimensional data sets. The aim of a PCA is to obtain a small number of principal components
320 (PC), which explain the variation of the data. Consequently, it generates an orthogonal
321 transformation of the raw data, which is easier to interpret than original data (Lo, Jiang, Chao, &
322 Chang, 2007). Principal component analysis of the data emphasized differences between the okra
323 pectins by separately grouping pectins from the different genotypes (**Figure 6**). In the plot, PC1
324 and PC2 describe 41.4 and 21.8 % of the variation between the different genotypes, respectively
325 (PC3 and PC4 describe a further 19.0 and 13.4 % of the variation between the samples). PC1 is
326 positively correlated with DM, arabinose, galactose and DA and negatively correlated with
327 galacturonic acid, k_H , intrinsic viscosity and molecular weight. As a result, Balabi is the most
328 different from other samples due to its low molecular weight and intrinsic viscosity and high
329 degree of methyl esterification and RG-I content. PC2 is positively correlated with protein,
330 glucose, molecular weight and intrinsic viscosity and negatively correlated with c^{**} , yield
331 galacturonic acid, and k_H . Therefore, Sengavi appears in the upper left corner due to its high
332 molecular weight and high protein content whilst the scores of the other four samples are highly
333 correlated with their galacturonic acid contents and the Huggins constant, respectively. Overall,
334 pectins from different genotypes are not very close to one another in the plot owing to the
335 structural differences between them. The present results show that knowledge of genetic
336 diversity along with the structural features of the extracted pectin could inform future breeding
337 strategies with the aim to obtain pectins with tailored functional properties.

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341 **4. Conclusion**

342 In the present work, the structural features of pectin extracted from six okra genotypes
343 were investigated. Regardless of the genotype, extracted okra pectin had low degree of
344 methylation (*i.e.*, LM-pectin) and high degree of acetylation. Despite these common structural
345 similarities, sugar molar ratios and variability in the HG and RG-I domains revealed a range of
346 backbone structures. Genotypes Asontem, Agbagoma and Sengavi show extensive branching
347 compared to the other three genotypes. In Asha and Balabi the abundance of RG-I domains
348 translates to “hairy” pectins whereas Penkruma is essentially a “smooth” pectin. Dilute solution
349 viscometry revealed two critical concentrations for all samples and steady shear measurements
350 showed that viscosity is not a simple function of Mw but other structural features need to be
351 considered. Principal component analysis demonstrated that pectins from different genotypes
352 present dissimilarities in their structural features. Results of the present work show that with
353 selection of the appropriate okra genotype it is possible to tailor the properties of pectin
354 depending on the desired functionality.

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356 **Acknowledgements**

357 The authors are grateful for the financial support from the Robert S. McNamara Fellowships
358 Program (RSM) Award and KNUST Research Fund (KReF).

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361 **References**

- 362 Alamri, M. S., Mohamed, A. A., & Hussain, S. (2012). Effects of alkaline-soluble okra gum on
363 rheological and thermal properties of systems with wheat or corn starch. *Food Hydrocolloids*, *30*,
364 541-551.
- 365 Alba, K. (2015). *Isolation, characterization and functional properties of okra pectin* University of
366 Huddersfield, Huddersfield.
- 367 Alba, K., & Kontogiorgos, V. (2017). Pectin at the oil-water interface: Relationship of molecular
368 composition and structure to functionality. *Food Hydrocolloids*, doi:
369 10.1016/j.foodhyd.2016.1007.1026.
- 370 Alba, K., Laws, A. P., & Kontogiorgos, V. (2015). Isolation and characterisation of acetylated LM-
371 pectins extracted from okra pods. *Food Hydrocolloids*, *43*, 726-735.
- 372 Alba, K., Sagis, L. M. C., & Kontogiorgos, V. (2016). Engineering of acidic O/W emulsions with pectin.
373 *Colloids and Surfaces B: Biointerfaces*, *145*, 301-308.
- 374 Archana, G., Sabina, K., Babuskin, S., Radhakrishnan, K., Fayidh, M. A., Babu, P. A. S., Sivarajan, M.,
375 & Sukumar, M. (2013). Preparation and characterization of mucilage polysaccharide for
376 biomedical applications. *Carbohydrate Polymers*, *98*, 89-94.
- 377 Bleton, J., Mejanelle, P., Sansoulet, J., Goursaud, S., & Tchaplal, A. (1996). Characterization of neutral
378 sugars and uronic acids after methanolysis and trimethylsilylation for recognition of plant gums.
379 *Journal of Chromatography A*, *720*, 27-49.
- 380 Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of
381 protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248-254.
- 382 Chapman, H. D., Morris, V. J., Selvendran, R. R., & O'Neill, M. A. (1987). Static and dynamic light-
383 scattering studies of pectic polysaccharides from the middle lamellae and primary cell walls of
384 cider apples. *Carbohydrate Research*, *165*, 53-68.
- 385 Curvale, R., Masuelli, M., & Padilla, A. P. (2008). Intrinsic viscosity of bovine serum albumin
386 conformers. *International Journal of Biological Macromolecules*, *42*, 133-137.
- 387 Denman, L. J., & Morris, G. A. (2015). An experimental design approach to the chemical characterisation
388 of pectin polysaccharides extracted from *Cucumis melo Inodorus*. *Carbohydrate Polymers*, *117*,
389 364-369.
- 390 Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for
391 determination of sugars and related substances. *Analytical Chemistry*, *28*, 350-356.
- 392 Filisetti-Cozzi, T. M. C. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference
393 from neutral sugars. *Analytical Biochemistry*, *197*, 157-162.
- 394 Gálvez-López, D., Laurens, F., Devaux, M. F., & Lahaye, M. (2012). Texture analysis in an apple
395 progeny through instrumental, sensory and histological phenotyping. *Euphytica*, *185*, 171-183.
- 396 Georgiadis, N., Ritzoulis, C., Sioura, G., Kornezou, P., Vasiliadou, C., & Tsiptsias, C. (2011).
397 Contribution of okra extracts to the stability and rheology of oil-in-water emulsions. *Food*
398 *Hydrocolloids*, *25*, 991-999.
- 399 Ghori, M. U., Alba, K., Smith, A. M., Conway, B. R., & Kontogiorgos, V. (2014). Okra extracts in
400 pharmaceutical and food applications. *Food Hydrocolloids*, *42*, 342-347.
- 401 Ghori, M. U., Mohammad, M. A., Rudrangi, S. R. S., Fleming, L. T., Merchant, H. A., Smith, A. M., &
402 Conway, B. R. (2017). Impact of purification on physicochemical, surface and functional
403 properties of okra biopolymer. *Food Hydrocolloids*, In press.
- 404 Hesarinejad, M. A., Razavi, S. M. A., & Koocheki, A. (2015). *Alyssum homolocarpum* seed gum: Dilute
405 solution and some physicochemical properties. *International Journal of Biological*
406 *Macromolecules*, *81*, 418-426.
- 407 Houben, K., Jolie, R. P., Fraeye, I., Van Loey, A. M., & Hendrickx, M. E. (2011). Comparative study of
408 the cell wall composition of broccoli, carrot, and tomato: Structural characterization of the
409 extractable pectins and hemicelluloses. *Carbohydrate Research*, *346*, 1105-1111.

410 Huggins, M. L. (1942). The Viscosity of Dilute Solutions of Long-Chain Molecules. IV. Dependence on
411 Concentration. *Journal of the American Chemical Society*, *64*, 2716-2718.

412 Irani, M., Razavi, S. M. A., Abdel-Aal, E.-S. M., Hucl, P., & Patterson, C. A. (2016). Dilute solution
413 properties of canary seed (*Phalaris canariensis*) starch in comparison to wheat starch.
414 *International Journal of Biological Macromolecules*, *87*, 123-129.

415 Kontogiorgos, V., Margelou, I., Georgiadis, N., & Ritzoulis, C. (2012). Rheological characterization of
416 okra pectins. *Food Hydrocolloids*, *29*, 356-362.

417 Kosman, E., & Leonard, K. J. (2005). Similarity coefficients for molecular markers in studies of genetic
418 relationships between individuals for haploid, diploid, and polyploid species. *Molecular Ecology*,
419 *14*, 415-424.

420 Lahaye, M., Devaux, M. F., Poole, M., Seymour, G. B., & Causse, M. (2013). Pericarp tissue
421 microstructure and cell wall polysaccharide chemistry are differently affected in lines of tomato
422 with contrasted firmness. *Postharvest Biology and Technology*, *76*, 83-90.

423 Lahaye, M., Falourd, X., Quemener, B., Devaux, M.-F., & Audergon, J.-M. (2014). Histological and cell
424 wall polysaccharide chemical variability among apricot varieties. *LWT - Food Science and*
425 *Technology*, *58*, 486-496.

426 Lahaye, M., Falourd, X., Quemener, B., Ralet, M. C., Howad, W., Dirlewanger, E., & Arús, P. (2012).
427 Cell Wall Polysaccharide Chemistry of Peach Genotypes with Contrasted Textures and Other
428 Fruit Traits. *Journal of Agricultural and Food Chemistry*, *60*, 6594-6605.

429 Lo, T. C. T., Jiang, Y. H., Chao, A. L. J., & Chang, C. A. (2007). Use of statistical methods to find the
430 polysaccharide structural characteristics and the relationships between monosaccharide
431 composition ratio and macrophage stimulatory activity of regionally different strains of *Lentinula*
432 *edodes*. *Analytica Chimica Acta*, *584*, 50-56.

433 Ma, X., & Pawlik, M. (2007). Intrinsic viscosities and Huggins constants of guar gum in alkali metal
434 chloride solutions. *Carbohydrate Polymers*, *70*, 15-24.

435 Manrique, G. D., & Lajolo, F. M. (2002). FT-IR spectroscopy as a tool for measuring degree of methyl
436 esterification in pectins isolated from ripening papaya fruit. *Postharvest Biology and Technology*,
437 *25*, 99-107.

438 McComb, E. A., & McCready, R. M. (1957). Determination of Acetyl in Pectin and in Acetylated
439 Carbohydrate Polymers. *Analytical Chemistry*, *29*, 819-821.

440 Monsoor, M. A., Kalapathy, U., & Proctor, A. (2001). Determination of polygalacturonic acid content in
441 pectin extracts by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry*,
442 *74*, 233-238.

443 Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., & Price, J. (1981). Concentration and shear
444 rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydrate Polymers*, *1*,
445 5-21.

446 Morris, G. A., de al Torre, J. G., Ortega, A., Castile, J., Smith, A., & Harding, S. E. (2008). Molecular
447 flexibility of citrus pectins by combined sedimentation and viscosity analysis. *Food*
448 *Hydrocolloids*, *22*, 1435-1442.

449 Morris, G. A., Foster, T. J., & Harding, S. E. (2000). The effect of the degree of esterification on the
450 hydrodynamic properties of citrus pectin. *Food Hydrocolloids*, *14*, 227-235.

451 Ndjouenkeu, R., Goycoolea, F. M., Morris, E. R., & Akingbala, J. O. (1996). Rheology of okra (*Hibiscus*
452 *esculentus* L.) and dika nut (*Irvingia gabonensis*) polysaccharides. *Carbohydrate Polymers*, *29*,
453 263-269.

454 Pereira, P. H. F., Oliveira, T. Í. S., Rosa, M. F., Cavalcante, F. L., Moates, G. K., Wellner, N., Waldron,
455 K. W., & Azeredo, H. M. C. (2016). Pectin extraction from pomegranate peels with citric acid.
456 *International Journal of Biological Macromolecules*, *88*, 373-379.

457 Perrier, X., & Jacquemoud-Collet, J. P. (2006). DARwin software In.
458 Resources, I. B. f. P. G. (1991). *Report of an International Workshop on Okra Genetic Resources: Held*
459 *at National Bureau for Plant Genetic Resources (NBPGR), New Delhi, India, 8-12 October 1990:*
460 IBPGR.

- 461 Samavati, V. (2013). Polysaccharide extraction from *Abelmoschus esculentus*: Optimization by response
462 surface methodology. *Carbohydrate Polymers*, *95*, 588-597.
- 463 Schafleitner, R., Kumar, S., Lin, C.-y., Hegde, S. G., & Ebert, A. (2013). The okra (*Abelmoschus*
464 *esculentus*) transcriptome as a source for gene sequence information and molecular markers for
465 diversity analysis. *Gene*, *517*, 27-36.
- 466 Schultz, T. H. (1965). Determination of the degree of esterification of pectin: Determination of the ester
467 methoxyl content of pectin by saponification and titration. In L. R. Whistler (Ed.), *Methods in*
468 *carbohydrate chemistry* (pp. 189-194). New York: Academic Press.
- 469 Sengkhamparn, N., Sagis, L. M. C., de Vries, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J.
470 (2010). Physicochemical properties of pectins from okra (*Abelmoschus esculentus* (L.) Moench).
471 *Food Hydrocolloids*, *24*, 35-41.
- 472 Sengkhamparn, N., Verhoef, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2009).
473 Characterisation of cell wall polysaccharides from okra (*Abelmoschus esculentus* (L.) Moench).
474 *Carbohydrate Research*, *344*, 1824-1832.
- 475 Sun, X. F., Xu, F., Sun, R. C., Geng, Z. C., Fowler, P., & Baird, M. S. (2005). Characteristics of degraded
476 hemicellulosic polymers obtained from steam exploded wheat straw. *Carbohydrate Polymers*, *60*,
477 15-26.
- 478 Vriesmann, L. C., de Mello Castanho Amboni, R. D., & de Oliveira Petkowicz, C. L. (2011). Cacao pod
479 husks (*Theobroma cacao* L.): Composition and hot-water-soluble pectins. *Industrial Crops and*
480 *Products*, *34*, 1173-1181.
- 481 Vriesmann, L. C., & de Oliveira Petkowicz, C. L. (2009). Polysaccharides from the pulp of cupuassu
482 (*Theobroma grandiflorum*): Structural characterization of a pectic fraction. *Carbohydrate*
483 *Polymers*, *77*, 72-79.
- 484 Wang, W., Ma, X., Jiang, P., Hu, L., Zhi, Z., Chen, J., Ding, T., Ye, X., & Liu, D. (2016).
485 Characterization of pectin from grapefruit peel: A comparison of ultrasound-assisted and
486 conventional heating extractions. *Food Hydrocolloids*, *61*, 730-739.
- 487 Westereng, B., Michaelsen, T. E., Samuelsen, A. B., & Knutsen, S. H. (2008). Effects of extraction
488 conditions on the chemical structure and biological activity of white cabbage pectin.
489 *Carbohydrate Polymers*, *72*, 32-42.
- 490 Woolfe, M. L., Chaplin, M. F., & Otchere, G. (1977). Studies on the mucilages extracted from okra fruits
491 (*Hibiscus esculentus* L.) and baobab leaves (*Adansonia digitata* L.) *Journal of the Science of*
492 *Food and Agriculture*, *28*, 519-529.
- 493 Zhang, P., Dong, S.-J., Ma, H.-H., Zhang, B.-X., Wang, Y.-F., & Hu, X.-M. (2015). Fractionation of corn
494 stover into cellulose, hemicellulose and lignin using a series of ionic liquids. *Industrial Crops and*
495 *Products*, *76*, 688-696.
- 496 Zheng, W., Zhao, T., Feng, W., Wang, W., Zou, Y., Zheng, D., Takase, M., Li, Q., Wu, H., Yang, L., &
497 Wu, X. (2014). Purification, characterization and immunomodulating activity of a polysaccharide
498 from flowers of *Abelmoschus esculentus*. *Carbohydrate Polymers*, *106*, 335-342.

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504 **FIGURE CAPTIONS**

505 **Figure 1:** Unrooted phylogenetic tree based on 41 alleles produced by 10 SSR markers. Tree
506 calculated using Jaccard's dissimilarity coefficient and the UPGMA clustering method. Figure
507 generated using FigTree (<http://tree.bio.ed.ac.uk>).

508 **Figure 2:** FT-IR spectra of pectin from all six different okra genotypes.

509 **Figure 3:** Typical a) ^1H -NMR, and b) ^{13}C -NMR spectra of okra pectin for all samples. Figure
510 shows spectra for the Balabi genotype.

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512 **Figure 4:** Double logarithmic plots of zero shear specific viscosity ($(\eta_{sp})_0$) against reduced
513 concentration ($c[\eta]$) for all samples. c^* is the concentration that demarcates the transition from
514 dilute to semi-dilute whereas c^{**} from semi-dilute to concentrated regime of the biopolymers.
515 The lines serve solely as a guide to the eye.

516 **Figure 5:** Viscosity dependence on shear rate of pectins from: (a) Asontem, and (b) comparison
517 of all samples at 4 % w/v.

518 **Figure 6:** Principal component analysis (PCA) plot for okra pectins from different genotypes.

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528 **Table 1:** Forward and reverse oligonucleotide sequences used for FLA. Sequences based on
 529 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	ACGAGAGTGAAGTGGAAGT	CTCCTCTTTCCTTTTTCCAT	3
AVRDC-Okra28	CCTCTTCATCCATCTTTTCA	GGAAGATGCTGTGAAGGTAG	3
AVRDC-Okra39	TGAGGTGATGATGTGAGAGA	TTGTAGATGAGGTTTGAACG	4
AVRDC-Okra52	AACACATCCTCATCCTCATC	ACCGGAAGCTATTTACATGA	4
AVRDC-Okra54	CGAAAAGGAAACTCAACAAC	TGAACCTTATTTTCCTCGTG	2
AVRDC-Okra56	GGCAACTTCGTAATTCCTA	TGAGTAAAAGTGGGGTCTGT	7
AVRDC-Okra64	AAGGAGGAGAAAGAGAAGGA	ATTTACTTGAGCAGCAGCAG	7
AVRDC-Okra89	TTTGAGTTCTTTCGTCCACT	GTATTTGGACATGGCGTTAT	3

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540 **Table 2:** Yield and chemical characteristics of isolated okra pectins (% dry weight basis)

Genotype	Pectin Yield	Protein	Total Carbohydrate	Methoxyl	D-GalA	Acetyl	DM	DA
Asha	14.6±1.2 ^a	5.5±3.1 ^a	86.3 ±2.0 ^a	1.92±0.01 ^b	63.4±1.1 ^a (64.7)	6.1±0.1 ^a	17.2±1.4 ^a	39.3±4.3 ^a
Penkruma	14.4±0.1 ^a	4.4±1.4 ^a	87.4 ±1.0 ^a	1.87±0.01 ^a	62.4±4.7 ^a (78.6)	3.0±0.2 ^d	17.0±0.1 ^a	19.9±0.5 ^c
Asontem	13.2±0.6 ^a	3.8±1.8 ^a	72.5 ±2.5 ^b	1.94±0.01 ^c	54.2±4.6 ^b (70.4)	5.3±0.3 ^b	20.4±1.8 ^{bc}	40.1±5.7 ^a
Agbagoma	14.2±0.1 ^a	5.4±2.8 ^a	66.2 ±1.0 ^b	1.91±0.01 ^b	51.9±3.4 ^b (73.1)	4.0±0.2 ^c	20.9 ±1.8 ^c	31.7±5.5 ^b
Sengavi	11.3±0.1 ^b	7.1±2.4 ^a	66.2 ±4.3 ^b	1.92±0.01 ^b	59.2±1.0 ^{ab} (71.9)	3.2±0.1 ^d	18.4±1.4 ^{ab}	22.4±3.3 ^c
Balabi	11.4±1.3 ^b	3.3±1.0 ^a	87.5 ±3.5 ^a	1.92±0.01 ^b	42.8 ±1.3 ^c (63.5)	3.9±0.1 ^c	25.5 ±1.8 ^d	37.9±5.8 ^{ab}

541 Numbers in parentheses indicate mol% of D-GalA. Means sharing the same letters in a column
 542 are not-significantly different ($p>0.05$)

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554 **Table 3:** Percentage neutral sugar composition on wet basis of pectin powder. In parentheses are
 555 mol% of each sugar. Sugar molar ratios of okra pectins are also shown as $R_1 = \text{GalA}/(\text{Rha} + \text{Ara}$
 556 $+ \text{Gal})$; $R_2 = \text{Rha}/\text{GalA}$; $R_3 = (\text{Ara} + \text{Gal})/\text{Rha}$; $R_4 = \text{Gal}/\text{Rha}$; $\text{HG} = \text{GalA} - \text{Rha}$; $\text{RG-I} = 2\text{Rha} +$
 557 $\text{Ara} + \text{Gal}$.

Genotype	D-Gal	L-Rha	L-Ara	D-Glc	R_1	R_2	R_3	R_4	HG	RG-I	HG/RG-I
Asha	16.6±0.3 (18.4)	8.9±1.5 (11.0)	1.7±0.4 (2.3)	0.8±0.1 (0.9)	2.0	0.17	1.9	1.7	53.7	42.7	1.3
Penkruma	5.9±1.4 (8.1)	4.3±1.5 (6.5)	1.5±0.5 (2.5)	0.7±0.1 (1.0)	4.6	0.08	1.6	1.2	72.1	23.6	3.1
Asontem	12.0±4.9 (16.9)	3.5±1.0 (5.5)	1.1±0.1 (1.9)	1.3±0.4 (1.8)	2.9	0.08	3.4	3.1	64.9	29.8	2.2
Agbagoma	10.4±2.3 (15.9)	2.7±1.9 (4.6)	1.1±0.1 (2.1)	0.4±0.1 (0.6)	3.2	0.06	3.9	3.5	68.5	27.2	2.5
Sengavi	11.2±0.5 (14.8)	4.0±1.7 (5.9)	1.2±0.3 (1.9)	1.7±0.5 (2.2)	3.2	0.08	2.8	2.5	66.0	28.5	2.3
Balabi	12.0±2.2 (19.3)	5.2±1.2 (9.3)	1.6±0.4 (3.2)	0.5±0.1 (0.8)	2.0	0.15	2.4	2.1	54.2	41.1	1.3

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567 **Table 4:** Molecular characteristics of isolated pectins from different okra genotypes

Genotypes	$[\eta]$ (dLg ⁻¹)	K_H	Slope			c^* (gdL ⁻¹)	c^{**} (gdL ⁻¹)	$c^*[\eta]$	$c^{**}[\eta]$	M_w (x 10 ³ gmol ⁻¹)	M_w/M_n
			1	2	3						
Asha	4.35	0.66	0.46	2.54	4.55	0.18	1.34	0.80	5.83	1202	1.34
Penkruma	4.35	0.73	0.55	2.07	5.52	0.17	1.37	0.72	5.95	893	1.20
Asontem	3.55	0.69	0.43	1.43	4.55	0.07	1.51	0.24	5.37	1233	1.35
Agbagoma	3.56	0.69	0.41	1.38	6.14	0.06	1.04	0.21	3.72	1419	1.18
Sengavi	5.10	0.55	0.46	2.32	5.65	0.18	1.65	0.90	8.39	1693	1.41
Balabi	2.91	0.37	0.48	1.45	3.35	0.15	1.94	0.44	5.65	791	1.50

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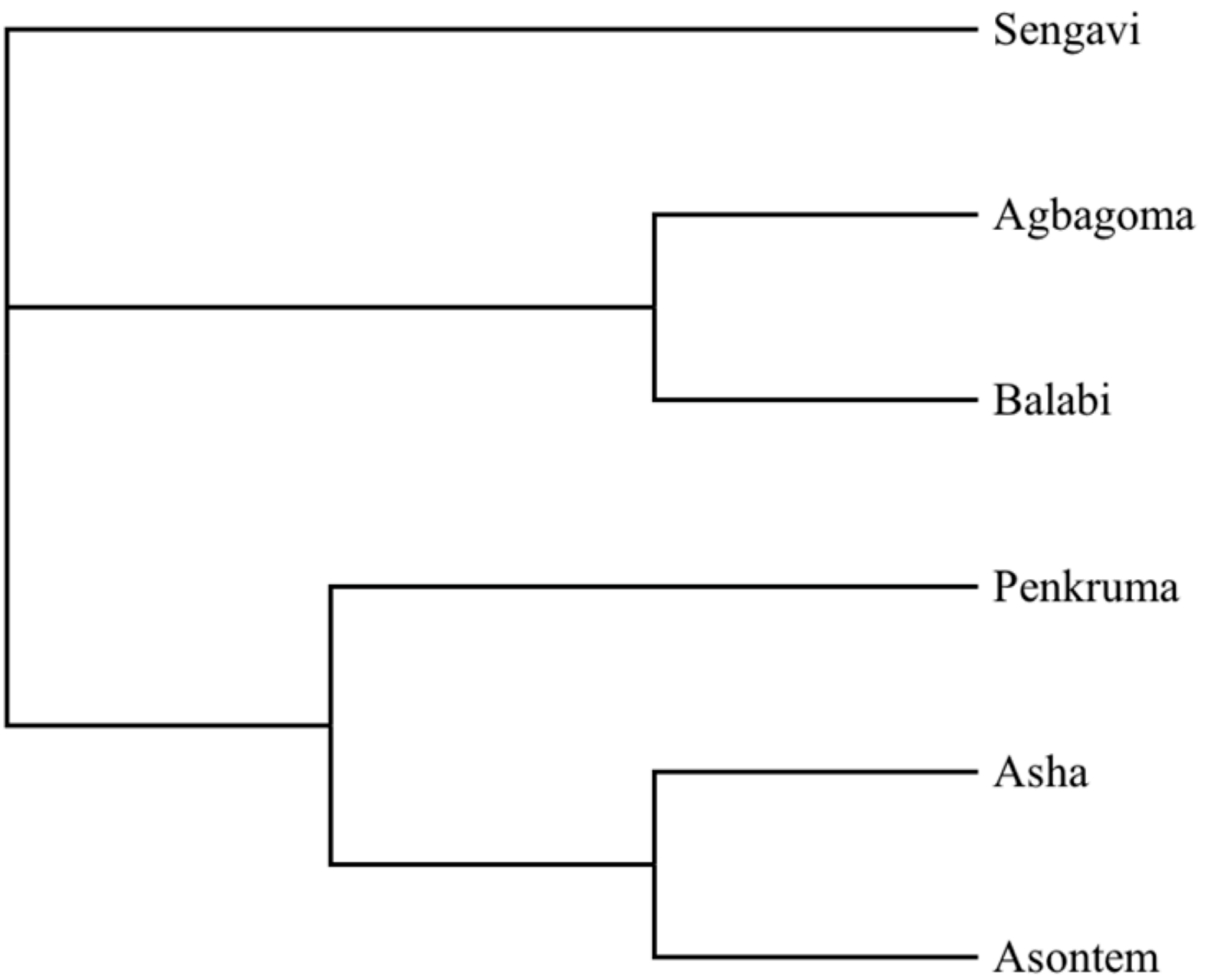
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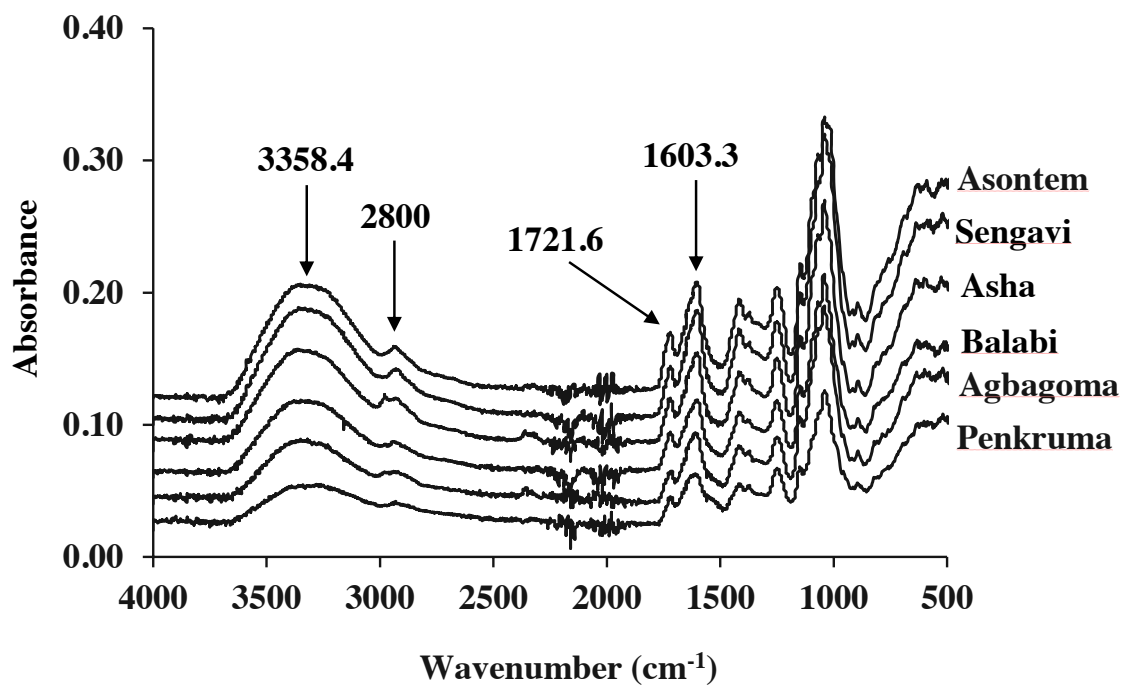
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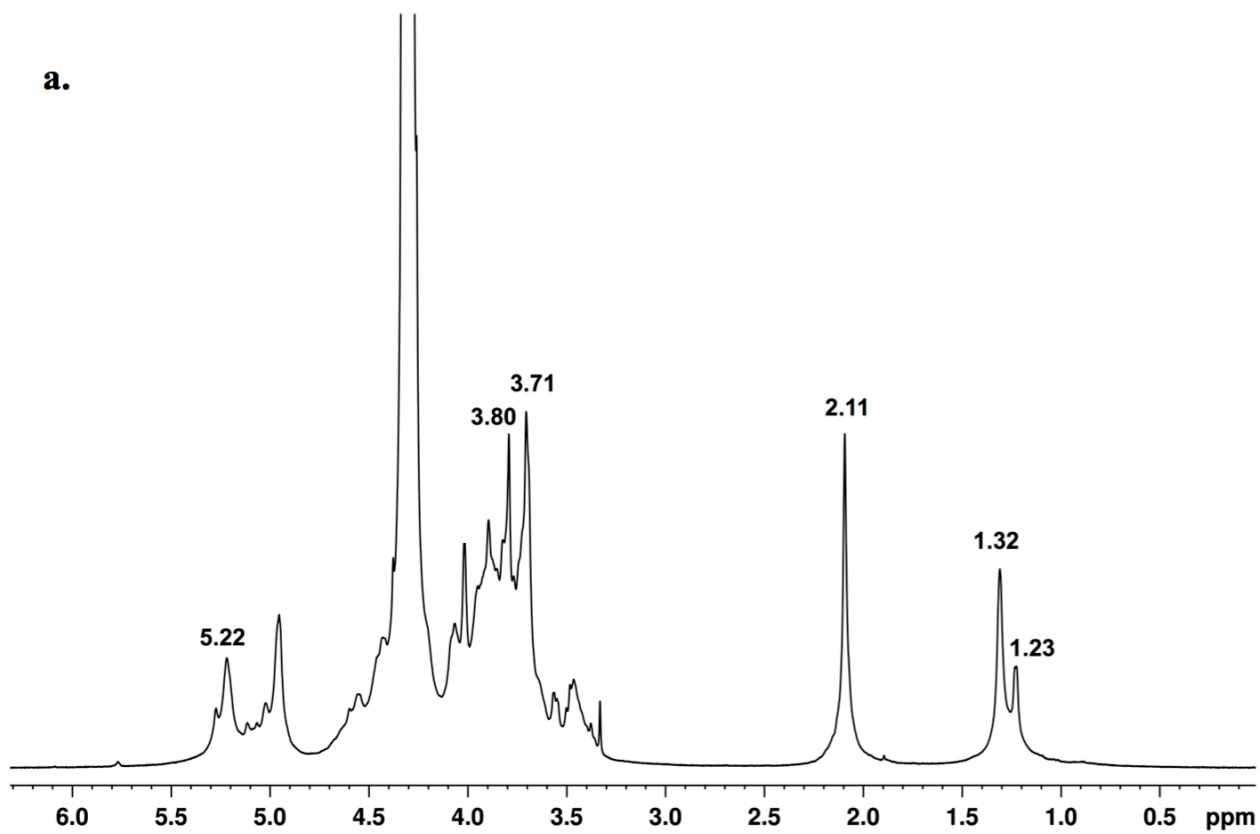
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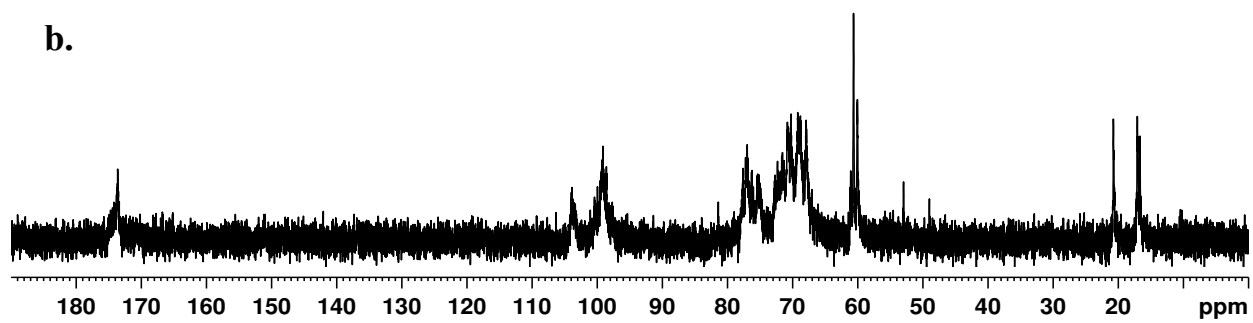


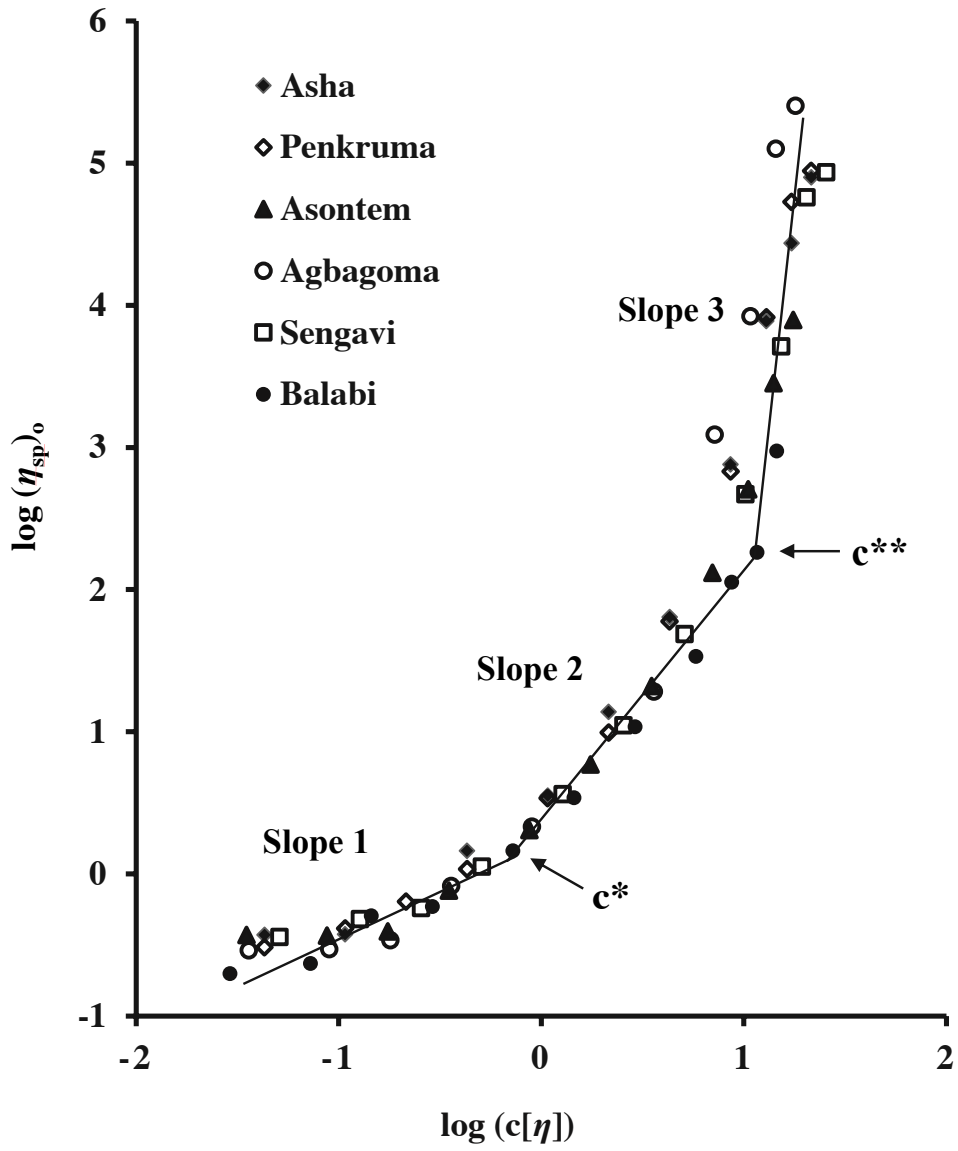


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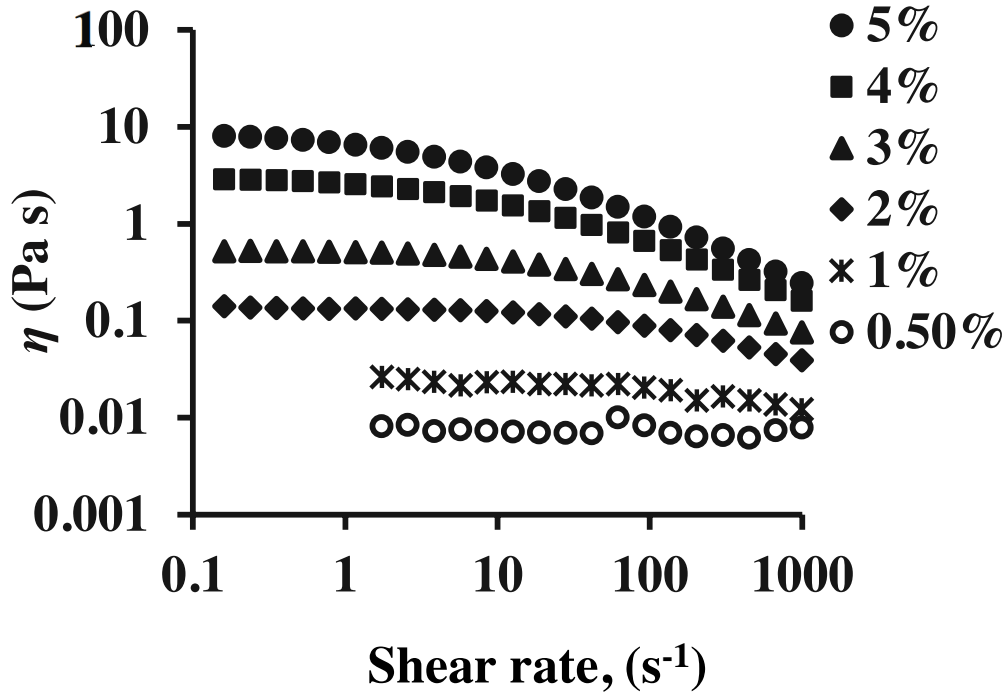


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