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PHYSICOCHEMICAL VARIABILITY OF PECTIN FROM DIFFERENT OKRA PHENOTYPE

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Abstract

Okra (Abelmoschus esculentus L.) is a readily available plant in Ghana and considered an abundant source of pectic polysaccharides which are useful ingredients for the functional food industry. Pectin extracts from eight different okra samples obtained from different sources were evaluated for their physicochemical and functional properties. The structural and molecular characteristics were analysed by means of Fourier transform infra-red (FTIR) spectroscopy, nuclear magnetic resonance spectroscopy (NMR) and size-exclusion coupled to multi-angle light scattering (SEC-MALS). The water absorption, oil absorption and emulsification capacities of the pectin extracts were determined. Results showed that the crude okra pectins had total carbohydrate contents in the range of 59.2 to 70.2% whereas protein content varied from 8.0 to 15.1%. The FTIR and ^{1}H-NMR spectra revealed similar structural features whereas the weight average molecular weight (Mw) ranged widely from 320×10^3 to 7600×10^3 gmol^{-1} in the order Pora < Akrofo < Asha < Asontem < Penkrumah (Techiman) < Sengavi < Penkruma (Kenkeso) < Agbagoma.The water/oil absorption capacity and emulsification capacity of the pectin isolates also varied depending on the geographical source. The relatively high galacturonic acid content of the pectins conferred hydrophilic characteristics that positively influenced the water absorption capacity of the polymers in solution. Pectin from the okra phenotypes Sengavi and Agbagoma shown to have low RG-I fractions demonstrated increased oil holding (378g/100g and 384g/100g respectively) and emulsification capacities (45%). The present findings indicate that the differences in pectin characteristics among the okra phenotypes have the potential to be exploited for different technological applications.

Introduction

Okra (*Abelmoschus spp.*) is a potential new source of natural polysaccharides which can be exploited industrially as functional ingredients in food and non-food products (Georgiadis et al., 2011). Natural gums and mucilage are preferred for commercial production of polysaccharides because of their low cost, availability and low toxicity (Alamri et al., 2012). Okra polysaccharides can be used as thickening agents, viscosity enhancers, gelling agents and texture modifiers (Alba et al., 2015). Mucilage polysaccharides are generally referred to as hydrocolloids due to their wide range functional properties which have positive technological applications (Archana et al., 2013). The thick and slimy texture is attributed to its polysaccharide content known as pectins (Alba et al., 2013; Ghori et al., 2014; Kontogiorgos et al., 2012; Samavati, 2013). Different varieties of okra are likely to have different mucilage yield and composition (Kpodo et al., 2017; Lahaye et al., 2014, 2012). Okra polysaccharides have been isolated on a laboratory scale and evaluated for a number of food and non-food applications (Kpodo et al., 2018). Research studies have employed different solvent protocols for the extraction of okra polysaccharides and these include water, sequential buffer systems, ethanol, methanol, acetone, phosphate buffer systems and mixture of solvent extraction systems (Alamri et al., 2012; Alba et al., 2015, 2013; Archana et al., 2013; Georgiadis et al., 2011; Kontogiorgos et al., 2012; Samavati, 2013; Sheu and Lai, 2012; Zheng et al., 2014). Factors that have been identified to influence the yield and composition of polysaccharides isolated are solvent used for extraction, pH and time of extraction (Alba et al., 2015; Kpodo et al., 2019). Isolation of polysaccharides have also been done on a laboratory scale using sequential solvent treatments (Alba et al., 2013; Georgiadis et al., 2011; Kontogiorgos et al., 2012; Sengkhamparn et al., 2009).

The physicochemical and functional properties of food polysaccharides are essential factors to consider in food processing, formulation of new food products or hydrocolloids systems (Datsomor et al., 2019; Kpodo et al., 2019). The origins of a plant material (and in this case, okra) from different geographical sources may influence its physiochemical and functional characteristics. The objective of the present study, therefore, was to evaluate the structural and functional properties of pectins obtained from okra in different geographical sources.

Materials and Methods

Materials and Sample Preparation

Soft and matured ($\sim$ 3 months old) okra pods (5–9 cm) of eight different okra samples were obtained from local farmers in five different sources (Northern, Volta, Brong Ahafo, Ashanti and Eastern regions) of Ghana for this study. The okra types used were namely; *Asha*, *Pora*, *Penkruma*, *Agbagoma*, *Asontem*, *Akrofo* and *Sengavi*. Two different samples of the *Penkruma* okra type were obtained from two different geographical zones (Ashanti and Brong Ahafo regions). Hence a total of eight samples were studied. Morphological variations among the okra types as regards stem, petiole, leaf and fruit

characteristics were scored using a standard international crop descriptor for okra (Resources, I.B.f.P.G., 1991). The pods were immediately frozen and kept at -20°C until required. The okra pod was dried and then subjected to extraction/isolation of the pectins. All chemicals used were of analytical grade.

Isolation of Okra Pectins

The dried and milled okra pods (20 g) were subjected to extraction using 0.1 M phosphate buffer (pH 6) as has been previously reported (Alba et al., 2015; Kpodo et al., 2017).

Protein and Total Carbohydrate Content Determination

Protein quantification was performed using the Bradford method (Bradford, 1976) whereas the total carbohydrate/polysaccharide content of the okra pectin powder was determined by means of the phenol-sulphuric acid assay (Dubois et al., 1956). All determinations were performed at least in triplicate.

Spectroscopy

Fourier transform infra-red (FTIR) spectra were obtained between 400 and 4000 cm^{-1} for all okra samples in Attenuated Total Reflection (ATR) mode at a resolution of 4 cm^{-1} using 128 scans (Nicolet 380, Thermo Scientific, UK). Spectral smoothing was applied using instrument software (OMNIC 3.1) (Kpodo et al., 2017).

For all the samples ^{1}H-NMR was conducted at 70°C with D_2O as the solvent using the Bruker Avance (500 MHz). The samples (2 mg) were dispersed in 2 mL deuterium oxide (D_2O) and then freeze dried. The freeze-dried samples were subsequently again dissolved in 600 μL D2O prior to performing the ^{1}H-NMR spectroscopy.^{1}H-NMR spectra were recorded with 64 scans at the same temperature (Kpodo et al., 2017).

Molecular Weight (Mw) Determination Using Size Exclusion Chromatography Coupled to Multi–Angle Light Scattering (SEC-MALS)

The molecular weights (MW) of the okra pectins from different sources were estimated using size exclusion chromatography coupled to multi–angle light scattering (SEC-MALS) at 25°C. Pectins were solubilised in 0.1M NaCl solution (2 $mg{\cdot}mL^{-1}$) at ambient room temperature with stirring overnight, filtered over 0.45 μm membrane filters. Samples were subsequently injected onto a SEC system (15 μm particle size, 25cm × 4mm, 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. The pectins were eluted with distilled water at a flow rate of 0.7 $mL{\cdot}min^{-1}$ (Alba et al., 2015). A differential index or refraction increment (dn/dc) of 0.147 mLg^{-1} typical for pectin (Alba et al., 2015; Morris et al., 2000) was used for all samples.

Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)

The WAC and OAC of the okra pectin powder were determined by dissolving 1.0 g of the mucilage powder in 10 mL of distilled water for the water holding capacity, and 10 mL of the refined oil for the oil holding capacity (Dossou, 2014; Noorlaila et al., 2015). The subsequent mixtures were vortexed for 2 min, centrifuged at 3000 rpm for 30 min and the supernatant drained off. The WAC/OAC were determined using equations 1 and 2 respectively:

$$WAC = \frac{Weight\ of\ absorbed\ water}{Weight\ of\ powder\ taken} \times 100 \quad (1)$$

$$OAC = \frac{Weight\ of\ absorbed\ oil}{Weight\ of\ powder\ taken} \times 100 \quad (2)$$

Emulsification Capacity

Each okra pectin sample (1.0 g) was dissolved in 50 mL distilled water and 50 mL sunflower oil (Archana et al., 2013). The mixture was hormogenized for 1 min and then centrifuged at 4000 rpm for 5 min. The emulsion capacity was calculated by dividing the volume of emulsified layer after centrifugation by the volume of the whole mixture as show in equations 3:

$$Emul.\ Capacity = \frac{Volume\ of\ emulsified\ layer}{Volume\ of\ mixture} \times 100 \quad (3)$$

where *Emul.* = Emulsification.

Emulsification Capacity

Data obtained was analysed using Statgraphics (Graphics Software System, STCC, Inc. USA). Statistical comparisons between the different okra genotypes were performed using analysis of variance (ANOVA) with a probability, $p < 0.05$.

Results and Discussion

Morphological Variations among Okra Types

Morphological variations among okra types were scored using the standard international crop descriptor for okra (Resources, I.B.f.P.G., 1991). The okra types showed relatively wide variations for all the morphological characteristics studied. Most of the okra plants had erect growth characteristic whilst leaf and stem colours were predominantly green. Petal or flower colour were predominantly yellow. Fruit were green to dark green, smooth and rough. The results showed that the fruit colour displayed different variations that ranged from green, with hairs to dark green (Table 1; Figure 1).

Figure 1. Morphological variations among okra fruit

Table 1. Stem, petiole, leaf and fruit characteristics

Type	StC	PtC	MLC	LRC	IPL	FC	FP
Asha	Green + Purple tinge	Purple	Green+Red Veins	Green	Red	Green	Little rough
Agbagoma	Green	Green	Green	Green	Green	Green	Little rough
Asontem	Green	Green	Green	Green	Green	Green	Smooth
Akrofo	Green	Green	Green	Green	Green	Green	Smooth
Sengavi	Green	Green + Red Veins	Green	Green	Red	Green	Little rough
Penkruma	Green+ Purple tinge	Purple	Green+Red Veins	Green	Red	Green+purple veins	Smooth

StC-stem colour; PtC-petiole colour; MLC-mature leaf colour; LSh-leaf shape; LRC-leaf rib colour; IPL; Intersection between petiole and leaf; FC-fruit colour; FP-fruit pubescene.

Protein and total carbohydrate contents of crude okra pectins

Okra mucilages are acidic polysaccharides often co-extracted with proteins. The okra polysaccharide is made up of galactose, rhamnose and galacturonic acid (Alba et al., 2015; Deters et al., 2005; Kpodo et al., 2017). The sugar composition of okra comprises of different types of polysaccharides including pectins, hemicelluloses such as xylan and xyloglycan and cellulose (Sengkhamparn et al., 2009). However the main polysaccharides in okra extracts are predominately pectins (Alba et al., 2015; Kpodo et al., 2017). Pectins extracted from the okra samples from different sources had varied purity (protein and carbohydrate content) although they were subjected to the same phosphate buffer extraction procedure (Alba et al., 2015) and experimental conditions (Table 2). Varietal differences, variations in geographical sources, agricultural practices and experimental conditions are considerable determinants that can influence polymer composition (Kpodo et al., 2017, 2019).

An evaluation of the purity of the crude pectin extracts showed protein content of 8.0 – 15.1% and total carbohydrate ranging from 58.4 to 70.1%. The purity levels of the pectins studied were lower than extracts obtained by (Alba et al., 2015) (protein, 4.3 – 6.3%; total carbohydrate, 70 – 81.8%) because the crude pectin obtained were not further purified by exhaustive dialysis. Crude polysaccharides have showed higher surface activity (Brummer et al., 2003) and produced very stable oil/water emulsion. The complex mixture of proteins and polysaccharides results in greater polymer hydrophobicity which increases surface activity (Alba et al., 2015; Kpodo et al., 2019). The total carbohydrate content of the different okra types generally increased with decreasing protein content.

Structural Features

FTIR spectroscopy (400 to 4000 cm^{-1}) was used to compare the pectin extracts from different okra types and the superposability of their infrared spectra confirmed that the okra types studied had similar functional groups (Figure 2). Pectins from all the different types of okra had peaks which corresponded to the O-H stretching absorption (3000 – 3500 cm^{-1}). This absorption band has been attributed to the inter- and intramolecular hydrogen bonding of the GalA backbone of okra pectins (Alba et al., 2015; Kpodo et al., 2017). The CH absorption which occurred in the region around 2850 – 2950 cm^{-1}, corresponds to CH, CH2 and CH3 stretching vibrations. The absence of this band in the other okra types (*Agbagoma*, *Asontem*, *Kpong* and *Sengavi*) could be attributed to the crude nature of the polysaccharide extracts, hence the need for further purification to make obvious bands sharper.

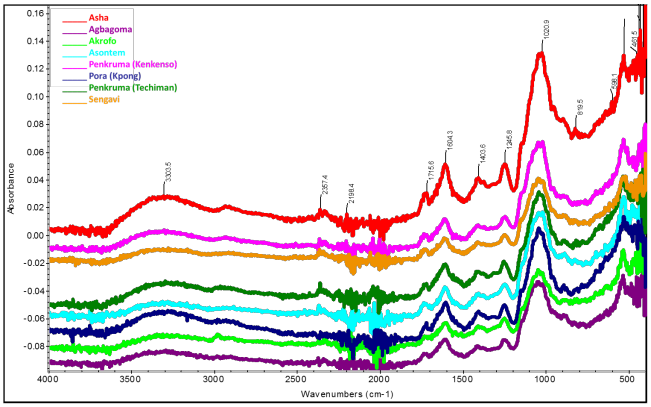

Figure 2. FTIR spectra of okra pectins from different sources

The region from 900 to 1200 cm^{-1} is typically referred to as the carbohydrate finger print region because most polysaccharides show peaks in this region. FTIR spectra of all the different crude okra types showed this carbohydrate fingerprint. Since these bands are also as a result of complex interacting vibrational modes, a band in this region cannot be assigned to a particular group of atoms (Manrique and Lajolo, 2002). Nonetheless it has been noted that the C−O and C−C groups of the glycosidic bonds have stretching vibrations in this region. The spectra of all the crude okra types revealed two critical peaks associated with the degree of esterification. A band that occurs approximately in the region of 1610 – 1630 cm^{-1} and thus corresponds to the symmetrical stretching vibration of the carboxylic group (COO^{-}). The second band which corresponds to the methyl esterified group ($COOCH_3$) occur within the region of 1720 – 1730cm^{-1}.

NMR spectroscopy was applied to investigate the structure of pectin. In the current study, ^{1}H-NMR spectra of all samples isolated revealed similar resonance patterns suggesting similarities in compositional characteristics of all pectins (Figure 3). A comparison of ^{1}H-NMR spectra of okra isolated (Figure 3) showed a high similarity between all spectra. A signal

Table 2. Purity and molecular weight characteristics of crude okra pectin from different geographical origins

Okra Types	Source/Region	Protein (%)	Total polysaccharide (%)	Mw (x10^6g mol^{-1})	Rg. (nm)
Asha	Ashanti	8.00 ± 0.01^a	70.16 ± 0.01^e	0.79 ± 0.18	136 ± 20
Pora (Kpong)	Northern	10.12 ± 0.01^b	68.20 ± 0.01^d	0.32 ± 0.04	130 ± 13
Penkruma (Kenkeso)	Ashanti	13.56 ± 0.01^d	58.36 ± 0.01^a	1.40 ± 0.20	140 ± 14
Agbagoma	Volta	14.73 ± 0.06^e	59.20 ± 0.01^a	7.60 ± 1.30	138 ± 15
Asontem	Eastern	11.10 ± 0.04^c	68.30 ± 0.01^d	0.83 ± 0.15	138 ± 17
Akrofo	Volta	14.83 ± 0.01^e	63.00 ± 0.01^b	0.42 ± 0.05	134 ± 11
Penkruma (Techiman)	Brong Ahafo	15.10 ± 0.04^b	64.53 ± 0.01^c	1.11 ± 0.10	133 ± 12
Sengavi	Volta	9.50 ± 0.05^b	59.20 ± 0.01^a	1.30 ± 0.20	137 ± 16

Values are mean ± SD of at least triplicate determinations. Mean values in a column with different letters are significantly different ($p < 0.05$)

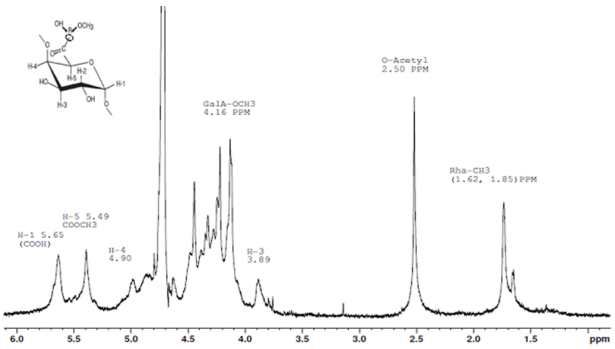

Figure 3. ^{1}H-NMR spectra of *Agbagoma* okra pectin showing specific linkages

at 4.16 ppm corresponds with methyl groups connecting to carboxyl groups of GalA (Alba et al., 2015). The signal at 2.50 ppm proves the presence of O-acetyl substituent which is similar to that reported in previous study for okra pods 2.10 ppm. The samples contained both unbranched α-1, 2-linked rhamnose (1.62 ppm) and branched α- 1, 2, 4-linked rhamnose (1.85 ppm). ^{1}H NMR spectra of all okra polysaccharides were comparable to the spectrum of okra pectins previously isolated by (Alba et al., 2015).

Molecular Weight

The pectins were analysed by high-performance size-exclusion chromatography (HPSEC) equipped with multi-angle laser light scattering (MALLS) and refractive index (RI) detectors. The weight-average molar mass values of the crude okra pectins ranged widely from 320×10^3 to 7600×10^3 $gmol^{-1}$ (Table 2). The high-MW of some pectin samples may be due to the presence of other aggregates, considering the crude nature of the samples. Pectins from the okra type Agbagoma (7600×10^3 $gmol^{-1}$) had a higher molecular weight value than the others although the same extraction method was employed for all samples. This high value can either be due to differences in origin of materials or the presence of other dissolved components such as excess cellulose. The molecular weights values obtained for most pectins studied were higher than values obtained by (Alba et al., 2015) which ranged from 640×10^3 to 767×10^3 $gmol^{-1}$. Molecular weights of pectins from all phenotypes studied were higher than okra pectins (50×10^3 to 60×10^3 $gmol^{-1}$) obtained from sequential extraction protocol (Kontogiorgos et al., 2012; Sengkhamparn et al., 2009) and also higher than pectins from sugar beet: 184×10^3 $gmol^{-1}$ (Guo et al., 2016).

Pectin samples used in this study were obtained from different sources. Differences in sample source/origin and genetic variation might account for the variations in the analysis. The wide diversity in molecular weight demonstrated by the pectin extracted from okra using the same experimental conditions but from varied sources, can be exploited to tailor pectin structure for potential multifunctional properties as food and pharmaceutical ingredients (Alba et al., 2015). This is because even a small difference in the structure and constitution of a molecule results in significant changes in the physicochemical properties (Gnanasambandam and Proctor, 2000). The z-average radius of gyration (Rg.z) of okra pectin studied was from 130 nm to 140 nm. The Rg.z values of the okra pectin are lower than reported values (593 nm and 671 nm) for other low methoxylated pectin (Golebiowski et al., 2020) but higher than the mean radius of gyration (34.8 nm to 39.2 nm) of sugar beet pectin (Lin et al., 2020).

Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)

The high water binding ability of these polymers have been attributed to the presence of many hydroxyl groups and galaturonic acid in the chemical structures (Li and Nie, 2016). The water holding capacity of pectin influences their characterization as functional ingredients in food and biomedical system (Archana et al., 2013). The WAC of different okra types studied varied in the range of 810g/100g to 963g/100g (Figure 4). The Akrofo okra type had the highest WAC whereas the WAC of the *Agbagoma* was the least. The varying water holding capacities of the okra pectins can be attributed to both genotypic differences and differences in sources. From previous studies (Kpodo et al., 2017) okra pectins have demonstrated high galaturonic acid content (51.9 to 63.4% w/w) which is a major component of the most abundant polymeric segment referred to as homogalacturonan (Kpodo et al., 2018). The relatively high galacturonic acid content conferred hydrophilic characteristics that increased the polymer ability to absorb and retain water (Qin et al., 2019). Due to the general high

WAC of the pectin studied they represent important functional ingredients as water immobilizers in food products and can act as thickeners and stabilizers in food products such as yoghurt, sauces and ice creams.

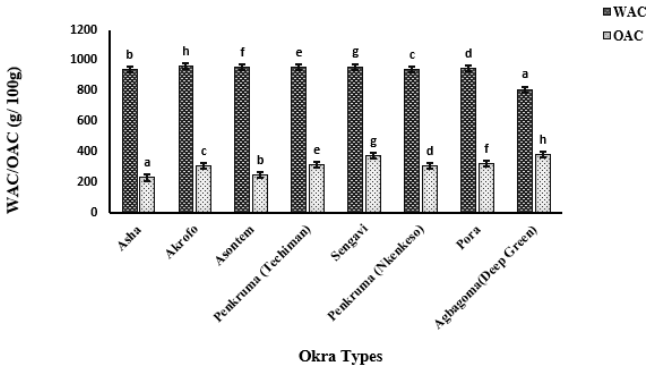

Figure 4. Water and oil absorption capacity (WAC/OAC) of okra pectins Different letters on bars with same patterns indicate significant differences ($p<0.05$)

Traditionally hydrocolloids are not considered as effective oil binders mainly due to their hydrophilicity and high molecular weight (Li and Nie, 2016), however okra pectins have been noted to differ from other polysaccharide hydrocolloids in terms of protein and acetyl content which impacts some hydrophobic characteristics when interacting with oil systems (Alba and Kontogiorgos, 2017). The OAC of okra pectins were appreciably high although considerably lower than corresponding WAC. The hydrophobic groups and the proteins in the okra pectin are expected to have contributed to this high oil holding capacity. The oil holding capacities of pectin from the different okra phenotypes were also different and ranged from 232g/100g to 384g/100g with *Agbagoma* recording the highest OAC value (Figure 4).

Emulsification Capacity

Proteins have been predominantly used as emulsifiers due to their low molecular weight and flexible molecular structure which confers an ability to easily adsorb on oil-water interfaces (Alba et al., 2013; Archana et al., 2013; Li and Nie, 2016). Gums such as Gum Arabic, Gum Ghatti and pectins from citrus peel and sugar beet have been known to impact surface activity and emulsification properties (Alba et al., 2013; Li and Nie, 2016). Pectin from the different okra types showed good emulsification capacity. The okra types *Sengavi* and *Agbagoma* had the highest emulsion capacity (45%) (Figure 5) and this result was comparable to values obtained by (Archana et al., 2013) (50 -54.75%) but however lower than the 79.87–85.83% obtained by (Noorlaila et al., 2015). Our previous studies (Kpodo et al., 2017, 2018) on the molecular characteristics of okra pectins showed that different polymers demonstrated diversity in the content of RG-I segment.

Pectin from the okra types *Sengavi* (28.5), *Agbagoma* (27.2) and *Penkruma* (23.6) were shown to have relatively lower RG-I fraction than *Asontem* (29.8) and *Asha* pectins (42.7).

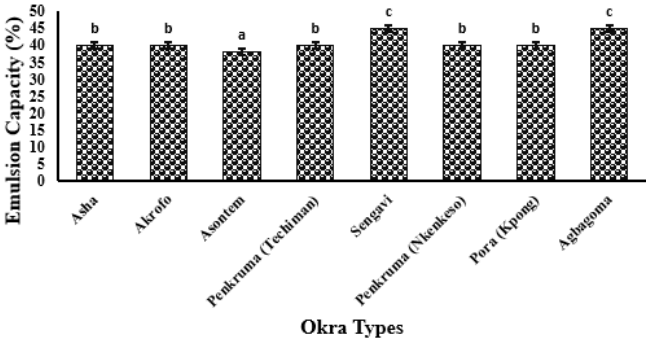

Figure 5. Emulsifying capacity (%) of crude okra pectins from different sources Different letters on bars with same patterns indicate significant differences ($p<0.05$)

RG-I segments have been shown to influence the stability of oil-water emulsion through the mechanism of steric stabilisation. Generally pectins with low RG-I content produce higher emulsification, whereas polymers with high RG-I segment confer greater chain flexibility, shortens the distance between droplets and contributes to less effective stearic stabilization. In this study, *Sengavi* and *Agbagoma* pectins with relatively lower RG-I segments demonstrated higher emulsion capacity (45%) than pectins from *Asha* (40%) and *Asontem* (38%) which also recorded least oil holding capacities of 232g/100g and 249g/100g respectively. These pectins (*Sengavi* and *Agbagoma*) can thus form structurally stable and induce non-separation of oil-water emulsions.

Conclusion

Pectins from eight different okra samples (*Abelmoschus* spp) obtained from different sources showed similar structural features but varied in terms of purity, weight-average molecular weight and functional properties (water/oil absorption capacity and emulsion capacity). The same okra genotype Penkruma obtained from different sources (Ashanti and Brong Ahafo regions) also demonstrated heterogeneity in chemical composition and macromolecular characteristics which subsequently affected pectin oil and water absorption capacities. Hence the same okra genotype obtained from different sources can produce pectins of varied chemical, macromolecular and functional characteristics. These findings suggest that depending on the source, okra pectin could be exploited as ingredient for different food and industrial applications and pectins from different sources may have the potential to be tailored to specific functions due their structure diversity.

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