Pectin isolation and characterization from six okra genotypes

F. M. Kpodo[a,b], J. K. Agbenorhevi[a], K. Alba[c], R. J. Bingham[c], I. N. Oduro[a] , G. A. Morris[d] and V. Kontogiorgos[c*]

[a]*Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[b]*Department of Nutrition and Dietetics, University of Health and Allied Sciences, Ho, Ghana*

[c]*Department of Biological Sciences, University of Huddersfield, HD1 3DH, UK*

[d] *Department of Chemical Sciences, University of Huddersfield, HD1 3DH, UK*

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 11–14 % resulting in pectin with galacturonic acid content between 43–63 %, 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 x 10^3 $gmol^{-1}$) 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.

Keywords: okra; isolation; characterization; genotypes; pectin

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, Ritzoulis, Sioura, Kornezou, Vasiliadou, & Tsioptsias, 2011; Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014; Ghori, Mohammad, Rudrangi, Fleming, Merchant, Smith, & Conway, 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, Sabina, Babuskin, Radhakrishnan, Fayidh, Babu, Sivarajan, & Sukumar, 2013; Georgiadis, et al., 2011; Samavati, 2013; Sengkhamparn, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009; Woolfe, Chaplin, & Otchere, 1977; Zheng, Zhao, Feng, Wang, Zou, Zheng, Takase, Li, Wu, Yang, & Wu, 2014). Isolated okra pectins are rich in rhamnogalacturonan-I (RG-I) segments with varying composition of side chains and molecular weights ranging from $10 - 767 \times 10^3$ 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, Quemener, Devaux, & Audergon, 2014; Lahaye, Falourd, Quemener, Ralet, Howad, Dirlewanger, & Arús, 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 ^{o}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 (Perrier, 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:

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

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, & Tchapla, 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

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 12800 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.

2.5. Molecular weight determination

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

2.6. Dilute solution viscometry and steady shear rheology

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

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

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

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

3. Results and discussion

3.1. Genetic diversity of okra samples

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

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

3.2. Chemical characterisation of okra pectins

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

rhamnose and arabinose (**Table 3**). Low glucose content indicates that the present isolation protocol results in pectin isolates with low amounts of co-extracted hemicelluloses, cellulosic oligomers or starch. The sugar molar ratios for the different pectins were calculated based on the sugar content determined experimentally. There are a number of different molar ratios (R_1 – R_4), which can be calculated from the monosaccharide composition (Denman & Morris, 2015; Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011) to reveal important structural information of pectins (**Table 3**). R_1 is the ratio of D-GalA to the neutral sugars that are present on the side chains and gives an estimate of the linearity of pectin (**Table 3**). Penkruma genotype was distinctively more linear compared to the rest of the samples. This is also confirmed by ratios R_2 and R_3 that represent the contribution of RG-I segments to the structure. In particular, greater R_2 values reveal greater proportion of RG-I chains whereas high R_3 values indicate greater branching of the RG-I segments. Asontem, Agbagoma and Sengavi show extensive branching compared to the other three samples. In addition to this, high R_4 values indicates long branches something that is particularly evident for samples Asontem and Agbagoma. The HG molar ratio of the okra pectins studied ranged from 53.7 to 72.1 % whereas the RG-I varied between 23.6 to 42.7 %. Penkruma is essentially a "smooth" pectin due to the predominance of HG regions in the structure. On the contrary, in genotypes Asha and Balabi the presence of RG-I units is particularly high revealing the abundance of "hairy" regions in these samples. These results show that it is possible to tailor the structure of the extracted pectin by selecting the appropriate genotype and create LM-pectins with an extensive spectrum of functionalities. As mentioned earlier, okra pectins have not shown potential as gelling agents due to extensive branching and 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, CH_2 and CH_3 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 CH_2, OH and $–CH_3CO$
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.

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

The ^{13}C NMR spectra of pectin from the different samples (**Figure 3b**) showed peaks around 173 to 173.64 ppm indicative of the carbonyl group of galacturonic acid. Signals occurring in the range of 50 to 53.04 ppm in the ^{13}C NMR spectra of the different okra pectins confirmed the presence of a methyl group esterified to the carboxyl group of the galacturonic acid. The $-OCH_3$ signal is similar to those observed for pectin from cacao pod husk (Vriesmann, de Mello Castanho Amboni, & de Oliveira Petkowicz, 2011), cupuassu (Vriesmann & de Oliveira Petkowicz, 2009) and white cabbage (Westereng, Michaelsen, Samuelsen, & Knutsen, 2008) and okra (Alba et al., 2015). Peaks in the range of 20.63 to 20.83 ppm can be assigned to acetyl groups and the signals ranging from 16.91 to 18.39 ppm corresponds to the methyl groups of rhamnose residue.

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 Mw
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 $x10^3$ g mol^{-1}
278 (Balabi) to 1693 $x10^3$ 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; Sengkhamparn,
282 Sagis, de Vries, Schols, Sajjaanantakul, & Voragen, 2010; Sengkhamparn, 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 Mw 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

addition of 0.1 M NaCl is a theta solvent (apart from Balabi) *i.e.,* pectin chains behave as ideal adopting random coil conformations. A double logarithmic plot of η_{sp} *versus* $c[\eta]$ was constructed in order to determine the critical coil overlap concentrations (c*, c**) *i.e.* the transition from dilute to semi-dilute (c*) and from semi-dilute to concentrated regime (c**) of the isolates. Double logarithmic plots of the polysaccharide extracts from the different okra genotypes superimposed closely (**Figure 4**). Pectins in this study demonstrated three different concentration regimes (dilute, semi-dilute and concentrated) and correspondingly showed three different slopes (**Table 4**). Similar observations have been done in a rheological study of okra gum, which demonstrated three regimes of concentration-dependence with slope 1 (1.17), slope 2 (2.1) and slope 3 (4.0) (Ndjouenkeu, et al., 1996). The transition at c* can be associated with the initial interaction between the individual coils of each polymer and has been noted to usually occur when $c^*[\eta] \approx 1$ whereas $c^{**}[\eta]$ varies between 2 and 10 (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). In the present investigation, results obtained are in agreement with the above generalization although Asontem and Agbagoma exhibited somewhat lower $c^*[\eta]$ values. Shear rate sweeps (0.01 to 1000 s^{-1}) reveal the viscosity dependence of okra pectin on shear rate at different concentrations for all samples (**Figure 5a**). Almost all pectins exhibited a transition from Newtonian to pseudoplastic flow behaviour at polymer concentration of about 1 % w/v, except Balabi that becomes pseudoplastic at about 2 % w/v. Samples exhibited decreasing viscosity in the order of Agbagoma > Sengavi > Asha > Penkruma > Asontem > Balabi **(Figure 5b)**. Closer examination of this order reveals that viscosity is not a simple function of Mw. It is difficult to draw a clear relationship between structure and viscosity but it appears that galacturonic acid content and RG-I regions play central role in the solution behaviour in the 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.
338
339
340

4. Conclusion

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

Acknowledgements

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

References

Alamri, M. S., Mohamed, A. A., & Hussain, S. (2012). Effects of alkaline-soluble okra gum on rheological and thermal properties of systems with wheat or corn starch. *Food Hydrocolloids, 30*, 541-551.

Alba, K. (2015). *Isolation, characterization and functional properties of okra pectin* University of Huddersfield, Huddersfield.

Alba, K., & Kontogiorgos, V. (2017). Pectin at the oil-water interface: Relationship of molecular composition and structure to functionality. *Food Hydrocolloids*, doi: 10.1016/j.foodhyd.2016.1007.1026.

Alba, K., Laws, A. P., & Kontogiorgos, V. (2015). Isolation and characterisation of acetylated LM-pectins extracted from okra pods. *Food Hydrocoilloids, 43*, 726-735.

Alba, K., Sagis, L. M. C., & Kontogiorgos, V. (2016). Engineering of acidic O/W emulsions with pectin. *Colloids and Surfaces B: Biointerfaces, 145*, 301-308.

Archana, G., Sabina, K., Babuskin, S., Radhakrishnan, K., Fayidh, M. A., Babu, P. A. S., Sivarajan, M., & Sukumar, M. (2013). Preparation and characterization of mucilage polysaccharide for biomedical applications. *Carbohydrate Polymers, 98*, 89-94.

Bleton, J., Mejanelle, P., Sansoulet, J., Goursaud, S., & Tchapla, A. (1996). Characterization of neutral sugars and uronic acids after methanolysis and trimethylsilylation for recognition of plant gums. *Journal of Chromatography A, 720*, 27-49.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248-254.

Chapman, H. D., Morris, V. J., Selvendran, R. R., & O'Neill, M. A. (1987). Static and dynamic light-scattering studies of pectic polysaccharides from the middle lamellae and primary cell walls of cider apples. *Carbohydrate Research, 165*, 53-68.

Curvale, R., Masuelli, M., & Padilla, A. P. (2008). Intrinsic viscosity of bovine serum albumin conformers. *International Journal of Biological Macromolecules, 42*, 133-137.

Denman, L. J., & Morris, G. A. (2015). An experimental design approach to the chemical characterisation of pectin polysaccharides extracted from Cucumis melo Inodorus. *Carbohydrate Polymers, 117*, 364-369.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350-356.

Filisetti-Cozzi, T. M. C. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry, 197*, 157-162.

Gálvez-López, D., Laurens, F., Devaux, M. F., & Lahaye, M. (2012). Texture analysis in an apple progeny through instrumental, sensory and histological phenotyping. *Euphytica, 185*, 171-183.

Georgiadis, N., Ritzoulis, C., Sioura, G., Kornezou, P., Vasiliadou, C., & Tsioptsias, C. (2011). Contribution of okra extracts to the stability and rheology of oil-in-water emulsions. *Food Hydrocolloids, 25*, 991-999.

Ghori, M. U., Alba, K., Smith, A. M., Conway, B. R., & Kontogiorgos, V. (2014). Okra extracts in pharmaceutical and food applications. *Food Hydrocolloids, 42*, 342-347.

Ghori, M. U., Mohammad, M. A., Rudrangi, S. R. S., Fleming, L. T., Merchant, H. A., Smith, A. M., & Conway, B. R. (2017). Impact of purification on physicochemical, surface and functional properties of okra biopolymer. *Food Hydrocolloids*, In press.

Hesarinejad, M. A., Razavi, S. M. A., & Koocheki, A. (2015). Alyssum homolocarpum seed gum: Dilute solution and some physicochemical properties. *International Journal of Biological Macromolecules, 81*, 418-426.

Houben, K., Jolie, R. P., Fraeye, I., Van Loey, A. M., & Hendrickx, M. E. (2011). Comparative study of the cell wall composition of broccoli, carrot, and tomato: Structural characterization of the extractable pectins and hemicelluloses. *Carbohydrate Research, 346*, 1105-1111.

Huggins, M. L. (1942). The Viscosity of Dilute Solutions of Long-Chain Molecules. IV. Dependence on Concentration. *Journal of the American Chemical Society, 64*, 2716-2718.
Irani, M., Razavi, S. M. A., Abdel-Aal, E.-S. M., Hucl, P., & Patterson, C. A. (2016). Dilute solution properties of canary seed (Phalaris canariensis) starch in comparison to wheat starch. *International Journal of Biological Macromolecules, 87*, 123-129.
Kontogiorgos, V., Margelou, I., Georgiadis, N., & Ritzoulis, C. (2012). Rheological characterization of okra pectins. *Food Hydrocolloids, 29*, 356-362.
Kosman, E., & Leonard, K. J. (2005). Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. *Molecular Ecology, 14*, 415-424.
Lahaye, M., Devaux, M. F., Poole, M., Seymour, G. B., & Causse, M. (2013). Pericarp tissue microstructure and cell wall polysaccharide chemistry are differently affected in lines of tomato with contrasted firmness. *Postharvest Biology and Technology, 76*, 83-90.
Lahaye, M., Falourd, X., Quemener, B., Devaux, M.-F., & Audergon, J.-M. (2014). Histological and cell wall polysaccharide chemical variability among apricot varieties. *LWT - Food Science and Technology, 58*, 486-496.
Lahaye, M., Falourd, X., Quemener, B., Ralet, M. C., Howad, W., Dirlewanger, E., & Arús, P. (2012). Cell Wall Polysaccharide Chemistry of Peach Genotypes with Contrasted Textures and Other Fruit Traits. *Journal of Agricultural and Food Chemistry, 60*, 6594-6605.
Lo, T. C. T., Jiang, Y. H., Chao, A. L. J., & Chang, C. A. (2007). Use of statistical methods to find the polysaccharide structural characteristics and the relationships between monosaccharide composition ratio and macrophage stimulatory activity of regionally different strains of Lentinula edodes. *Analytica Chimica Acta, 584*, 50-56.
Ma, X., & Pawlik, M. (2007). Intrinsic viscosities and Huggins constants of guar gum in alkali metal chloride solutions. *Carbohydrate Polymers, 70*, 15-24.
Manrique, G. D., & Lajolo, F. M. (2002). FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biology and Technology, 25*, 99-107.
McComb, E. A., & McCready, R. M. (1957). Determination of Acetyl in Pectin and in Acetylated Carbohydrate Polymers. *Analytical Chemistry, 29*, 819-821.
Monsoor, M. A., Kalapathy, U., & Proctor, A. (2001). Determination of polygalacturonic acid content in pectin extracts by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry, 74*, 233-238.
Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., & Price, J. (1981). Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydrate Polymers, 1*, 5-21.
Morris, G. A., de al Torre, J. G., Ortega, A., Castile, J., Smith, A., & Harding, S. E. (2008). Molecular flexibility of citrus pectins by combined sedimentation and viscosity analysis. *Food Hydrocolloids, 22*, 1435-1442.
Morris, G. A., Foster, T. J., & Harding, S. E. (2000). The effect of the degree of esterification on the hydrodynamic properties of citrus pectin. *Food Hydrocolloids, 14*, 227-235.
Ndjouenkeu, R., Goycoolea, F. M., Morris, E. R., & Akingbala, J. O. (1996). Rheology of okra (Hibiscus esculentus L.) and dika nut (Irvingia gabonensis) polysaccharides. *Carbohydrate Polymers, 29*, 263-269.
Pereira, P. H. F., Oliveira, T. Í. S., Rosa, M. F., Cavalcante, F. L., Moates, G. K., Wellner, N., Waldron, K. W., & Azeredo, H. M. C. (2016). Pectin extraction from pomegranate peels with citric acid. *International Journal of Biological Macromolecules, 88*, 373-379.
Perrier, X., & Jacquemoud-Collet, J. P. (2006). DARwin software In.
Resources, I. B. f. P. G. (1991). *Report of an International Workshop on Okra Genetic Resources: Held at National Bureau for Plant Genetic Resources (NBPGR), New Delhi, India, 8-12 October 1990*: IBPGR.

Samavati, V. (2013). Polysaccharide extraction from Abelmoschus esculentus: Optimization by response surface methodology. *Carbohydrate Polymers, 95*, 588-597.

Schafleitner, R., Kumar, S., Lin, C.-y., Hegde, S. G., & Ebert, A. (2013). The okra (Abelmoschus esculentus) transcriptome as a source for gene sequence information and molecular markers for diversity analysis. *Gene, 517*, 27-36.

Schultz, T. H. (1965). Determination of the degree of esterification of pectin: Determination of the ester methoxyl content of pectin by saponification andtitration. In L. R. Whistler (Ed.), *Methods in carbohydrate chemistry* (pp. 189-194). New York: Academic Press.

Sengkhamparn, N., Sagis, L. M. C., de Vries, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2010). Physicochemical properties of pectins from okra (Abelmoschus esculentus (L.) Moench). *Food Hydrocolloids, 24*, 35-41.

Sengkhamparn, N., Verhoef, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2009). Characterisation of cell wall polysaccharides from okra (Abelmoschus esculentus (L.) Moench). *Carbohydrate Research, 344*, 1824-1832.

Sun, X. F., Xu, F., Sun, R. C., Geng, Z. C., Fowler, P., & Baird, M. S. (2005). Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. *Carbohydrate Polymers, 60*, 15-26.

Vriesmann, L. C., de Mello Castanho Amboni, R. D., & de Oliveira Petkowicz, C. L. (2011). Cacao pod husks (Theobroma cacao L.): Composition and hot-water-soluble pectins. *Industrial Crops and Products, 34*, 1173-1181.

Vriesmann, L. C., & de Oliveira Petkowicz, C. L. (2009). Polysaccharides from the pulp of cupuassu (Theobroma grandiflorum): Structural characterization of a pectic fraction. *Carbohydrate Polymers, 77*, 72-79.

Wang, W., Ma, X., Jiang, P., Hu, L., Zhi, Z., Chen, J., Ding, T., Ye, X., & Liu, D. (2016). Characterization of pectin from grapefruit peel: A comparison of ultrasound-assisted and conventional heating extractions. *Food Hydrocolloids, 61*, 730-739.

Westereng, B., Michaelsen, T. E., Samuelsen, A. B., & Knutsen, S. H. (2008). Effects of extraction conditions on the chemical structure and biological activity of white cabbage pectin. *Carbohydrate Polymers, 72*, 32-42.

Woolfe, M. L., Chaplin, M. F., & Otchere, G. (1977). Studies on the mucilages extracted from okra fruits (Hibiscus esculentus L.) and baobab leaves (Adansonia digitata L.) *Journal of the Science of Food and Agriculture, 28*, 519-529.

Zhang, P., Dong, S.-J., Ma, H.-H., Zhang, B.-X., Wang, Y.-F., & Hu, X.-M. (2015). Fractionation of corn stover into cellulose, hemicellulose and lignin using a series of ionic liquids. *Industrial Crops and Products, 76*, 688-696.

Zheng, W., Zhao, T., Feng, W., Wang, W., Zou, Y., Zheng, D., Takase, M., Li, Q., Wu, H., Yang, L., & Wu, X. (2014). Purification, characterization and immunomodulating activity of a polysaccharide from flowers of Abelmoschus esculentus. *Carbohydrate Polymers, 106*, 335-342.

FIGURE CAPTIONS

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

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

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

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

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

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

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

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}

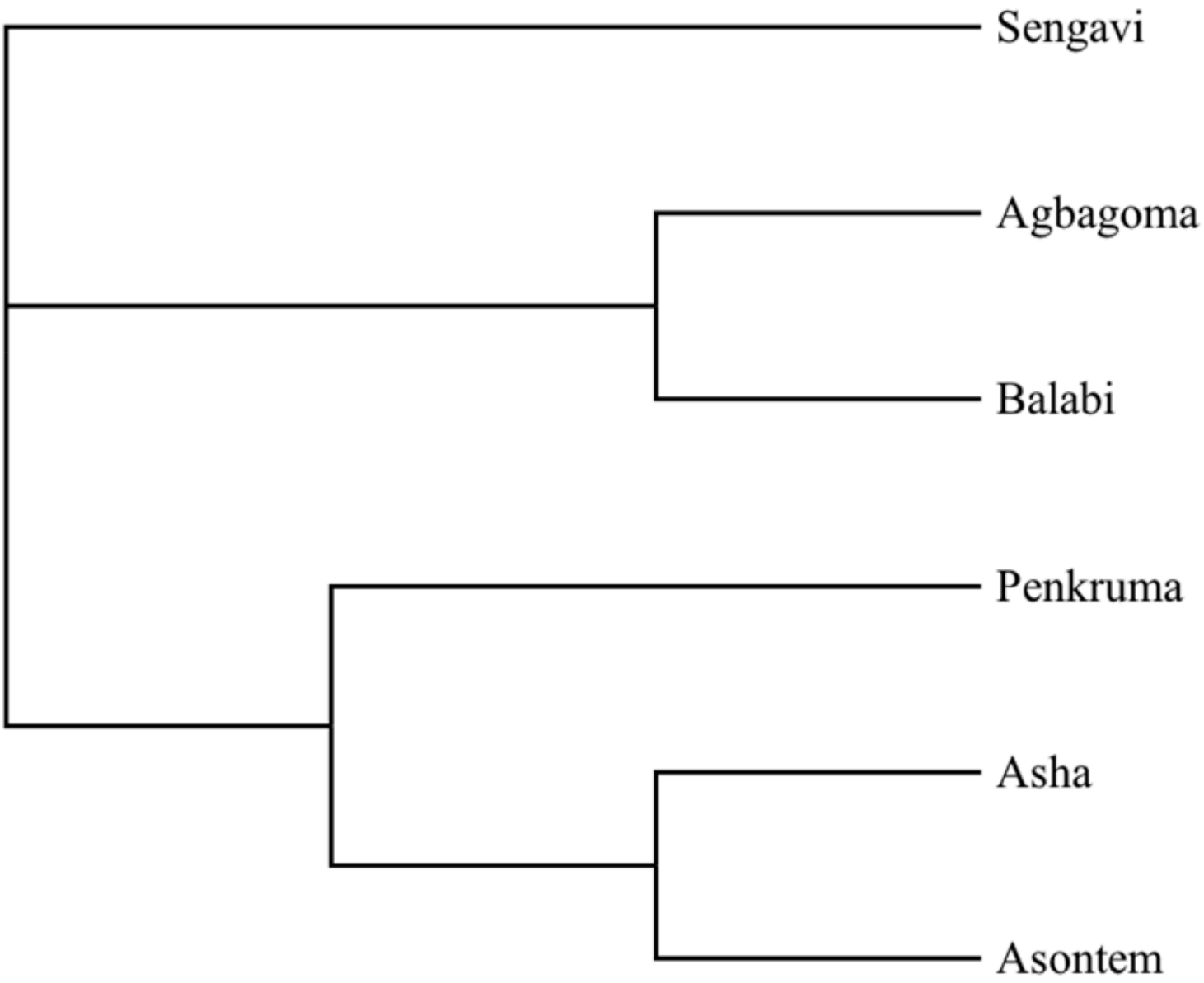
Numbers in parentheses indicate mol% of D-GalA. Means sharing the same letters in a column are not-significantly different (p>0.05)

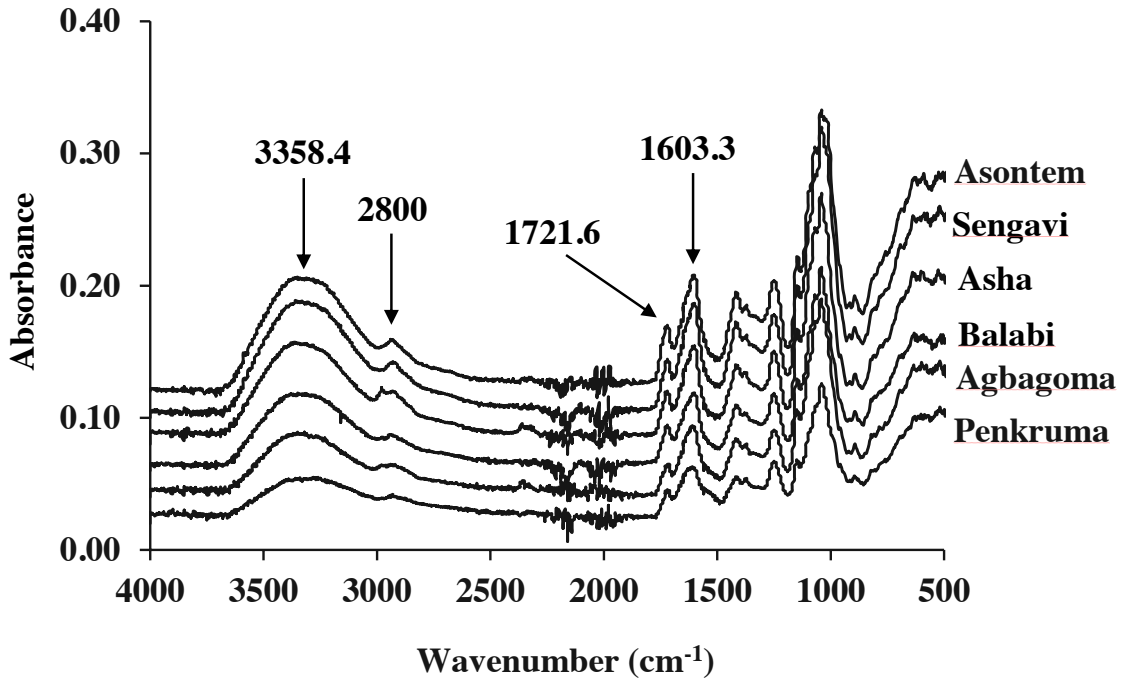
Table 3: Percentage neutral sugar composition on wet basis of pectin powder. In parentheses are mol% of each sugar. Sugar molar ratios of okra pectins are also shown as R_1 = GalA/ (Rha + Ara + Gal); R_2 = Rha/GalA; R_3 = (Ara + Gal)/Rha; R_4 = Gal/Rha; HG = GalA – Rha; RG-I = 2Rha + Ara + Gal.

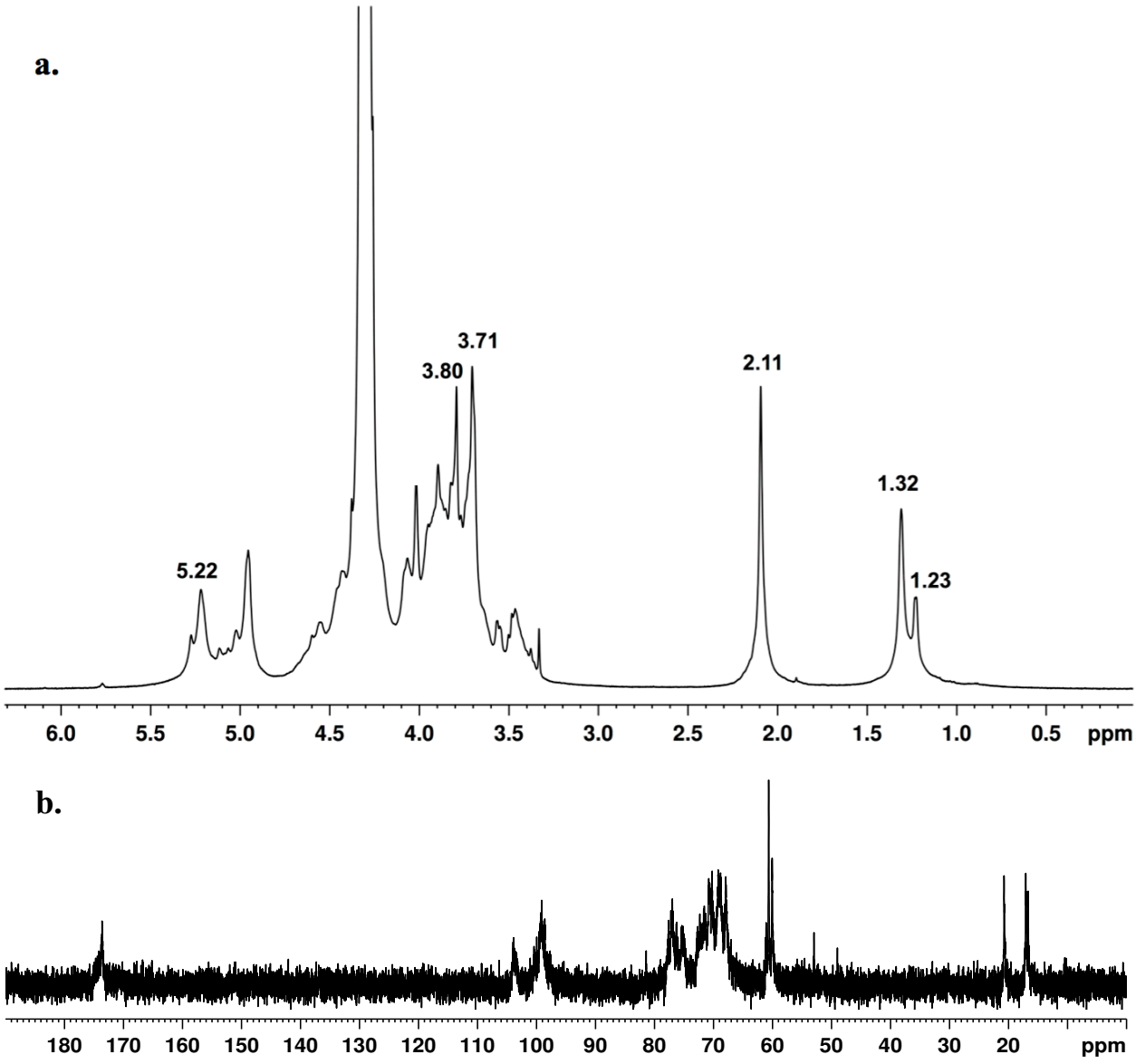
Genotype	D-Gal	L-Rha	L-Ara	D-Glc	R_1	R_2	R_3	R_4	HG	RG-I	HG/RG-I
Asha	16.6±0.3 (18.4)	8.9±1.5 (11.0)	1.7±0.4 (2.3)	0.8±0.1 (0.9)	2.0	0.17	1.9	1.7	53.7	42.7	1.3
Penkruma	5.9±1.4 (8.1)	4.3±1.5 (6.5)	1.5±0.5 (2.5)	0.7±0.1 (1.0)	4.6	0.08	1.6	1.2	72.1	23.6	3.1
Asontem	12.0±4.9 (16.9)	3.5±1.0 (5.5)	1.1±0.1 (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

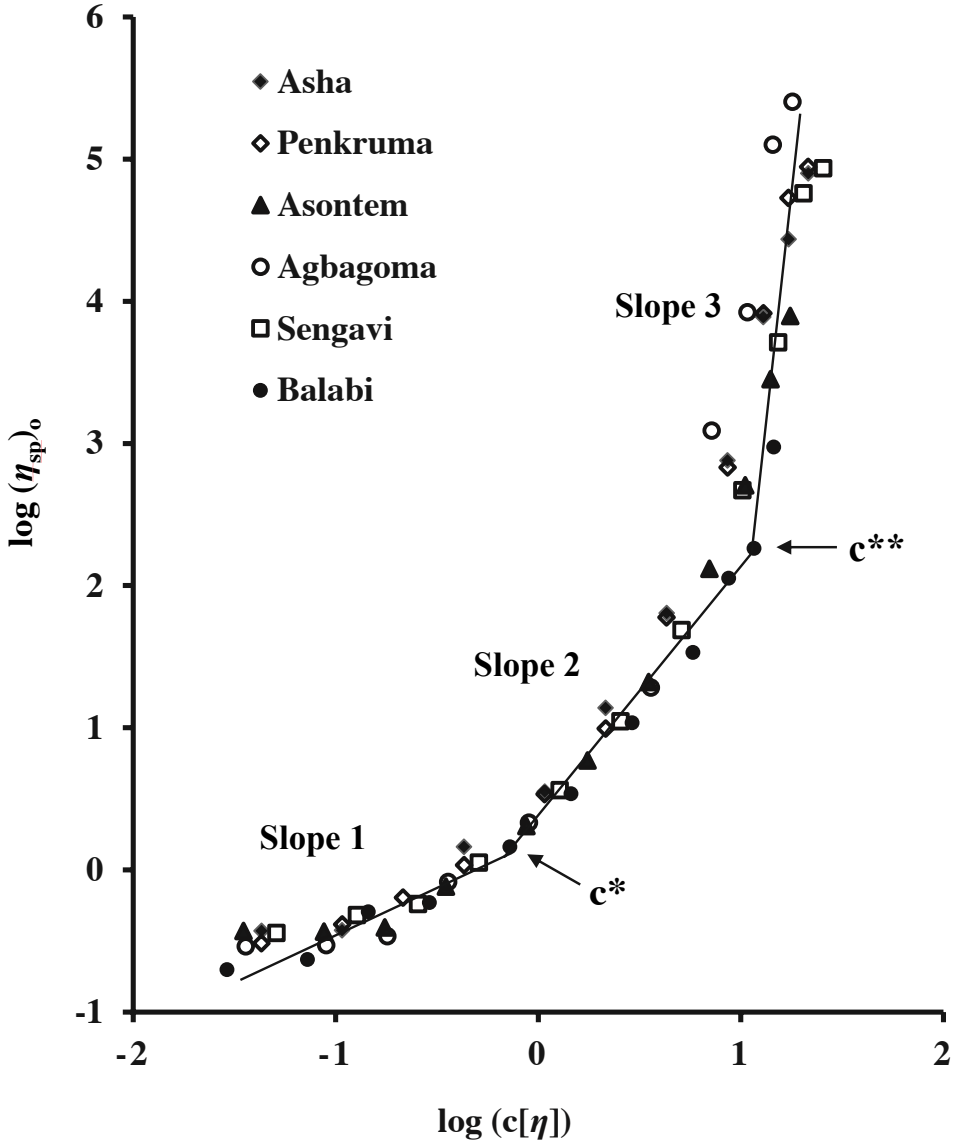
Table 4: Molecular characteristics of isolated pectins from different okra genotypes

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

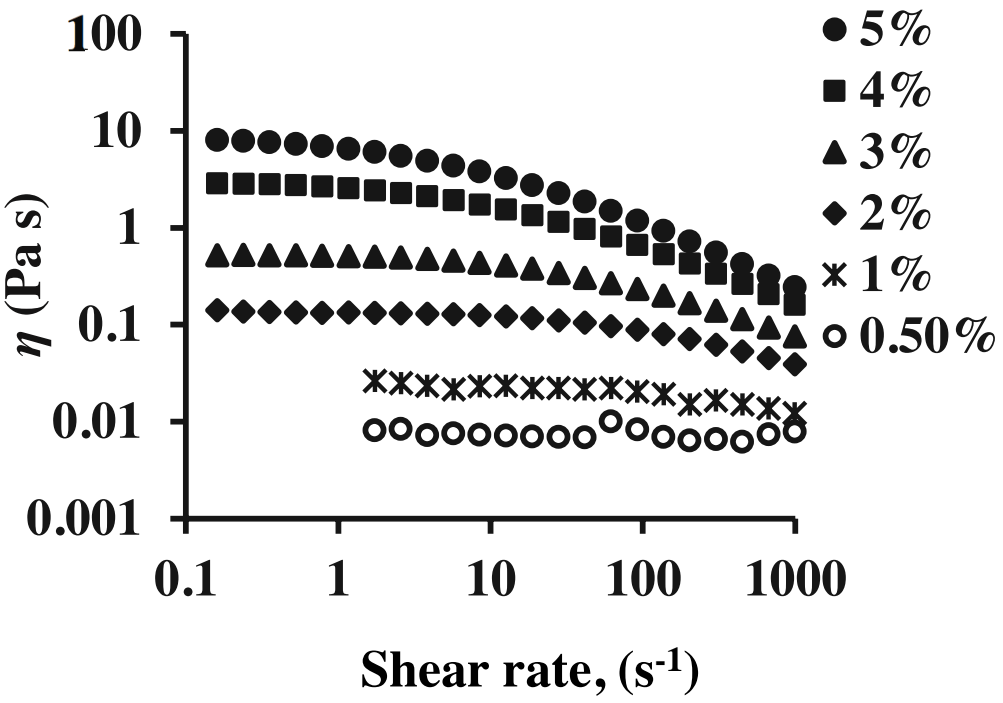

Sengavi
Agbagoma
Balabi
Penkruma
Asha
Asontem


0.40
0.30
0.20
0.10
0.00
Absorbance
3358.4
2800
1721.6
1603.3
Asontem
Sengavi
Asha
Balabi
Agbagoma
Penkruma
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)

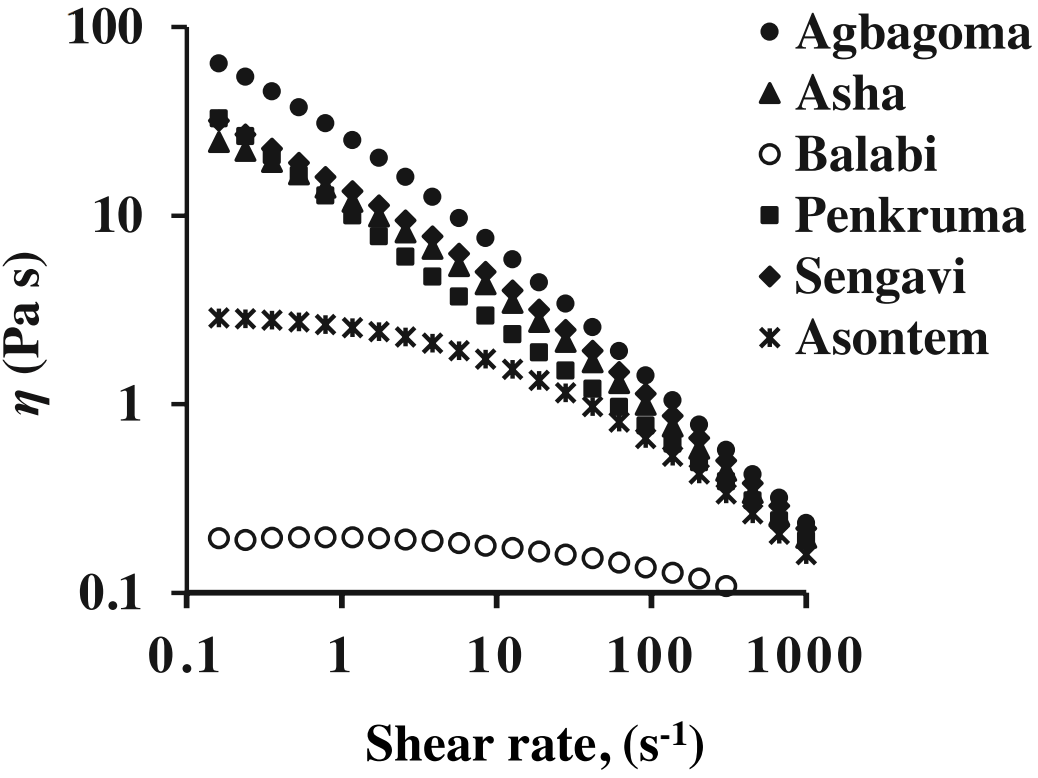

a.
5.22
3.80
3.71
2.11
1.32
1.23
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
b.
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

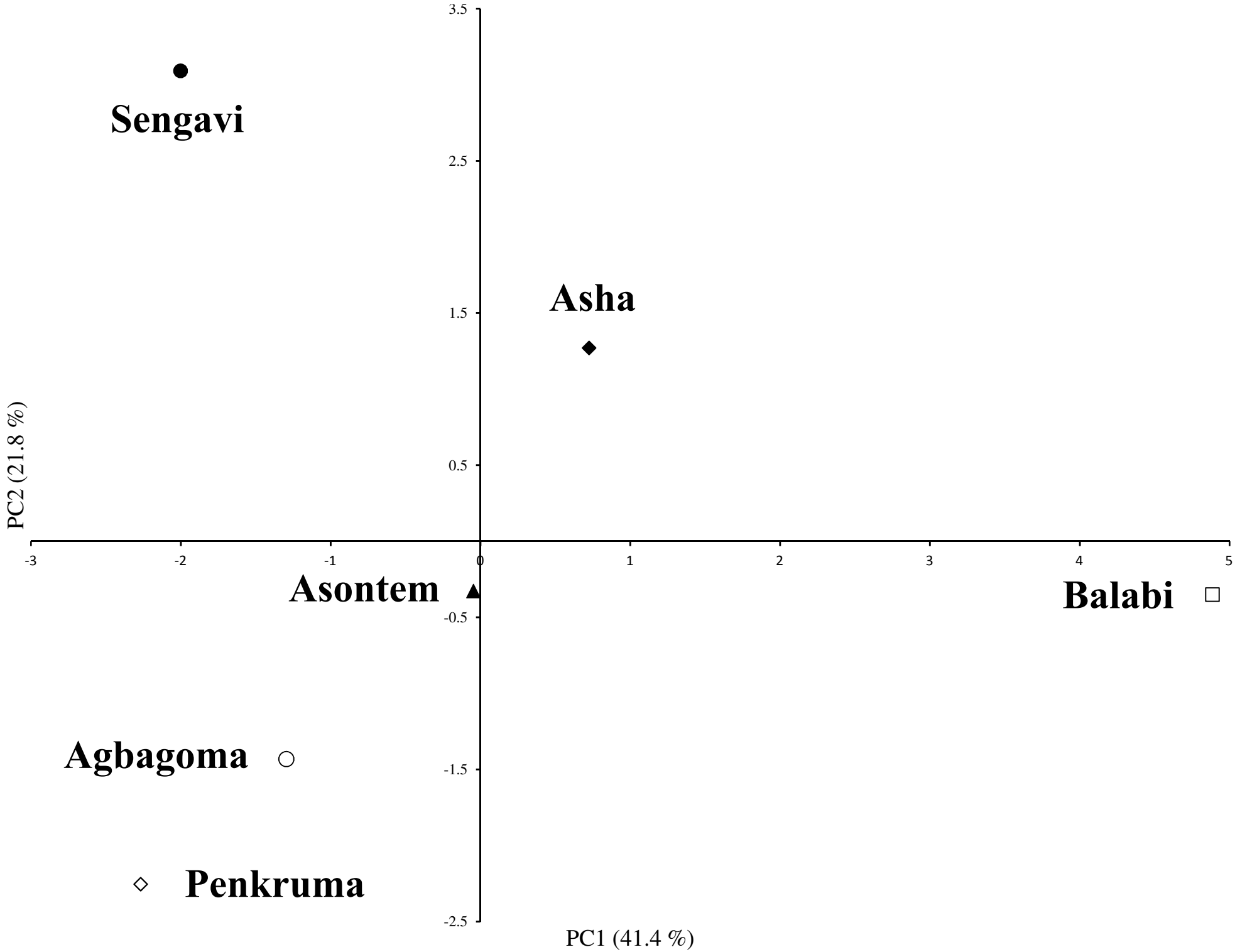

Asha
Penkruma
Asontem
Agbagoma
Sengavi
Balabi
Slope 1
Slope 2
Slope 3
c*
c**
log $(\eta_{sp})_o$
log (c[η])
6
5
4
3
2
1
0
-1
-2
-1
0
1
2

a.


100
10
1
0.1
0.01
0.001
η (Pa s)
0.1
1
10
100
1000
Shear rate, (s-1)
5%
4%
3%
2%
1%
0.50%

b.


100
10
1
0.1
η (Pa s)
0.1
1
10
100
1000
Shear rate, (s-1)
Agbagoma
Asha
Balabi
Penkruma
Sengavi
Asontem


Sengavi
Asha
Asontem
Balabi
Agbagoma
Penkruma
PC2 (21.8 %)
PC1 (41.4 %)
3.5
2.5
1.5
0.5
-0.5
-1.5
-2.5
-3
-2
-1
0
1
2
3
4
5