PHYSICOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF PECTIN EXTRACTS FROM DIFFERENT OKRA GENOTYPES

By

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DECLARATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge and belief, it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgement is made in the thesis.

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ABSTRACT

Okra (*Abelmoschus spp.*) is a plant of the Malvaceae family cultivated for its immature pods. The pectin content is responsible for the slimy texture of okra extracts and is of major technological interest for food, non-food and medicinal applications. A preliminary study on isolation of pectins from eight different okra types obtained from different geographical zones in Ghana revealed that the okra pods had considerable pectin yield and different physicochemical properties which can be exploited for their potential applications. Six different okra (*Abelmoschus* spp) genotypes were then cultivated under the same environmental conditions and their genetic diversity determined using fragment length analysis (FLA) of ten simple sequence repeat (SSR) markers. Pectins were extracted from the pods of the different okra genotypes using 0.1 M phosphate buffer (pH 6). Physicochemical, functional and rheological characteristics of the pectins were evaluated using FT-IR and NMR spectroscopy, Size-exclusion chromatography (SEC) and dilute solution viscometry. Neutral sugars composition was evaluated using GC-MS. Emulsifying properties of the pectins were determined by analyzing the interfacial protein and pectin concentrations, emulsion rheology, particle size distribution, zeta potential and the emulsion morphology using fluorescence microscopy. Genotypic studies confirmed unique genotypes for each okra sample used with each SSR markers detecting an average of 4.1 alleles. Physicochemical results showed that okra genotypes had pectin yields between 11–14 %. Resultant pectins were all low methoxyl pectin with varied degree of methylation (17–25 %), total carbohydrate (66.2–87.5 %), protein (3.3–7.1 %), sugar composition, degree of acetylation (20– 40 %), GalA content (43–63 %) and weightaverage molar mass (700–1700 x 10^3 g mol^{-1}). FT-IR and NMR spectroscopy showed structural similarities between the pectins from the different genotypes. Intrinsic viscosity of the okra pectins ranged from 2.91 to 5.10 dL g^{-1}. Huggins constant values calculated for okra pectins ranged from 0.3 to 0.7 revealing flexible chain conformation. Coil overlap plots revealed three concentration regimes with two critical concentrations. Stability of emulsions to creaming varied from 0.8 to 16.9 % and surface coverage of pectins (0.6 – 3.6 mg m^{-2}) were relatively higher than that of the proteins (0.3-1.0 mg m^{-2}). The okra pectins demonstrated good emulsifying abilities with initial surface weighted mean diameter ($d_{3,2}$) ranging between 1.3 and 3.7 μm. Emulsion destabilization occurred in all samples after five days of storage and the viscosity of samples remained constant with aging. The differences between genetic variations have potential applications in the food industry as emulsifying agents.

CONTRIBUTION TO KNOWLEDGE

The following key findings have been established from this study:

- Okra pectin has low degree of methylation and high degree of acetylation irrespective of the genotype
- Okra pectin from the different genotypes present diversity in the amount of rhamnogalacturonan I (RG-I) and homogalacturonan (HG) components
- Pectin from different okra genotypes exhibit flexible chain conformation and varied flow behaviour
- Prevalence of rhamnogalacturonan I (RG-I) segments and length of branching influenced emulsion stability
- Branches with intermediate length with molar ratio of (Ara + Gal)/Rha ranging between 2 and 3 exhibit the optimum emulsification capacity

Publication from the work:

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God bless you all.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of Study

Okra, *Abelmoschus esculentus L*. belongs to the Malvaceace family and is a widely consumed vegetable in tropical and subtropical countries (Eze and Akubor, 2012). It is known by many names such as *Gombo* (French), *Miyan-gro* (Hausa) *La* (Djerma), *Layre* (Fulani), *Gan* (Bambara), *Kandia* (Manding), *Nkruma* (Akan) and *Fetri* (Ewe) (Kumar *et al.*, 2010). The plant is cultivated for its fruits which are used as vegetables both in the green and dried state (Jideani and Bello, 2009). The most dominant okra producing countries include India, Nigeria, Pakistan, Ghana and Egypt (Eze and Akubor, 2012), although the okra plant is now grown in many other areas such as Thailand and the southern states of the USA. Nigeria is the largest producer of okra (2,039,500 t) in West Africa, followed by Cote d'Ivoire and Ghana (FAOSTAT, 2014). Brong Ahafo, Ashanti, Northern, Volta, Greater Accra and Central regions are the largest producers of okra in Ghana (NARP, 1993). Okra is the fourth popular vegetable after tomatoes, peppers and garden eggs (Tweneboah, 1998).

Okra polysaccharide consists mainly of cellulosic material and a polymer of uronic acids and sugar (the portion that imparts the ropiness in aqueous solution). Okra contains large quantities of polysaccharides which are responsible for the viscosity of the aqueous suspension (Eze and Akubor, 2012). The high mucilage content of the okra fruit is particularly desirable in soups and gravies (Jideani and Bello, 2009). Okra mucilage is water soluble and produces viscous, shear thinning, viscoelastic solutions in water (Meister and Anderle, 1983). Different cultivars of okra produce different yield of mucilage

(Dayana and Hayati, 2012). The mucilaginous polysaccharide isolated from okra pods have been studied to various extents, however each report shows considerable compositional and structural variation. Okra polysaccharide is an acidic polysaccharide that is made up of galactose, rhamnose and galaturonic acids (Deter *et al.*, 2005). The sugar composition of okra is mainly glucose, galactose and galacturonic acid (Sengkhamparn *et al.*, 2009). The structural elements of okra polysaccharide consist of a repeating unit of alternating α 1→2 linked rhamnosyl and α 1→4 linked galacturonic acid residues with a disaccharide side chain of β 1→4 linked galactosyl moieties attached to half of the L-rhamnosyl residues (Sengkhamparn *et al.*, 2009). Although scientific reports on the use of okra in food systems is very scarce, okra mucilage has some medicinal and industrial applications. The more viscous and slimy property of okra is used in stews, soups and gravies for its thickening effect (food thickener) (Eze and Akubor, 2012). Okra mucilage can also be used as stabilizers and emulsifiers in food systems (Dayana and Hayati, 2012). Okra polysaccharides can be used as egg white substitute in chocolate bar cookies and in chocolate frozen dairy desserts.

1.2 Statement of the Problem

The pectin content of okra is of major technological interest for food, non-food and medicinal applications. Despite the potential of the crop, the market value of okra in Ghana decreases when in season due to limited use in soup/stew preparation. The crop is underutilized in Ghana and most African countries, partly due to limited knowledge on genotypes required for other food, non-food and medicinal uses. 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 and Kontogiorgos, 2016). Okra pectins have been previously isolated by following different extraction protocols (Alamri *et al.*, 2012; Alba *et al*., 2015; Archana *et al*., 2013; Georgiadis *et al*., 2011; Samavati, 2013; Sengkhamparn *et al*., 2009; Woolfe *et al*., 1977; Zheng *et al*., 2014). Although the effect of extraction conditions on structural and macromolecular characteristics of okra polysaccharides is well investigated and understood, there is limited information on the impact of different okra genotypes on those features. 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. The understanding of the impact of each structural parameter (neutral sugar composition, degree of methylation and acetylation) of cell wall polysaccharides may serve as the basis for breeding okra genotypes with specific functional properties. Isolation procedure and genotypic differences can impact functional properties of the okra pectin isolates.

1.3 Research Justification

In order to provide critical information for the application of okra polysaccharides in food systems, the physicochemical, functional and rheological characteristics of different genotypes of okra consumed in Ghana needs to be studied. Functional and rheological properties of food polysaccharides are essential factors to consider in food processing or in the formulation of new food products. This knowledge will influence potential applications of okra polysaccharides in the food industry since a particular functional property is especially useful for the manufacture and stability of novel food products. Different cultivars of a plant have different physicochemical, functional and rheological

characteristics, hence different genotypes of okra might have different polysaccharide content, composition and yield. The study thus determined and compared the polysaccharide yield and composition of different genotypes of okra consumed in Ghana. This will elucidate the suitability of using a particular genotype in producing a specific behaviour in a food system. The research work also explored the emulsifying properties of okra polysaccharides. Incorporating okra polysaccharide in the production of food products has the potential to increase the utilization and production of okra. Aside this okra is readily available and inexpensive hence research into its unique properties and utilization in novel food systems could decrease the cost of such food products and subsequently assure food security. The need to also explore other sources of high quality food polysaccharides and other food applications of okra polysaccharides in food systems has made it imperative to study the physicochemical and functional properties of the polysaccharide extracts. The study will enhance the possibility of developing a food product incorporating okra polysaccharide for the Ghanaian market.

1.4 Aim:

The aim of the study was to isolate pectins from different okra genotypes and evaluate their physicochemical, rheological and emulsifying properties.

1.5 Specific Objectives:

The specific objectives of the study are to:

1. Extract pectin from okra in different geographical locations of Ghana and assess their yield, structural, chemical and functional properties.
2. Isolate pectin from different okra genotypes cultivated under the same environmental conditions and evaluate their physicochemical and structural

properties.

3. Investigate the rheological behaviour of pectin from different okra genotypes.
4. Investigate the emulsifying characteristics of pectin from the different okra genotypes.

CHAPTER 2

LITERATURE REVIEW

2.1 Production of Okra and Okra Cultivars in Ghana

Okra, *Abelmoschus esculentus* L. (Moench) is a hot climate vegetable mainly grown for its young pods and leaves. It is an economically important crop cultivated in tropical and sub-tropical parts of the world. Okra is easily grown because it tolerates environmental stress, flowers and produces fruit. Most cultivars of okra flowers 35-60 days after planting and the flowers are normally self-pollinated or are pollinated by insects (Martin, 1982;

Hamon, 1989). Okra pods permitted to mature 4-8 days give a gum product having ropiness values approximately three times greater than those of pods permitted to mature 11-12 days (Whistler and BeMiller, 1993). The use of pods of not more than 8 days maturity and use directly after picking is preferred (Whistler and BeMiller, 1993). World okra production has been estimated at 4.8 million tons with India (70 %) being the largest producer of the crop. Other important okra producing countries include Nigeria (15 %), Parkistan (2 %), Ghana (2 %), Egypt (1.7 %) and Iraq (1.7 %) (Gulsen *et al*., 2007). Nigeria is the largest producer of okra in West Africa, followed by Cote d'Ivoire and Ghana (FAOSTAT, 2014). The vegetable is known by several names in West and Central Africa and some of these names are *Gombo* (French), *Miyan-gro* (Hausa), *La* (Djerma), *Layre* (Fulani), *Gan* (Bambara), *Kandia* (Manding), *Nkruma* (Akan) and *Fetri* (Ewe) (Kumar *et al.*, 2010). In Ghana, Brong Ahafo, Ashanti, Northern, Volta, Greater Accra and Central regions are the largest producers of okra (NARP, 1993).

Okra belongs to a family of plants called *Malvaceae* and genus - *Abelmoschus*. Wild and cultivated species of the genus *Abelmoschus* documented include *A. moschatus*, *A. manihot, A. esculentus, A. tuberculatus, A. ficulneus, A. crinitus, A. angulosus* and *A. caillei* (Siemonsma, 1982). Some genotypes/accessions of okra morphologically characterized in Ghana are *Nkrumahene, Asontem, Nkrumatia, Nsafitaa, Debo, Atuogya, Muomi, Asontem-Gar, Awole Nkruma, Labadi, Legon fingers, Spinless, Voltui, Awoale Nkruma, Agbodrofe, Agbodroga, Fetri, New york, Ngruma, Nkruma hwam, KNUST, Nkruma tenten, Tech Nkruma, Nsapan, Asante abe, Bekwaso, Wune mana, Mamolega, Gyeabatan, Pora, Atuogya-tiatia, Sheo mana, Ason-wen, Atuogya-Asante, Gbodro-wild, Dikaba, Atuogya-tenten* (Ahiakpa *et al.*, 2013; Oppong-Sekyere *et al.*, 2012). Most studies

performed on genotypes of okra cultivated in Ghana, characterized varieties based on morphological markers (phenotypic characterization), however characterization based on DNA variations using biochemical or molecular markers such as Random Amplified Polymorphic DNA (RAPD), Restriction fragment length Polymorphism (RFLP), Simple Sequence Repeat (SSR) to confirm morphological characteristics are lacking.

2.2 Nutritional Value and Proximate Composition of Okra

Okra is cultivated for its immature pods usually consumed in the form of soups and stews and as a vegetable. As a vegetable the pods are usually used as thickeners in stews or soups, and both fresh and dried fruits are used. The fruit pods are picked for cooking, canning, and freezing when they are young and tender. In West Africa, okra leaves are also consumed and the stem is used for fibre and rope (Whistler and BeMiller, 1993). Okra is considered a health food due to its high fibre, mineral and vitamin content (Kumar *et al.*, 2010). The leaves and fruits of the okra plant contain valuable proteins, although the protein content of the leaves is higher than that of the fruits (Nwachukwu *et al*., 2014). Literature results of the proximate nutrient composition of okra is as shown in Tables 2.1, 2.2 and 2.3.

Table 2.1: Proximate composition of different okra varieties (%)

Variety	Moisture	Crude protein	Crude Fibre	Crude Fat	Ash	Carbohydrate
Fadaman Kubanni	18.63	13.15	29.76	9.73	7.35	51.13
Baoule (sun dried)	7.28	17.15	7.83	2.02	9.61	63.92

Dioula (sun dried)	7.33	15.75	9.07	2.17	9.50	65.24
Benin	88.73	14.87	10.63	9.67	8.26	-
Auchi	87.59	13.61	10.15	9.82	7.19	-
Ikaro	90.13	16.27	11.18	9.03	9.63	-
Akure	89.02	15.17	10.93	9.97	8.56	-
Okene	89.63	15.77	11.63	10.57	9.16	-
Lokoja	88.35	14.41	10.28	9.22	7.89	-

Sources: (Ekwumemgbo *et al.*, 2014; Nwachukwu *et al*., 2014; Kouassi *et al.*, 2013; Eze and Akubor, 2012; Adetuyi *et al*., 2011)

Table 2.2: Proximate composition of different parts of the okra plant (%)

Plant Part	Moisture	Crude protein	Crude Fibre	Crude Fat	Ash	Carbohydrate
Leaf	82.60	4.81	1.13	0.19	2.44	8.83
Young fruit	88.47	2.56	0.37	0.18	1.38	7.05
Mature fruit	82.25	2.51	2.44	0.46	1.17	11.16

Sources: (Ekwumemgbo *et al.*, 2014; Nwachukwu *et al*., 2014; Kouassi *et al.*, 2013; Eze and Akubor, 2012; Adetuyi *et al*., 2011)

Table 2.3: Effect of processing method on the proximate composition of okra fruit (%)

Sample	Moisture	Crude protein	Crude Fibre	Crude Fat	Ash	Carbohydrate
Fresh Sample	89.09	20.7	8.34	4.12	7.7	59.85

Blanched dried	oven	10.64	25.12	6.22	2.44	10.53	55.68
Blanched dried	sun	15.35	25.34	6.06	1.91	10.05	56.63

Sources: (Ekwumemgbo *et al.*, 2014; Nwachukwu *et al*., 2014; Kouassi *et al.*, 2013; Eze and Akubor, 2012; Adetuyi *et al*., 2011)

Okra is a rich source of various vitamins that are supplied to the body when consumed in adequate amounts. The provision of vitamins to the body is an important contribution of vegetables towards human nutrition. Okra is a good source of vitamins A, B, C and folate (Sami *et al.*, 2014; Kumar *et al.*, 2013). In terms of mineral content, green tender fruits of okra are highly nutritious containing 107 mg Calcium and 8.9 mg Iron (Thampi and Indira, 2000). Okra is also an excellent source of iron, phosphorus and iodine which is required for the control of goitre. The antioxidant properties of okra has prompted research into the crop in reducing diseases and improving health. Okra is known for being high in antioxidants (Kumar *et al.*, 2013). Studies have proved that substantial antioxidants (phenol and flavonoids) are present in okra (Arapitsas, 2008). The okra pods are also rich in beta carotene, xanthin and lutein (Kaur *et al.*, 2013). Consumption of the vegetable could boost the immune system in the body and minimize the occurrence of diseases.

2.3 Polysaccharides

Carbohydrates are one of the major constituents and largest proportion of organic compounds in plants (Bhat and Tharanathan, 1987). Some functions of carbohydrates in plants and animals include; formation of structural component in plants (cellulose), source of energy (starch), preservation of tissue desiccation (gums and mucilages), stimulate

insulin secretion and decreases plasma fatty acid contents in animals. Plant carbohydrates can be divided into mono-, di-, oligo- and polysaccharides. Plant cell wall polysaccharides are among the most complex carbohydrates since their structures are characterized by a variety of details essential for their characterization and elucidation of their functional roles (Mikshina *et al.*, 2012). Polysaccharides are abundant in nature and are generally found in almost all living organisms- cells of crustaceans and insects, cell walls of bacteria, yeast and fungi, stems, leaves and seeds of plants, and body fluids of animals (Singh *et al*., 2012).

2.3.1 Okra Polysaccharides

The okra plant is cultivated for its pods which contains a thick and slimy mucilage when immature. Okra mucilages are acidic polysaccharides mixed with proteins. The mucilage obtained from the pods produces stringiness when dispersed in water and hence it is used as a thickening agent to impart characteristic viscosity and smoothness known as the okra effect (Alistar *et al*., 2006). Okra polysaccharides consist of galactose, rhamnose and galacturonic acid (Deters *et al.,* 2005). The sugar composition of okra comprises of different types of polysaccharides which includes pectin, hemicellulose such as xylan and xyloglycan, and cellulose (Sengkhamparn *et al.*, 2009). The polysaccharides in okra extracts are predominately pectins (Ghori *et al.*, 2014).

Pectin is a complex structural polysaccharide which is a constituent of the cell walls of fruits and vegetables. They are primarily found in the cell walls of plants in combination with other polysaccharides like cellulose, hemicelluloses and lignin (Alistar *et al.*, 2006). Pectins are the main structural material of plants and its biological functions include protection of plants against withering and drought. In fruits and vegetables they act as dietary fibre which aid the prevention of diseases such as diabetes and colectral cancer

(Yapo and Koffi, 2008). Pectins are also used as gelling and thickening agents or as a source of stabilizer for acidic milk drinks or oil-in-water (o/w) formulations (Maxwell *et al.*, 2012). Pectin extracts from cell walls are a family of polysaccharides with common features (Ridley *et al*., 2001). Pectin substances are the most complex in terms of structural organization and functionality among polysaccharides (Yapo, 2011). Pectins are acidic heteropolysaccharides with three main constituents which include homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Ghori *et al.*, 2014; (Willats *et al*., 2006). Homogalacturonan is a homopolymer composed of α(1→4) linked α-D-galacturonic acid (GalA) residues containing methyl esterified carboxyl groups (Figure 2.1) and partially acetylated hydroxyl groups (Ghori *et al.*, 2014; Maxwell *et al.*, 2012). The RG-I are highly branched structures with neutral sugar (mainly α-L-arabinose and β-D-galactose) side chains (arabinans, galactans and arabinogalactans) attached to the rhamnose residue (Maxwell *et al.*, 2012). The branches are repetition of α(1→4) linked αD-galacturonic acid and of α(1→2) linked α-L-rhamnose units (Ghori *et al.*, 2014; Vincken *et al.*, 2003). The RG-II segment comprises of a diverse group of monomer sugar units, hence its structure is complex (Ghori *et al.*, 2014; Willats *et al.*, 2006). Pectin extracts can also contain xylogalacturonan (XG) and rhamnogalacturonan (RGH). The presence of free carboxyl group in the uronic acid residue of pectins make its solution exhibit acidic pH (Figure 2.1). Depending on the pH and temperature of the aqueous environment, pectins may undergo chemical modification, hence are not stable (Alistar *et al*., 2006).

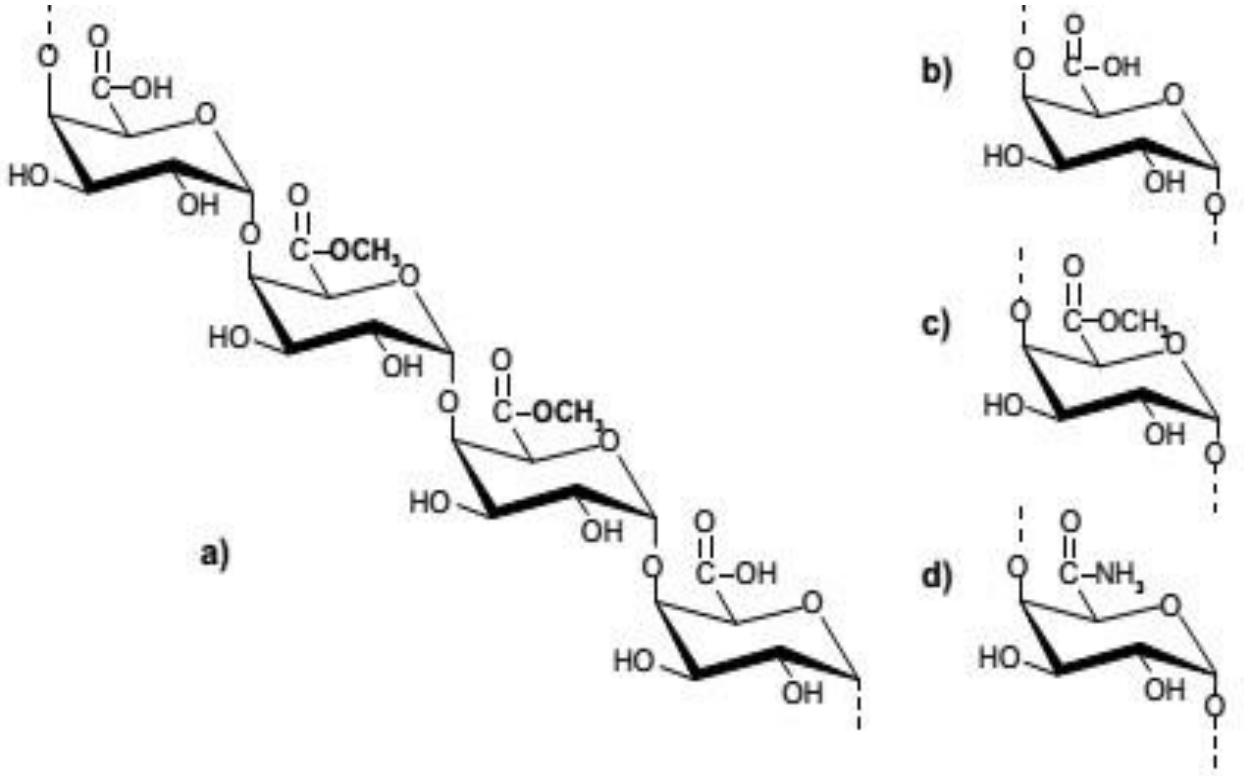


Figure 2.1: a. Repeating segment of pectin molecule and functional group; b. Carboxyl; c. Ester; d. Amide in pectin chain Source: (Sundar Raj *et al.*, 2012)

2.4 Chemical Composition of Okra Polysaccharides

The cell wall of Okra is made up of different types of polysaccharides which include pectin, hemicelluloses (xylan and xyloglucan) and cellulose. The chemical structure of okra polysaccharide is as shown in Figure 2.2. The main sugars present in okra extracts are galactose, galacturonic acid and rhamnose but their specific ratios differ in literature. The ratios of galactose to rhamnose to galactouronic acid as suggested by Sengkhamparn *et al.* (2009) was 1.3:1.0:1.3 in sodium acetate buffer and 4.5:1.0:0.6 in EDTA and sodium acetate buffer. Lengsfeld *et al.* (2004) reported a ratio of 0.9:1.0:0.6 in an okra polysaccharide water extract. Wu *et al.* (1995) and Woolfe *et al.* (1977) reported ratios of 0.4:0.27:0.24 and 1.0:0.1:1.3 respectively in water extracts precipitated with alcohol.

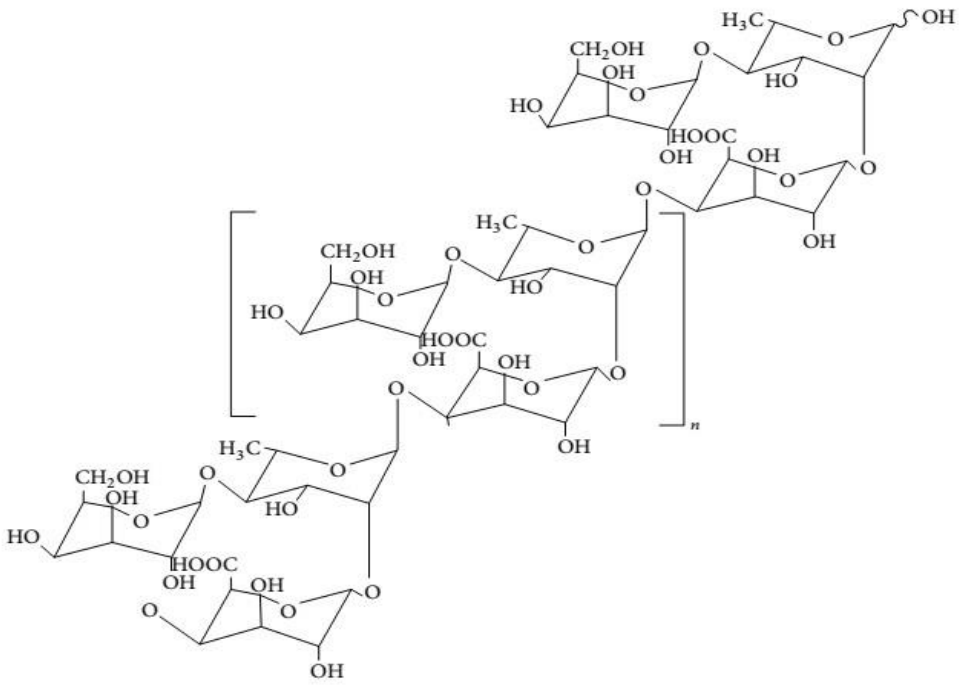


Figure 2.2: Chemical structure of okra polysaccharide as proposed by Zaharuddin *et al.* (2014) and Mishra *et al*. (2008)

Determinations of the monosaccharide constituents of okra have given widely varied results probably more because of differences in purity than differences in source (Whistler and BeMiller, 1993). However the basic structure of the major component would appear to be that of a rhamnogalacturonogalactan, commonly called rhamnogalacturonan (Whistler and BeMiller, 1993). From methylation analysis and partial degradation, Tomoda *et al.* (1980) suggested that the major component of okra gum has a main chain of -> 4)-aGalpA(l -» 2) a-L-Rhmp (l -» in which half of the L-rhamnopyranosyl units possess branches of /3Galp (l -» 4) Galp on 0-4.

2.5 Structural Composition of Okra Polysaccharides

The polysaccharides of plants are complex structures characterised by a variety of details (Mikshina *et al.*, 2012) hence research studies on specific plants such as okra is relevant in

the elucidation of these complex structural details and their useful functional roles. The present research work investigated the detailed structure of okra polysaccharides from different okra genotypes. Structural features of the polysaccharides were extensively studied to establish co-relation among the structures of the different okra genotypes. Structure clarification of the different genotype polysaccharides was to provide a basis for the application of a particular genotypes in food systems based on its unique characteristics. Different genotypes of okra (*Abelmoschus esculentus)* might have different mucilage yield or content. The composition of okra mucilage has been a subject of controversy due to genotype differences coupled with variations in the extraction procedure or the uncertain homogeneity of resulting preparations (Bhat and Tharanathan, 1986). Since food structure is of prime importance in all aspects of functionality (Nielsen, 1998), this study investigated the structural details of different okra genotypes using homogeneous extraction methods and conditions to establish structural variations which will subsequently inform its specific food and other applications.

To understand the structural details of okra pectin from different varieties, this study employed NMR and FTIR spectrometry techniques, molecular weight determination techniques, mass spectrometry of lower molecular weight upon hydrolysis of side chains and backbones to analyse the whole polysaccharide structure (Mikshina *et al.*, 2012). "Nuclear magnetic resonance is a branch of spectroscopy that is useful for analysis because of the exquisite sensitivity of magnetic atomic nuclei (nuclear spins) to their environment. NMR measure the magnetic properties of spins. This magnetic behaviour is determined by molecular and ionic structure, motion, and interactions. These in turn are determined by chemical composition, distributions of mass among different phases (solid, viscous,

liquid), molecular mobility (rotational and translational diffusion), and chemical and physical change in food materials. Nuclei and electrons that are magnetic occur naturally in all matter, and thus can be observed easily in foods via their resonance with electromagnetic radiation. Because magnetic resonance "sees" the interior of the samples, it is usually unnecessary to disrupt the sample beyond shaping it to fit in the magnet" (Nielsen, 1998). NMR spectroscopy analysis done by Sengkhamparn *et al.* (2009) showed that okra contained RG- I structures. NMR spectra of the flax cell wall also demonstrated that the polymer is built as a rhamnogalacturonan I (Mikshina *et al.*, 2012).

2.6 Rheological Characteristics of Okra Polysaccharides

The Greek word "rheos" is synonymous to the term flow, hence rheology is the study of the flow and deformation of matter (Picout and Ross-Murphy, 2003).

Polysaccharides determine rheological behaviour due to the large volume these macromolecules occupy in an aqueous system (Kontogiorgos *et al.*, 2012). Plant derived non-starch polysaccharides help modify texture and rheological properties and improve the stability of food (Alamri *et al*., 2013). Xu *et al*. (2006) noted that dispersion of polysaccharides in water improve the gelation and thickening properties of aqueous food systems. The viscoelastic characteristic of okra water extracts is primarily due to the presence of its polysaccharides (Georgiadis *et al.*, 2011). Although the rheology of okra has been a subject of limited studies (Meister *et al*., 1983; Ndjouenkeu *et al.*, 1996; Sengkhamparn *et al.*, 2010; Kontogiorgos *et al.*, 2012; Alba *et al.*, 2013; Alba *et al*., 2015), understanding the rheological behaviour of okra polysaccharide extracts from different genotypes is critical for possible applications in both food, non-food and pharmaceutical products.

2.6.1 Viscosity

2.6.1.1 Viscosity of Polymer Molecules

Viscosity is an important property that affects mouth feel, the texture and some processing factors of foods (Yu *et al*., 2007). Consumer acceptance of liquid based semisolid type foods is usually dependent on the viscosity and consistency of the product (Fennema, 1996). Viscosity is defined as the measure of a fluid's resistance to motion upon the application of a shearing stress (Barbosa-canovas *et al.*, 1996). The viscosity of a solution relates to its resistance to flow under an applied force (or shear stress); hence the presence or absence of polymer molecules within a solution directly affects the flow properties of a fluid. Polymer molecules in solution resist the flow properties of a solution by either impeding the movement of the micro molecules of the solvent or forming interactive intermolecular bonds with components of the solvent system. This situation produces a thickening effect referred to as "viscous". The higher the amount of polymer in a solution the thicker and more viscous the solution. Hence a measure of the viscosity of a solution is an indirect measure of the molecular weight of polymers present in the solution. Higher molecular weight polymers will occupy more space (hydrodynamic volume) in solution, have more interactions with solvent molecules and ultimately increase solution viscosity.

2.6.1.2 Shear Stress and Shear Rate

As regards the relationship between shear stress and shear rate; for an ideal solution, the shear stress – force per unit area, is directly proportional to the shear rate (that is the velocity gradient between the layers or the liquid (dv/dy). This is expressed as:

$$\frac{F}{\mathrm{A}} = \eta \frac{dv}{\mathrm{dy}}$$

Or

$$\dot{\gamma} = -\frac{dv}{\mathrm{dy}}$$

Definition of Variables:

F- Force; A- Area; $\dot{\gamma}$ = Shear rate; dv/dy = Shear rate; η = Viscosity coefficient

The proportionality constant (η) known as the viscosity coefficient or dynamic viscosity is defined as the ratio of the shear stress and shear rate. Fluids that obey this linear expression between shear stress and shear rate are called Newtonian fluids (Fennema, 1996; Barbosacanovas *et al.*, 1996). Most molecular solutions and food materials do not display Newtonian behaviour, especially at high molecule concentrations. For these systems either the viscosity coefficient decreases when the shear rate increases (shear thinning/pseudoplastic behaviour) or viscosity coefficient increases as shear rate increases (shear thickening/ dilatant behaviour).

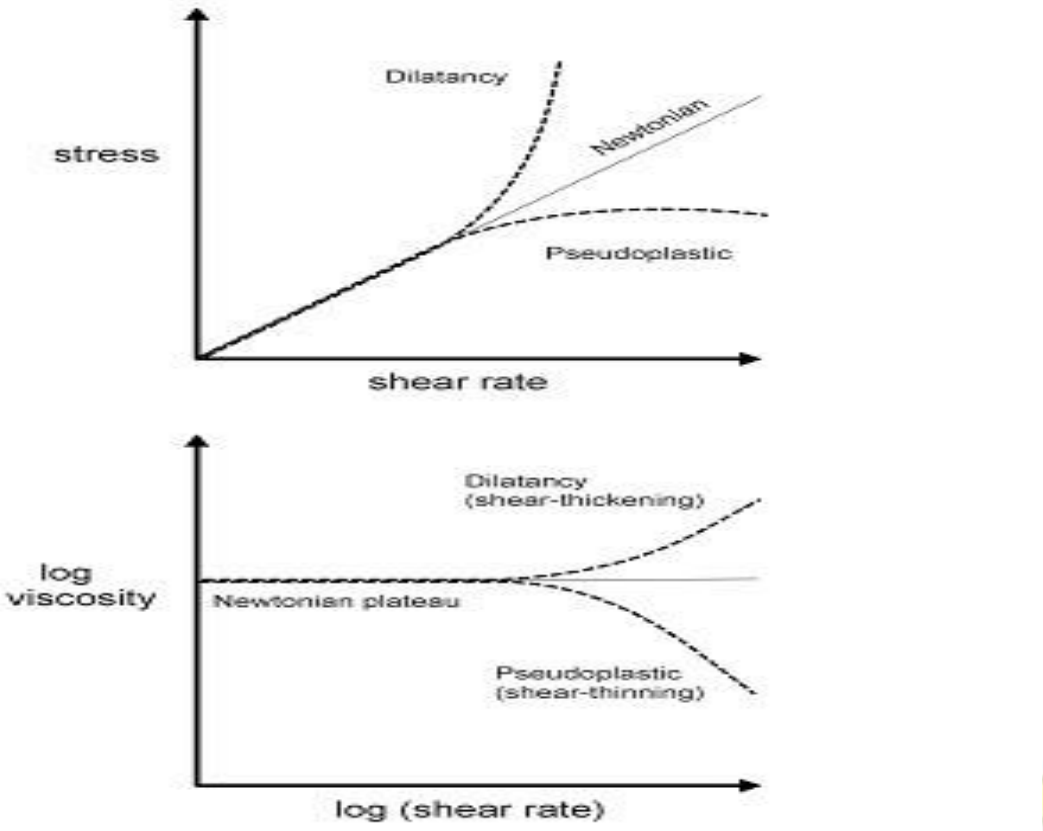


Figure 2.3: Flow curves of different fluids (Picout and Ross-Murphy, 2003)

Pseudoplastic or shear thinning behaviour follows the relationship:

$$F/A = m(dv/dr)^n$$

where m is the consistency coefficient and n is an exponent known as the flow behaviour index. The pseudoplastic behaviour of such solutions arises because of the tendency of molecules to orient their major axes in the direction of flow (Figure 2.3). Okra polysaccharide solutions have been found to be pseudoplastic and viscoelastic (Whistler and BeMiller, 1993; Sengkhamparn *et al.*, 2010). These polysaccharides when extracted in water can result in highly viscous solutions with slimy appearance (Georgiadis *et al.*, 2011). Polymer concentration plays a critical role in the rheology of solutions because in dilute solutions the individual polymer molecules are isolated coils, whiles in concentrated

solutions the total hydrodynamic volume of the individual chains can exceed the volume of the solution (Chamberlain and Rao, 2000; Xu *et al*., 2006).

2.6.1.3 Expressions of Viscosity

The viscosity of fluids can be expressed in different forms given that η_o is the viscosity of the pure solvent, η is the viscosity of the solution and c is concentration:

- Relative Viscosity: It is the viscosity of the polymer solution relative to the viscosity of the pure solvent.

$$\eta_r = \frac{\eta}{\eta_o}$$

- Specific Viscosity: It is the difference in the viscosity of the polymer solution and the pure solvent relative to the viscosity of the pure solvent

$$\eta_{sp} = \frac{\eta - \eta_o}{\eta_o} = \eta_r - 1$$

- Inherent Viscosity: It is expressed by dividing the relative viscosity by the concentration of the solution

$$\eta_i = \frac{\ln \eta_r}{c}$$

- Intrinsic Viscosity: It is expressed by dividing the specific viscosity by the concentration of the solution.

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}$$

2.6.1.4 Dilute Solution Viscometry - Intrinsic Viscosity

Dilute solutions of pectins are characterized by negligible interactions between pectin chains, hence intrinsic viscosity of the biopolymers is dependent only on the dimension of the polymer chain: $[\eta]$ = lim c→0 (η_{sp}/c); η_{sp} – Specific viscosity, C-

Concentration of polymer (Alistar *et al*., 2006). In dilute solute solutions polymers separate from each other and pectin conformation is mainly affected by pectin-solvent interactions and not pectin-pectin interaction (Hua *et al.*, 2014). The viscosity of the solution is affected by environmental conditions, however when the concentration of pectin molecules is increased polymers aggregate to form a three dimensional structure leading to the formation of a gel (Hua *et al.*, 2014). Dilute pectin solutions show a Newtonian behaviour up to approximately 0.5 % depending on the pH and ionic strength of the solution. Viscosity of very dilute solutions increase with increasing pH in the range pH 2-3 and pH 6-7 (Alistar *et al*., 2006).

Intrinsic viscosity is a measure of the hydrodynamic volume of macromolecules in dilute solutions (Xu *et al*., 2006). It usually reflects the hydrodynamic volume of individual polymer coils, and when multiplied by concentration gives an index of the total degree of space occupancy (Ndjouenkeu *et al.*, 1996). Intrinsic viscosity measurements are of importance in biopolymer characterization and hence are used to characterize the volume occupied by individual polymer molecules in isolation (Kontogiorgos *et al.*, 2012).

2.6.1.5 Experimental Determination of Intrinsic Viscosity

Intrinsic viscosity experiments are performed using a glassware referred to as Ubbelohde capillary viscometer (Figure 2.4).

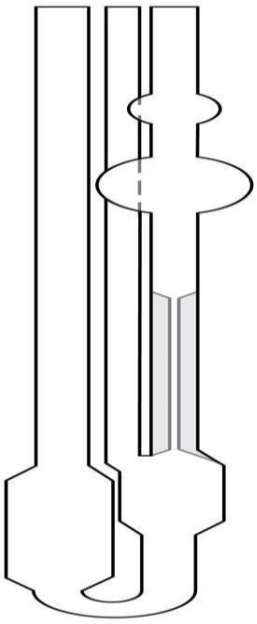


Figure 2.4: Schematic representation of a Ubbelohde capillary viscometer- relative viscosity of solvent in capillary (Harding, 1997)

The time taken for polymer solution to flow through the tube (efflux time) is measured at varying dilute concentration regimes and used as an index for intrinsic viscosity. If kinetic energy terms are ignored, the efflux time is proportional to the solution viscosity and inversely proportional to the density according to the equation (Harding, 1997):

$$t_{solvent} = \frac{\eta_{solvent}}{\rho_{solvent}}$$

$$t_{solution} = \frac{\eta_{solution}}{\rho_{solution}}$$

Relative and specific viscosities are determined by comparing the flow time of the solution to the flow time of the pure solvent at varying concentrations. η_{sp}/c and In η_r/c are then plotted

as a function of concentration. Intrinsic viscosity of the polymer solution is measured by the extrapolation of both curves to zero concentration (Temperature- 20 ^{o}C; pH-7). The curves extrapolate to the same η but approach zero concentration with different slopes (Figure 2.5)

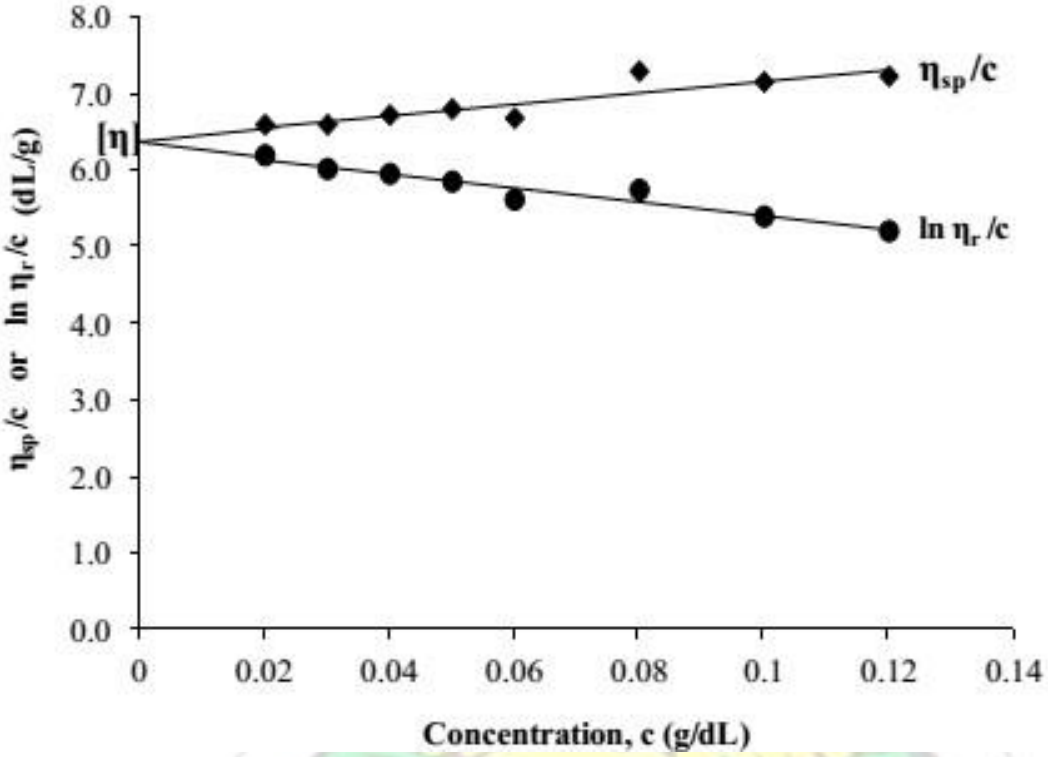


Figure 2.5: Huggins and Kraemer plot of dilute β-glucan polymer solution (Agbenorhevi, 2011)

2.6.1.6 Huggins and Kraemer Equations

The Huggins and Kreamer equations are used to determine the intrinsic viscosity of a polymer solution by a double extrapolation of their separate linear plots.

$$\frac{\eta_{SP}}{C} = [\eta] + d_{Huggins}\, C = [\eta] + k'[\eta]^2 C \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Huggins, 1942)}$$

$$\frac{\ln(\eta_r)}{C} = [\eta] + d_{Kraemer}\, C = [\eta] + k''[\eta]^2 C \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Kraemer, 1938)}$$

Where η_{sp} – specific viscosity, η_r- relative viscosity, C- polymer concentration (mg dL^{-1}), k'-Huggins constant and k''-Kraemer constant. dHuggins and dKraemer are slopes of the Huggins and the Kraemer plots respectively.

The Huggins and Kraemer constants are obtained using the following equations;

$$k' = \frac{d_{Huggins}}{[\eta]^2}$$

$$k'' = \frac{d_{Kraemer}}{[\eta]^2}$$

The Huggins and Kraemer constants are related by the following equation

$$k' = k'' + 0.5$$

Depending on the flexibility of polymers, the Huggins constant ranges from 0.2 to 0.8 for flexible chains and approximately 2 for uncharged spheres (Kontogiorgos *et al.*, 2012; Harding, 1997). Huggins constant values for okra pectins ranges from 0.43 to 0.58 reflecting a flexible chain conformation (Kontogiorgos *et al.*, 2012). The Kraemer equation is an empirical equation which expresses concentration dependence on the relative viscosity of a polymer in dilute solution. A plot of In η_r/c versus c is a straight line with the intercept at c = 0 being the intrinsic viscosity. The Kraemer constant is a value which is dependent on the polymer/solvent/temperature under consideration. Usually it has values in ranges less than that of similar Huggins constant.

2.6.1.7 Factors that Influence the Viscosity of Polysaccharide Solutions

Viscosity of polysaccharide solutions is influenced by factors such as molecular weight, stiffness, charge and charge density, polymer conformation/structure and pH

(Phillips and Williams, 2009; Alba *et al.*, 2013; Kontogiorgos *et al.*, 2012; Xu *et al.*, 2006; Alistar *et al.*, 2006). Depending on the plant source, stage of ripening and extraction method, pectins exhibit heterogeinity in molecular weight. Pectins have lower molecular weight and intrinsic viscosities when compared with other polysaccharides like xanthan gum, guar or locust bean gum (Alistar *et al.*, 2006). This attribute of lower viscosities in comparison with other hydrocolloids can be exploited for food applications.

As regards factors associated with charge and charge density, okra gum contains a substantial proportion of charged galacturonate residues which influence its inter and intra molecular interactions and consequently affects its viscosity (Ndjouenkeu *et al.*, 1996; Kontogiorgos *et al.*, 2012). The larger the amount of galacturonic acid residues on a pectin chain the greater the repulsive forces between the residues, hence polymers tend to expand and occupy a larger hydrodynamic volume (Figure 2.6). However polymers that contain lower amounts of the galacturonic acid residues coil up via hydrophobic interactions and occupy less hydrodynamic volume in solution (Figure 2.7).

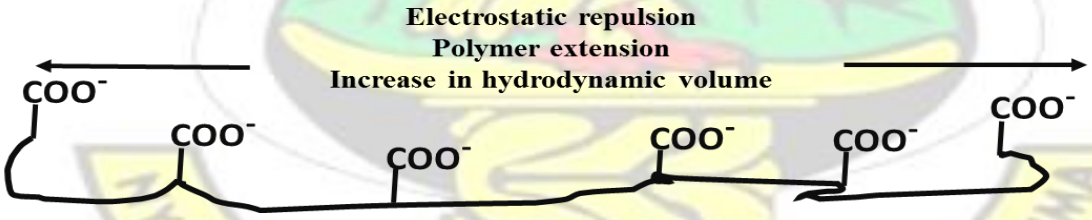


Figure 2.6: Schematic of intra-molecular electrostatic repulsion within individual coil which extend chain length (Ndjouenkeu *et al.*, 1996; Kontogiorgos *et al.*, 2012)

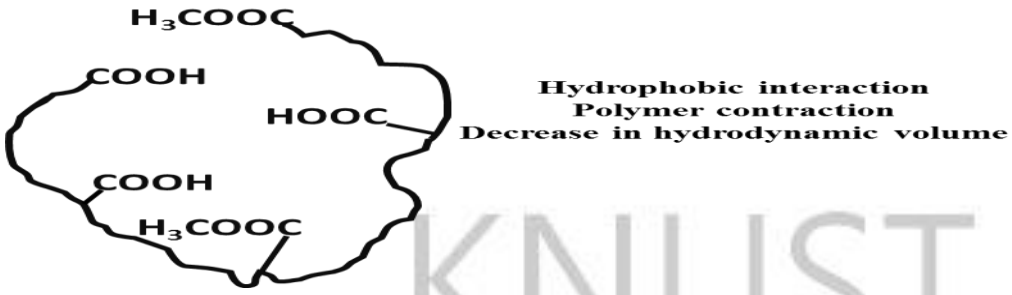


Figure 2.7: Schematic of intra-molecular hydrophobic interaction within individual coil which contract chain (Ndjouenkeu *et al.*, 1996; Kontogiorgos *et al.*, 2012)

Also when pectins are in water the individual coils are expanded by inter- and intramolecular electrostatic repulsion (Figure 2.8a). Addition of salt however screens these repulsions (Figure 2.8b) and allow coils to contract to a more compact conformation with consequent reduction in intrinsic viscosity (Ndjouenkeu *et al.*, 1996). Hence under electrostatic screening provided by high NaCl concentration, the intrinsic viscosity reflects the volume occupied by the chains in the absence of inter polymer interactions (Kontogiorgos *et al.*, 2012). The extent of coil expansion of a polymer with counterions in a water medium decreases as the concentration of the polymer increase (Ndjouenkeu *et al.*, 1996).

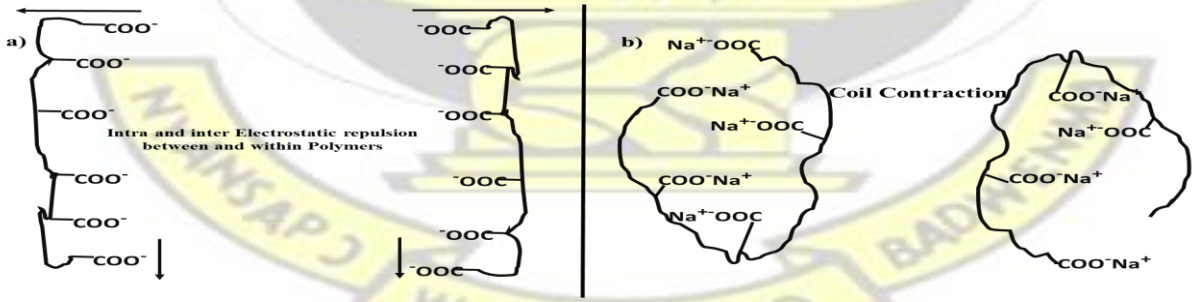


Figure 2.8: Schematic of electrostatic interactions between pectin chains. a. Intra and inter electrostatic repulsion between and within polymers b. Electrostatic screening caused by presence of NaCl salt which contract coil (Ndjouenkeu *et al.*, 1996; Kontogiorgos *et al.*, 2012)

Different rheological techniques agree that interactions between polymeric chains (Figure 2.9) occur as the concentration of the polymer increase and this influence the flow behaviour of okra pectins (Kontogiorgos *et al.*, 2012).

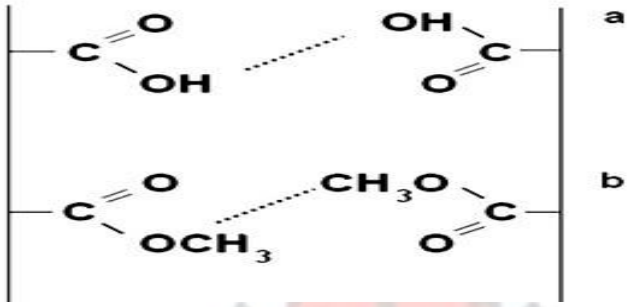


Figure 2.9: Structure formation mechanisms between pectin chains. a. Hydrogen bonds between undissociated carboxyl groups b. Hydrophobic interactions. Source: (Kastner *et al*., 2012)

Kontogiorgos *et al.* (2012) further noted that okra pectins in a medium that consist of 0.1M NaCl and pH 7, produce relatively low viscous solutions at high concentration, hence okra pectins may not be able to form gels at industrially significant concentration. However since gelation of polyelectrolytes depends on conditions such as ionic strength, pH, and the presence of specific cations (such as Ca^{2+}) further investigation is required to elucidate the gelation properties of okra polysaccharides (Kontogiorgos *et al.*, 2012). pH can also influence the viscosity of polysaccharides in solutions because it has been reported that pectins extracted at higher pH may have greater degree of methylation and contribute to stronger intermolecular interactions (Pagan *et al.*, 1999; Alba *et al.*, 2013). Considering molecular structure and its influence on viscosity, Sengkhamparn *et al.* (2010) stated that the hydrophobic acetylated rhamnogalacturonan I (RG-I) molecules of okra pectins increase viscosity to a higher extent than acetylated galacturonic acid (GalA) residues.

2.7 Functional and Physicochemical Characteristics of Okra Polysaccharides

Functional properties are those physical and chemical properties that influence the behaviour of polymers in food systems during processing, storage, cooking and consumption (Moure *et al.,* 2006). Proteins impart desirable functional properties like water and oil absorption capacities, viscosity, emulsification, gelation, foam formation and whipping capacity to food systems (Moure *et al.*, 2006; Yu *et al*., 2007). Polysaccharides because of their hydrophilic character are normally not considered as useful emulsifying agents (Alba *et al.*, 2013). Nevertheless acetylated pectins from sugar beet possess greater surface activity and hence is capable of stabilizing o/w emulsions (Alba *et al.*, 2013). Similarly acidic, random coiled and viscous okra pectins differ substantially from other plant pectins in terms of protein and acetyl content indicative of their hydrophobic characteristic and hence could also be used as effective emulsifying agents. Alba *et al.* (2013) further illustrated that okra extracts at different pHs (pH 6 and pH 4) showed good emulsification abilities but varied efficiencies in long term emulsion stability due to differences in pH of extraction medium, resulting in variations in the structure as well as amino acids in the extracts. Okra pectin extracted at pH 6.0 also showed potential as emulsifiers in acidic environment producing fine emulsion with stability against coarsening (Ghori *et al.*, 2014; Alba *et al*., 2013).

2.8 Gelation Properties of Pectins

Pectin is used in food and non-food systems mainly due to its gelling and thickening abilities (Ström *et al*., 2014). It is applied as a gelling agent in jam and jellies, confectionary products and bakery filling. Aside these food applications, pectins can also be used as stabilizers and thickeners in acidified milk and yoghurt products. Depending on the degree

of methylation which influences gelling characteristics of the polymer, pectins can be classified as high methoxy pectins (HMP) and low methoxy pectins (LMP) (Ström *et al*., 2014). The most important environmental factors that trigger and accelerate gelation of the pectin biopolymer include pH, calcium ions and soluble solids (sugar) (Kastner *et al*., 2012). These factors however have different and partly opposite effect on the gelling process of the different types of pectins (Kastner *et al*., 2012).

2.8.1 High Methoxy Pectins (HMP)

High methoxy pectins are biopolymers which contain >50 % of methyl esters in their molecular structure and gel under acidic conditions in the presence of high sugar concentration (Ström *et al*., 2014; Kastner *et al*., 2012; Fraeye *et al*., 2009). The mechanism of gelling in HMP is explained by hydrophobic interactions formed by higher temperatures and hydrogen bonds formed between undissociated carboxyl groups at lower temperatures (Kastner *et al*., 2012). Calcium does not influence the gelation of HMP, however a study conducted by Yang *et al.* (2013) indicated the possibility of calcium cations triggering and accelerating the gelation of high methoxy pectin. Since the mechanism of gelation of HMP by calcium is not clear, studies have to be conducted to establish the exact role of calcium in this gelation process.

2.8.2 Low Methoxy Pectins (LMP)

Low methoxy pectins are pectin biopolymers which contain <50 % methyl esters and gel in the presence of multivalent ions under acidic conditions according to the egg box model (Ström *et al*., 2014). Gels can also be formed in the presence of 0.2M monovalent cations (Ström *et al*., 2014). The gelling mechanism is explained by interactions through ionic inter-chain associations (Cardoso *et al*., 2003). High amount of

free carboxylic acid groups in low methoxy pectin enables the polymer to interact with divalent ions such as calcium which results in a continuous gel network system (Fraeye *et al.*, 2009). Figure 2.10 shows calcium gel networks generated via junction zones (Ngouémazong *et al.*, 2012).

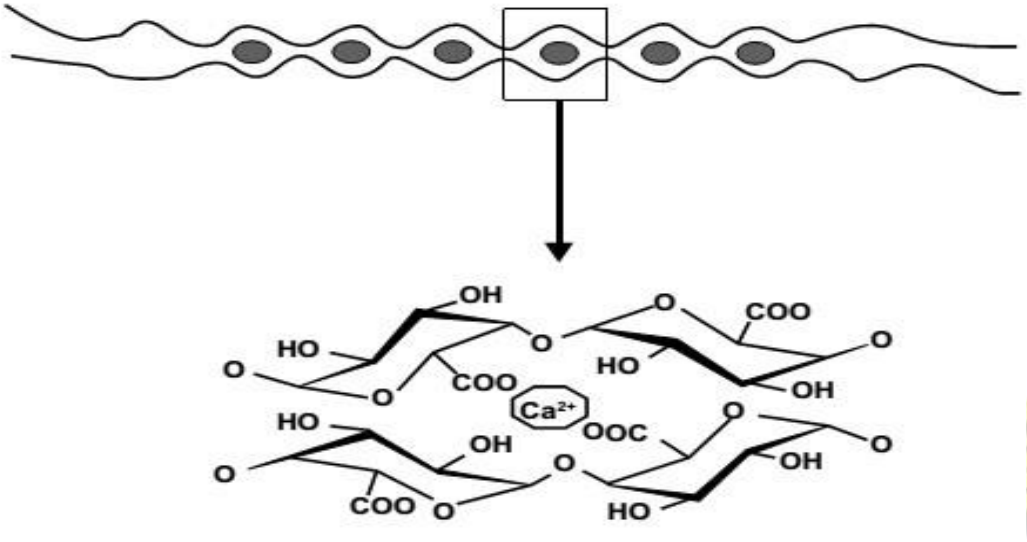


Figure 2.10: Schematic of calcium binding to polygalacturonate sequences: 'egg box dimer' and 'egg box cavity'. Source: (Sundar Raj *et al.*, 2012)

The calcium ions enter the cavities provided by the participating polygalacturonic sequences and adopt a zigzag conformation (Li *et al.*, 2013). The egg box model describes pectin chains in which a number of adjacent residues that are non-methoxylated chelate Ca^{2+} ions (Fraeye *et al.*, 2009). However the exact number of successive non-methoxylated GalA residues needed to form 'egg box' configuration had been estimated to be 6 - 13 (Luzio and Cameron, 2008), 9 (Liners *et al*., 1992), 20 (Braccini and Perez, 2001). LMP gels can be formed with or without sugar and over a wide range of pH (Hua *et al.*, 2014). Although higher sugar

concentration is not essential for gelation in LMP, its presence could support the binding of water and promote close interactions between neighbouring molecules (Kastner *et al*., 2012). Electrostatic attractions between LMP polymers are stronger when the pectin is fully charged at pH > 4.5, hence gels can be formed in the presence of calcium ions at high pHs (Capel *et al.*, 2006; Li *et al.*, 2013). A decrease in the pH of LMP solution decreases charge density which correspondingly decrease the sensitivity of LMP to calcium ions (Hua *et al.*, 2014). The presence of amide or acetyl group on the LMP chain differently influence polymer interaction with calcium ions. Whereas amidated LMP require less Ca^{2+} for gelling because hydrogen bond between amide groups promote gel formation at low pH ≤ 3.5, the acetyl groups of pectins (pectins that are naturally acetylated) decrease the binding strength of the biopolymer to calcium ions (Hua *et al.*, 2014). Okra pectins are highly acetylated hence unable to gel in the presence of calcium. Hence the possibility of deacytylation of pectins from okra and subsequent gelling in the presence of calcium can be considered in future studies.

2.9 Applications of Okra Polysaccharides

2.9.1 Food Application of Okra Polysaccharides

Okra polysaccharides have useful applications in food systems due to its characteristic physicochemical, functional and rheological properties. The polysaccharides can potentially replace egg white and fat in chocolate bars or frozen desserts (RomanchikCerpovicz *et al*., 2006). Okra polysaccharides have unique water holding capacity and solubility properties that can strengthen dough used for bakery-cakes and cookies. Alamri *et al*. (2012) reported that okra extracts increased firmness of bread loaf due to water migration from crump to crust. The polymer extracts have shown good emulsion stability properties under acidic environments and hence have potential applications in fruit drinks and acidified dairy products (Ghori *et al.*, 2014; Alba *et al.*,

2013; Kontogiorgos *et al.*, 2012; Ndjouenkeu *et al.*, 1996). The inclusion of okra powder in ojojo-a fried yam snack significantly increased the viscosity and dispersability of the reconstituted yam flours and also the sensory attributes of fried yam products subsequently developed (Shittu and Olaitan, 2011).

2.9.2 Pharmaceutical Applications of Okra Polysaccharides

Natural gums and mucilage have been widely useful in drug formulations as a thickener, emulsifier, stabilizer, gelling agent, granulating agent, suspending agent, binder, film former, disintergrant and as sustained release matrix (Sangwan *et al.*, 2011). The preference of natural gum over the synthetic in drug formulations is because products from the natural polymer are economical, readily available, non-toxic, capable of chemical modification, biodegradable and biocompatible (Avachat *et al*., 2011; Sangwan *et al.*, 2011; Jain *et al*., 2007). Okra gum in drug formulations serves as a binder and shows a faster onset and higher ability of plastic deformation than drug containing gelatin (Avachat *et al*., 2011). Okra gum produce tablets with extended disintegration times (Avachat *et al*., 2011). The polysaccharides have been successfully used to create modified release drug formulations due to its excellent swelling and dissolution properties. Hence it is used as hydrophilic matrix tablets which acts as oral drug delivery vehicle for controlled drug release applications (Ghori *et al.*, 2014).

2.9.3 Physiological Benefits of Okra Polysaccharides

Investigations conducted on the effects of okra polysaccharide on metabolic disorders of mice found that the polysaccharides lowered body weight and glucose levels, improved glucose tolerance and decreased serum cholesterol levels in high fat diets fed to the mice (Fan *et al.*, 2013). Amin (2011) also reported that the mucilaginous textural

properties of okra impacts health properties by mopping up unhealthy cholesterol, toxin, and mucous waste from the intestinal tracts. The polymer acts as a laxative that can heal ulcer, reduce acid reflux and generally promote cardiovascular and gastrointestinal health (Amin, 2011). Fresh extracts of okra have been used to inhibit *Helicobacter pylori* because the polysaccharide possesses anti-adhesive properties that interrupt the adhesion of *H. pylori* to human stomach tissue (Messing *et al.*, 2014). Plant derived non-toxic polysaccharide from okra could also be used as potential antitumor immunomodulatory agent in health food and pharmaceutical therapy due to its thick and slimy property (Zheng *et al.*, 2014). Kumar *et al*. (2013) summarized these physiological benefits of okra as follows:

- Okra contain special fibre which could be used to manage blood glucose levels.
- Mucilage in okra mops bad cholesterol and toxic substances in the body.
- Purgative properties of the polysaccharide are useful in bowel purification.
- The fibre contain valuable nutrients for intestinal microorganisms.
- Okra is an effective remedy for ulcers; it is used to counteract the acids.
- Okra is applied as treatment for pulmonary inflammations and bowel irritation.

CHAPTER 3 PHYSICO-CHEMICAL DIVERSITY OF OKRA PECTINS FROM DIFFERENT

GEOGRAPHICAL LOCATIONS OF GHANA

3.1 Introduction

Okra (*Abelmoschus* spp.) is cultivated throughout the tropical, sub-tropical and temperate regions of the world including the shores of the Mediterranean Sea. Okra has

high economic value, good nutritional and functional properties. The polysaccharide content known as pectin is responsible for the slimy texture of okra extracts and is of major technological interest for food, non-food and pharmaceutical/medicinal applications (Ghori *et al.*, 2014; Alba *et al.*, 2013). Okra polysaccharides can be used as emulsifiers, thickeners, stabilizers and as egg white and fat substitute in cookies, chocolate bars, fruit drinks and dairy products. Okra is a potential source of natural polysaccharides which can be exploited industrially as functional ingredients in food and non-food applications (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.*, 2013). 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). Water extracts of okra are thick and slimy hence are used in stews and soups mainly as thickeners and viscosity enhancers. The thick and slimy texture is attributed to its polysaccharides known as pectins (Samavati, 2013; Kontogiorgos *et al.*, 2012; Alba *et al.*, 2013; Ghori *et al.*, 2014). Different varieties of okra are likely to have different mucilage yield and composition. Okra polysaccharides have been isolated on a laboratory scale and evaluated for a number of food and non-food applications (Ndjouenkeu *et al.*, 1996). Research studies have employed different solvent protocols for the extraction of okra polysaccharides and this include water, sequential buffer systems, ethanol, methanol, acetone, phosphate buffer systems and mixture of solvent extraction systems (Alba *et al*., 2015; Zheng *et al.*, 2014; Alba *et al.*, 2013; Archana *et al.*, 2013; Samavati, 2013; Alamri *et al.*, 2013; Kontogiorgos *et al.*, 2012; Sheu and Lai, 2012; Georgiadis *et al.*, 2011; Ameena *et al.*, 2010; Sengkhamparn *et al.*, 2010; Ndjouenkeu *et al.*, 1996; Woolfe *et al*.,

1977). 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). Isolation of polysaccharides have also been done on a laboratory scale using sequential solvent treatments (Sengkhamparn *et al.*, 2010; Georgiadis *et al.*, 2011; Kontogiorgos *et al.*, 2012; Alba *et al.*, 2013).

The physicochemical and functional properties of food polysaccharides are essential factors to consider in food processing and formulation of new food products. Genotypes of a plant material (and in this case, okra) from different geographical zones may have different physicochemical properties which influences functional characteristics. To our knowledge, pectin isolates from okra in different geographical zones have not been characterized for their potential applications. The objective of the present study, therefore, was to evaluate the effect of okra obtained from different geographical locations on pectin yield and physicochemical properties.

3.2 Material and Methods

3.2.1 Collection of Okra Types from Different Ecological Zones

Soft and mature okra pods (5 - 9 cm) of seven (7) different okra types which were approximately 3 months old were obtained from local farmers in five different geographical areas (Northern, Volta, Brong Ahafo, Ashanti and Eastern regions) of Ghana for the study. The okra types used were- *Asha and Penkruma* (Ashanti region); *Agbagoma, Akrofo and Sengevi* (Volta region); *Pora* (Northern region); Asontem (Eastern region); Penkruma (Brong Ahafo region). Two different samples of the okra type *Penkruma* were obtained from two different geographical areas; Ashanti and Brong Ahafo regions. Hence a total of Eight (8) samples were studied. The pods were immediately frozen and kept at 20 ^{o}C until further preparations were done.

3.2.2 Preparation of Okra Pods

The Okra fruits were separated into three parts: pod, calyx and seed. The okra pods were freeze dried and then subjected to further analysis. The okra pods were freeze dried to maintain material in the original state prior to pectin extraction. The freeze dried material (20 g) was subjected to extraction procedure as shown in Figure 3.1 (Alba *et al*., 2015).

3.2.3 Preparation of Lipid Free Freeze-dried Material

Freeze dried okra powder (20 g) was defatted with Petroleum ether (1 g:10 ml) by placing the okra-ether mixture on a rotar shaker (120 rpm, 25 ^{o}C) for 4 h. Extraction of the freeze dried okra powder with petroleum ether was to free the material from lipids prior to isolation of the okra pectins.

3.2.4 Aqueous Extraction of Pectin

The defatted okra powder was subjected to aqueous extraction with 0.1 M phosphate buffer (1 g freeze-dried defatted powder:30 ml buffer solution), pH 6 at 80 ^{o}C for 1 h. Aqueous extraction of the polysaccharides at a temperature of 80 ^{o}C and low pH of 6 was to extract the water soluble fraction. Studies have established that the strong bonds between protopectin and other cell wall materials are cleaved at high temperatures and low pH, hence extraction of pectin under these conditions of temperature and pH results in high percentage yield (Alba *et al.*, 2015; Samavati, 2013). Alba *et al*. (2015) noted that at 80 ^{o}C the solubilisation of insoluble protopectins are facilitated. After extraction, the soluble polymer was separated from the insoluble residue by centrifugation (3000 rpm for 10 min at 25 ^{o}C) (Alba *et al.*, 2015). The insoluble residue was subsequently subjected to a repeated aqueous extraction protocol (0.1 M phosphate buffer, pH 6, 80 ^{o}C for 1 h, 1:15

ml) and the supernatant separated by centrifugation (3000 rpm for 10 min at 25 ^{o}C) (Alba *et al*., 2015). The second aqueous extraction stage was to maximize pectin yield and ensure that a high amount of insoluble pectins are solubilized and extracted. The solubilized protopectins collected as supernatant in the two aqueous extraction stages were concentrated by rotary evaporation at 80 ^{o}C (Alba *et al*., 2015).

3.2.5 Alcohol Extraction of Pectin

Solubilized okra protopectins were subjected to alcohol extraction using 96 % (v/v) ethanol kept at 40 ^{o}C for 1 h (1:2). Extraction with aqueous alcohol is to remove proteins and some polar compounds. Pectin substances were precipitated with the 96 % (v/v) ethanol. The extraction with ethanol was followed by washing using isopropanol and then freeze-dried (Alba *et al*., 2015).

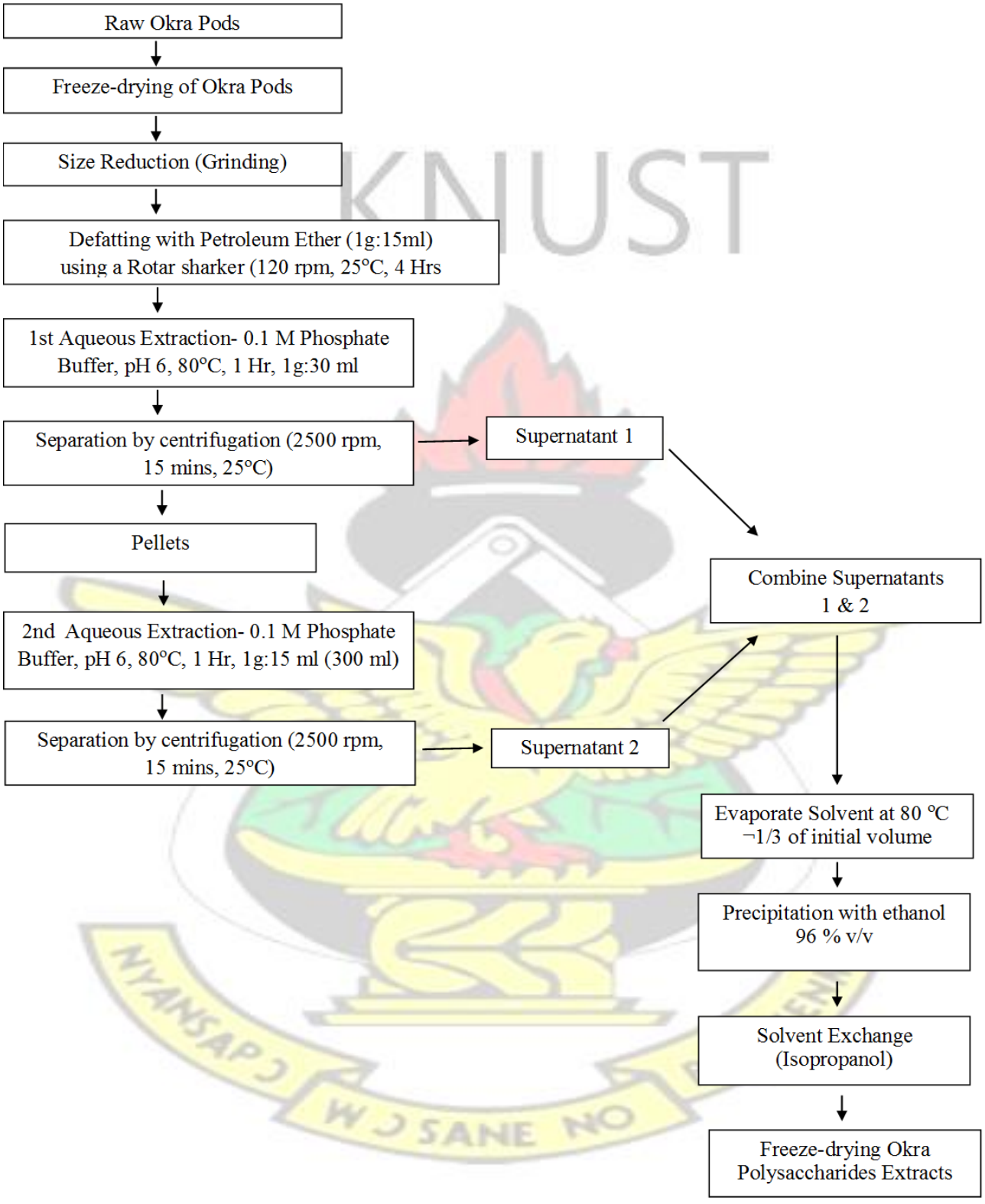


Figure 3.1: Protocol for isolating polysaccharides from okra pods (Alba *et al*., 2015)

3.2.6 Polysaccharide Yield Determination

The percentage yield was calculated based on the amount of dry powder sample used for the extraction process and the amount of dry soluble polysaccharide obtained after extraction (Archana *et al.*, 2013; Samavati, 2013). The percentage yield (w/w) was calculated using the formula:

$$\text{Percentage extraction yield} = \frac{\text{Weight of freeze dried polysaccharide}}{\text{Weight of dried okra powder}} x\ 100$$

3.2.7 Protein Content Determination

Protein quantification was done using the Lowry method (Lowry *et al.*, 1951). A standard calibration curve was generated with Bovine Serum Albumin (BSA) standards (200 -3200 µg ml^{-1}) and used to estimate the protein content of samples (Appendix 3.1). The blank (0 µg ml^{-1}) was made using distilled water and dye reagent. Each standard and blank (100 µl) were pipetted into separate clean test tubes. Lowry reagent (100 µl) was added to each tube, vortexed and incubated for 10 min at room temperature. FolinCiocalteau phenol reagent (50 µl) was then added to the solution. The resulting solution was vortexed and incubated at room temperature for 30 min (samples were not incubated for more than 1 h). Absorbance readings were then taken at 650 nm using a nanospectrophotometer (Nano drop Technologies, ND-1000UV/VIS, 9231, USA).

3.2.7.1 Preparation of Okra Sample for Protein Assay

Freeze dried okra polysaccharide extract (1 mg) was weighed and dissolved in 1 ml of distilled water. The mixture was warmed at 40 ^{o}C until pectins dissolved. Samples (100 µl)

were pipetted into separate clean test tubes. Lowry reagent (100 µl) was added to each tube, vortexed and incubated for 10 min at room temperature. Folin-Ciocalteau phenol reagent (50 µl) was then added to the solution (Lowry *et al.,* 1951). The resulting solution was vortexed and incubated at room temperature for 30 min (samples were not incubated for more than 1 h). Absorbance readings were then taken at 650 nm using a nanospectrophotometer (Nano drop Technologies, ND-1000UV/VIS, 9231, USA).

3.2.8 Total Carbohydrate Content Determination

Total carbohydrate was estimated using the phenol-sulphuric acid assay (Dubois *et al.,* 1956). A standard calibration curve was generated with galactose (0 - 40 µg ml^{-1}) and used to estimate the total carbohydrate content of samples (Appendix 3.2). The blank (0 µg ml^{-1}) was made using distilled water, concentrated sulphuric acid and 5 % phenol. Each standard (50 µl) and blank (50 µl) were pipetted into separate clean test tubes. Concentrated sulphuric acid (150 µl) was added to each tube. Phenol (5 %; 30 µl) was subsequently added and the resulting solution was vortexed and incubated for 5 min at 90 ^{o}C in a static water bath. The solution was then allowed to cool to room temperature and absorbance readings were then taken at 490 nm using a UV-VI spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, USA).

3.2.8.1 Preparation of Okra Sample

Freeze dried okra polysaccharide extract (10 mg) was weighed and dissolved in 100 ml of distilled water. The mixture was warmed at 40 ^{o}C until complete solubilisation of the polysaccharides. The solubilized polysaccharide solution (2 ml) of each genotype was pipetted into separate clean test tubes. Concentrated sulphuric acid (6 ml) was added to each tube. Phenol (5 %; 1.2 ml) was subsequently added and the resulting solution was

vortexed and incubated for 5 min at 90 ^{o}C in a static water bath. The solution was then allowed to cool to room temperature and absorbance readings were then taken at 490 nm using a UV-VI spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, USA).

3.2.9 Functional Characteristics

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

The WAC and OAC of the mucilage powder from the different genotypes were determined by dissolving 1.0 g of the mucilage powder in 10 ml of distilled water for the water absorption capacity, and 10 ml of sunflower oil for the oil absorption capacity (Noorlaila *et al.*, 2015). The mixture was vortexed for 2 minutes, centrifuged at 3000 rpm for 30 minutes and the supernatant drained off. The WAC/OAC were determined using the formula:

$$\text{WAC/OAC} = \frac{\text{Weight of absorbed water/oil}}{\text{Weight of powder taken}} x\ 100$$

3.2.9.2 Emulsion Capacity

The emulsifying capacity of the mucilage from the different okra genotypes were determined. Each sample (1.0 g) was dissolved in 50 ml distilled water and 50 ml refined oil (Archana *et al.*, 2013). The mixture was hormogenized for 1 minute 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:

$$\text{Emulsion Capacity} = \frac{\text{Volume of emulsified layer}}{\text{Volume of mixture}} x\ 100$$

3.2.10 Structural Analysis

3.2.10.1 FT-IR Determination

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).

3.2.10.2 ^{1}H NMR Spectroscopy

For all the samples ^{1}H NMR was conducted at 70 °C with D_2O as the solvent using Bruker AV 500 spectrometer (Bruker Co., Switzerland) at 500 MHz ^{1}H. The samples (2 mg) were dispersed in 2 mL deuterium oxide (D_2O) and then freeze dried. The freeze-dried samples were subsequently dissolved in 600 µL D_2O prior to performing the ^{1}H NMR Spectroscopy.

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

The molecular weight (MW) of the okra polysaccharides from different genotypes was estimated using Size Exclusion Chromatography Coupled to Multi – Angle Light Scattering (SEC-MALS) Detector at 25 °C. Isolated pectins were solubilised in 0.1 M NaCl solution (2 mg 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, 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. The pectins were eluted with distilled water at a flow rate of 0.7 mL min^{-1} (Alba *et al*., 2015).

3.2.11 Data Analysis

3.2.11.1 Statistical Analysis

Data obtained were analysed using Statgraphics (Centurion XV, Graphics Software System, STCC, Inc. USA) at $p < 0.05$. Data was reported as averages of triplicate and analysed using one-way analysis of variance (ANOVA) to compare the means of all determined parameters followed by LSD post hoc multiple comparisons.

3.3 Results and Discussion

3.3.1 Polysaccharide Yield, Protein and Total Carbohydrate Content

The okra pectins had yields of 11.25 – 14.59 % and an evaluation of the purity level of the crude polysaccharide extract showed protein content between 8 – 15.10 % and total polysaccharide ranging from 58 – 70.1 % (Table 3.1). Okra mucilages are acidic polysaccharides (Deters *et al*., 2005), and comprise of different types of polysaccharides which includes pectin, hemicellulose such as xylan and xyloglycan, and cellulose (Sengkhamparn *et al.*, 2009). However the main polysaccharides in okra extracts are predominately pectins (Ghori *et al.*, 2014). The pectin yield (%) of the crude extracts from the okra types studied were higher than yields reported for extracts using acetone-8.6 %, methanol-0.28 %, distilled water containing 1 % sodium metabisulphite-1.46 % (Noorlaila *et al.*, 2015; Archana *et al.*, 2013) but comparable with yields ranging from 8.8 % to 15.7 % obtained by Alba *et al*. (2015) and Samavati (2013) for okra pectins extracted using similar isolation protocol.

Table 3.1: Polysaccharide yield, protein and total carbohydrate content of okra pectin extracts

Okra Types	Region	Yield (%)	Protein (%)	Total Carbohydrate (%)
Asha	Ashanti	14.59 (1.2)^{b}	8.00 (0.01)^{a}	70.16 (0.01)^{e}

Pora (Kpong)	Northern	14.37 (0.5)[b]	10.12 (0.01)[b]	68.20 (0.01)[d]
Penkruma (Kenkeso)	Ashanti	14.35 (0.1)[b]	13.56 (0.01)[d]	58.36 (0.01)[a]
Agbagoma	Volta	14.17 (0.1)[b]	14.73 (0.06)[e]	59.20 (0.01)[a]
Asontem	Eastern	13.20 (0.6)[b]	11.10 (0.04)[c]	68.30 (0.01)[d]
Akrofo	Volta	11.40 (1.2)[a]	14.83 (0.01)[e]	63.00 (0.01)[b]
Penkruma (Techiman)	Brong Ahafo	11.25 (0.2)[a]	15.10 (0.04)[e]	64.53 (0.01)[c]
Sengevi	Volta	11.25 (0.3)[a]	9.50 (0.05)[b]	59.20 (0.01)[a]

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

The purity levels of the polysaccharides studied were lower than extracts obtained by Alba *et al.* (2015) (protein- 4.3-6.3 %; total carbohydrate- 70-81.8 %), because the crude polysaccharides obtained were not further purified by exhaustive dialysis. Polysaccharides extracts of okra types from different ecological zones showed varied polysaccharide yield and purity (protein and carbohydrate content) although subjected to the same hot acid buffer extraction procedure (Alba *et al*., 2015) and experimental conditions (Table 3.1). Varietal differences, variations in geographical zones and agricultural practices contributed to the differences in polysaccharide yield and composition.

3.3.2 Functional characteristics

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

Hydrocolloids such as pectins have gained extensive technological and biomedical applications due to their ability to function as thickening, emulsifying, stabilizing, binding

and gelling agents in both food and non-food systems. The high water binding ability of these polymers have been attributed to the presence of many hydroxyl groups in the chemical structures (Li and Nie, 2016). Okra pectins have the ability to effectively bind water due to its polysaccharide content resulting in viscous and slimy water extracts (Georgiadis *et al.*, 2011; Archana *et al.*, 2013). The water absorption capacity of pectin influences their characterization as functional ingredients in food and biomedical system (Archana *et al.*, 2013). The WAC of the different okra types were significantly different ($p<0.05$) and ranged from 810 g/100 g to 963 g/100 g (Figure 3.2). The okra type *Akrofo* had the highest WAC whereas the WAC of *Agbagoma* was the least. The varying water absorption capacities of the okra pectins can be attributed to genotypic differences or differences in the ecological zones from which the pods were obtained. Due to the high WAC of the pectins 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.

Traditionally hydrocolloids are not considered as effective oil binders mainly due to their hydrophilicity and high molecular weight (Li and Nie, 2016), however okra pectin 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, 2016). The OAC of okra pectins were appreciably high although considerably and comparatively lower than corresponding WAC. The

hydrophobic groups and the proteins in the okra pectin might have contributed to this high oil absorption capacity. The oil absorption capacities of pectin from the different okra types

were also significantly different ($p<0.05$) and ranged from 232 g/100 g to 384 g/100 g with *Agbagoma* recording the highest OAC value (Figure 3.2).

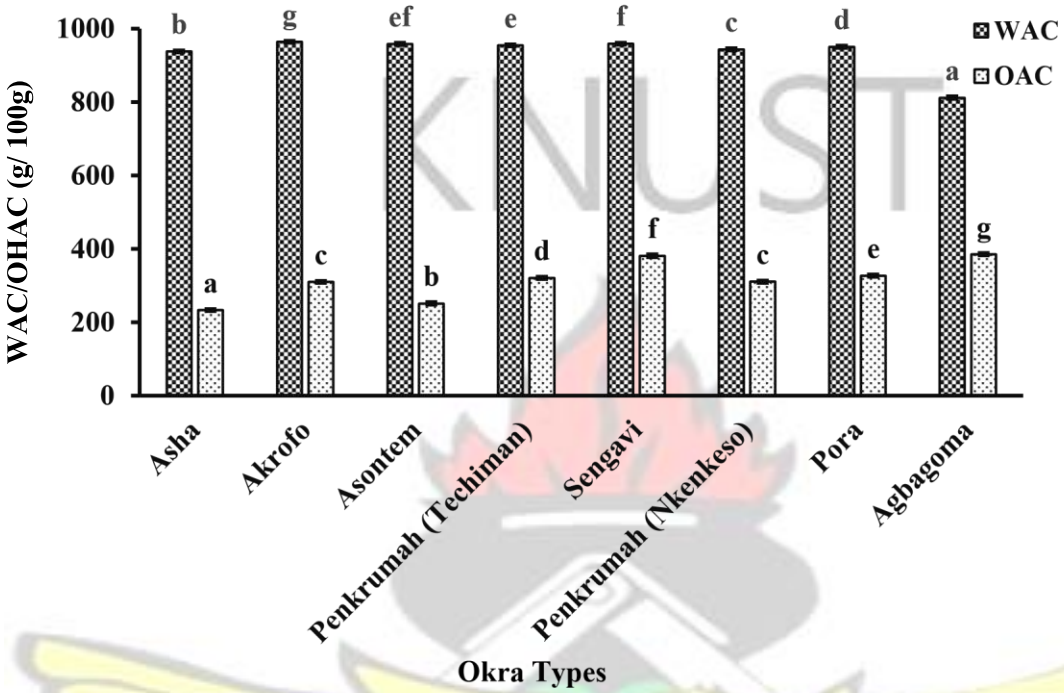


Figure 3.2: Water and oil absorption capacity of pectin from different okra types Different letters on bars with same patterns indicate significant differences ($p<0.05$)

3.3.2.2 Emulsion 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 at oilwater interfaces (Alba *et al.*, 2013; Archana *et al.*, 2013; Li and Nie, 2016). Some few gums such as gum Arabic, gum Ghatti and pectins from citrus peel and sugar beet have been known to impact surface activity and emulsification property (Alba *et al.*, 2013; Li and Nie, 2016). Pectin from the different okra types showed good emulsifying capacity after centrifugation. The okra types *Sengavi* and *Agbagoma* had the highest emulsion capacity (45 %) (Figures 3.3) 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). The emulsion capacities of *Sengavi* and *Agbagoma* were significantly different ($p<0.05$) from the other okra types studied. These pectins can thus form structurally stable and induce non-separation of oil-water emulsions (Schmidt *et al.*, 2015; Alba *et al.*, 2013).

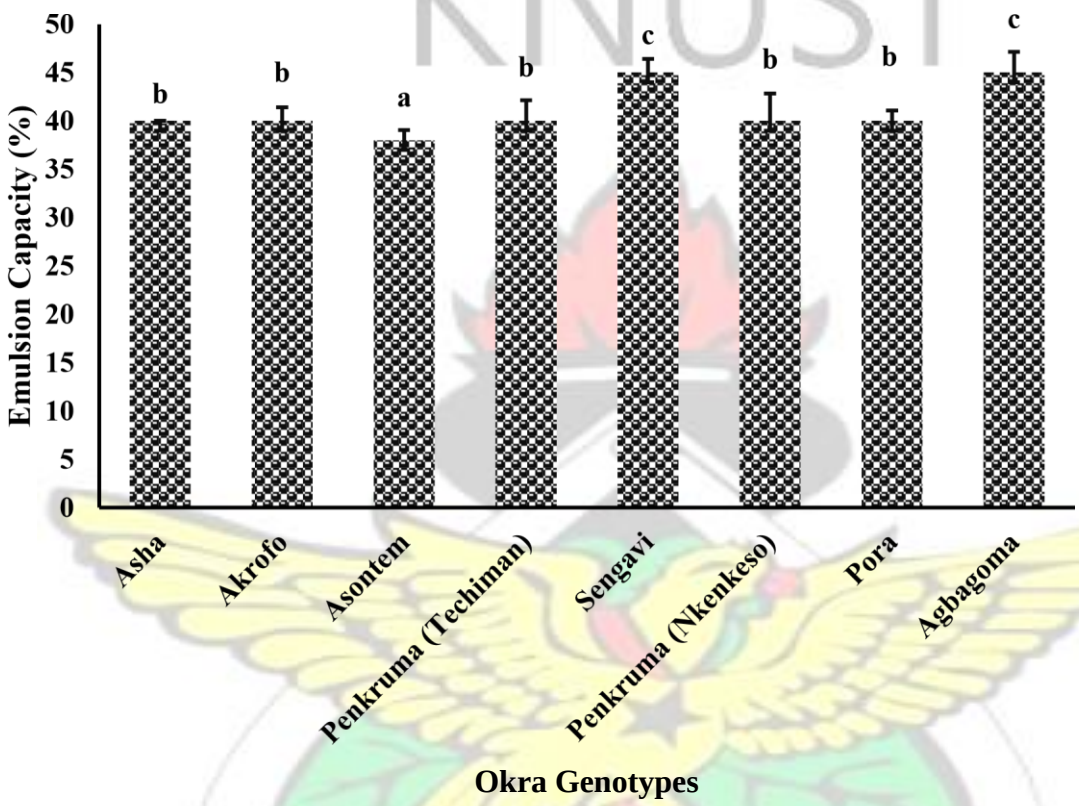


Figure 3.3: Emulsifying capacity (%) of pectin from different okra types,
Different letters on bars with same patterns indicate significant differences ($p<0.05$)

3.3.3 Structural Analysis

3.3.3.1 Fourier-Transform Infrared (FT-IR) Spectroscopy

Fourier transform infrared spectroscopy is a type of analytical technique in which a sample is irradiated with polychromatic radiation and the entire range of frequencies is simultaneously recorded to produce an intergerogram. A mathematical treatment called the

Fourier Transform is subsequently used to convert the results into a typical IR spectrum. FTIR spectroscopy (900 to 4000 cm^{-1}) was used to compare polysaccharide extracts from different okra types and the superposability of their infrared spectra confirmed that the extracted pectin from the okra types had similar functional groups hence belonged to *Abelmoschus esculentus* family (Figure 3.4).

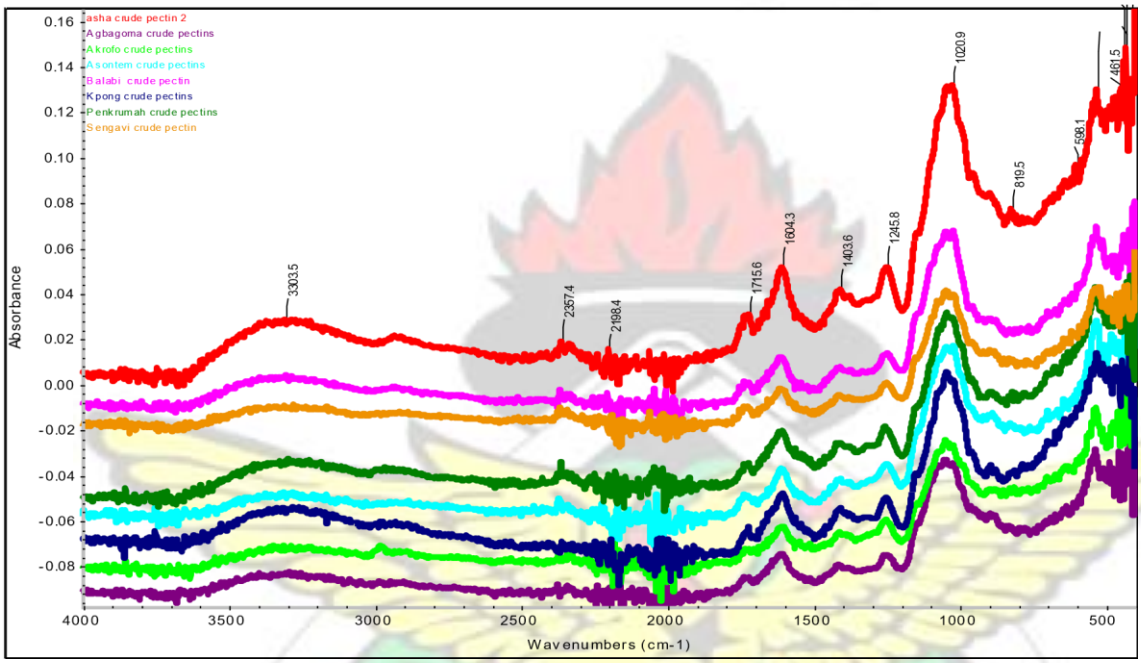


Figure 3.4: FTIR spectra of crude okra pectins

All the different types of okra had peaks which corresponded to the O-H stretching absorption (3000 – 3500 cm^{-1}). This absorption band had been attributed to the inter- and intra- molecular hydrogen bonding of the GalA backbone of okra pectins (Alba *et al*., 2015). The CH absorption which occurred in the region around 2850 – 2950 cm^{-1}, corresponds to CH, CH_2 and CH_3 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. 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 finger print. 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 1720-1730 cm^{-1}.

3.3.3.2 ^{1}H Nuclear Magnetic Resonance Analysis

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 3.5). A comparison of ^{1}H-NMR spectra of okra isolated (Figure 3.5) shows a high similarity between all spectra. A signal at 4.16 ppm (figure 4.7) was assigned to 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 (Figure 3.6). 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 polysaccharides previously isolated by Alba *et al*. (2015).

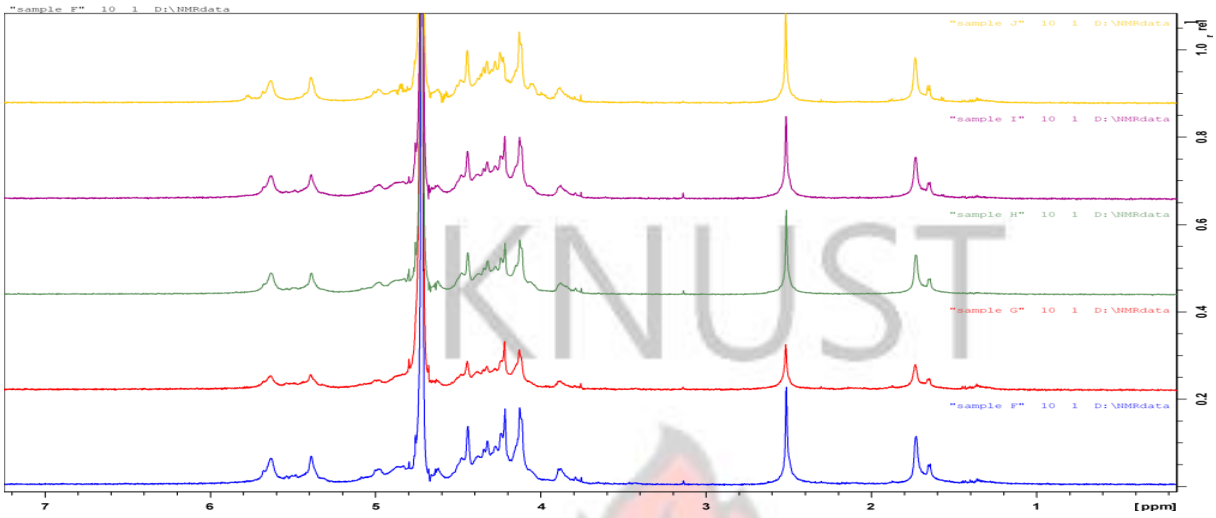


Figure 3.5: ^{1}H NMR of crude okra pectins from *Asha, Sengavi, Asontem, Penkruma* (Techiman) and *Agbagoma*

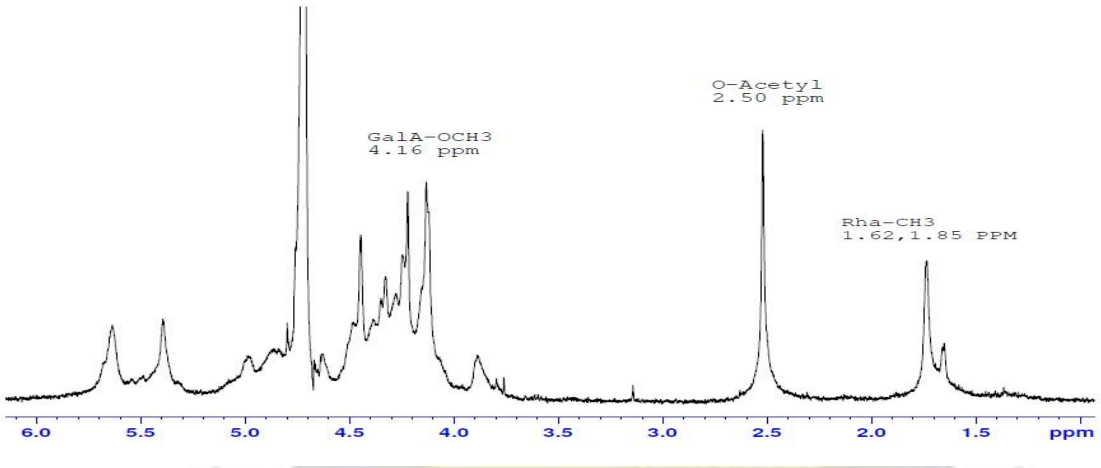


Figure 3.6: ^{1}H NMR of okra pectin from *Sengavi* showing specific linkages

3.3.3.3 Pectin Molecular Weight (Mw) Determination

The molecular weight of the pectins were analysed by high-performance sizeexclusion chromatography (HPSEC) equipped with multi-angle light scattering (MALS) and refractive index (RI) detectors. The weight-average molar mass values of the crude okra pectins ranged widely from 320 x 10^3 to 7600 x 10^3 g mol^{-1} (Table 3.2). The

high-MW of some pectin samples may be due to the presence of other aggregates, considering the crude nature of the samples.

Table 3.2: Molecular weight of extracted pectin from different okra types

Okra Types	Mw (g mol^{-1})	Rg.z (nm)
Asha	$0.79 \pm 0.18 \times 10^6$	136 ± 20
Pora	$0.32 \pm 4 \times 10^6$	130 ± 13
Penkruma (Nkenkeso)	$1.40 \pm 0.2 \times 10^6$	140 ± 14
Agbagoma	$7.60 \pm 1.3 \times 10^6$	138 ± 15
Asontem	$0.83 \pm 0.15 \times 10^6$	138 ± 17
Akrofo	$0.42 \pm 5 \times 10^6$	134 ± 11
Penkruma (Techiman)	$1.11 \pm 0.1 \times 10^6$	133 ± 12
Sengavi	$1.30 \pm 0.2 \times 10^6$	137 ± 16

Pectins from the okra type *Agbagoma* (7600×10^3 g mol^{-1}) had a relatively 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 x 10^3 to 767 x 10^3 g mol^{-1}. Molecular weights of pectins from all genotypes studied were higher than okra pectins (50 x 10^3 to 60 x 10^3 g

mol^{-1}) obtained from sequential extraction protocol (Kontogiorgos *et al.*, 2012; Sengkhamparn, *et al.*, 2009) and also higher than pectins from sugar beet- 184 x 10^3 g mol^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. This is because even a small difference in the structure and constitution of a molecule results in significant changes in the physico-chemical properties (Gnanasambandam and Proctor, 2000).

3.4 Conclusion

Pectins from eight different okra types (*Abelmoschus* spp) obtained from different ecological zones in Ghana were extracted using 0.1 M phosphate buffer (pH 6). The resultant pectin samples were then characterized by yield, purity, Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR) and sizeexclusion coupled to multi-angle light scattering (SEC-MALS). The functional properties of the different pectins were also investigated using standard procedures. Results showed that the okra types used had yields of 10-15 % pectin and there were differences in terms of their yield, purity, weight-average molar mass and functional properties (water/oil holding capacity and emulsion capacity). Okra pectins however had similar FTIR and NMR spectra with slight differences. The differences among the different okra types have the potential to be engineered in a number of potential applications. The present results indicate that pectin can be extracted from local okra types and exploited in potential food applications.

CHAPTER 4

PHYSICO-CHEMICAL EVALUATION OF PECTIN ISOLATES FROM DIFFERENT OKRA GENOTYPES

4.1 Introduction

Okra is a potential source of natural polysaccharides which can be exploited industrially as functional ingredients in food and non-food applications (Georgiadis *et al.*, 2011). Factors that have been identified to influence the yield and composition of polysaccharides isolated are origin of pod, solvent used for extraction, pH and time of extraction (Alba *et al*., 2015). Isolation of polysaccharides have been done on a laboratory scale using sequential solvent treatments (Sengkhamparn *et al.*, 2009; Georgiadis *et al.*, 2011; Kontogiorgos *et al.*, 2012; Alba *et al.*, 2013). Sequential solvent treatment was however preceded by the production of an alcohol insoluble solid (AIS). Okra polysaccharide yields (g/100 g of AIS) and protein content expressed as a percentage of AIS, obtained by researchers using the sequential extraction protocol have been summarized (Table 4.1). In order to facilitate the process of isolating polysaccharides from okra and develop a process that can be commercially and industrially feasible, alternative convenient protocols which employs a single hot acid extraction protocol have been suggested by other studies (Kontogiorgos *et al.*, 2012; Alba *et al.*, 2013; Alba *et al*., 2015). It has been realized that high yielding pectins can be obtained using extraction solvents of low pH and high temperature (Alba *et al*., 2015). A drawback that have been identified with extraction using chelating agents and alkaline is that it is laborious to remove residual chelates, and alkaline extraction reduces the length and degree of acetylation and methylation by β-elimination (Alba *et al*., 2015).

Chapters 4 and 5 have been published as: Kpodo, F.M., Agbenorhevi, J.K., Alba, K., Bingham, R. J., Oduro, I.N., Morris, G.A. and Kontogiorgos, V. (2017). Pectin isolation and characterization from six okra genotypes, *Food Hydrocolloids* 72, 323–330

Table 4.1: Percentage yield and protein content of okra polysaccharide from selected studies

Type of soluble solids	Sengkhamparn *et al*. (2009)		Georgiadis *et al*. (2011)	Kontogiorgos *et al*. (2012)
	Yield (%)	Protein (%)	Protein (%)	Protein (%)
AIS	5.8	15.8	ND	ND
HBSS	11.2	3.5	6.7	6.7
CHSS	4.8	10.5	31	31
DASS	13.2	16.6	11.4	11.4
CASS	4.1	13.2	ND	ND

AIS-alcohol insoluble solids; HBSS- hot buffer soluble solids; CHSS- chelating agent soluble solids; DASS- dilute alkali soluble solids; CASS- concentrate alkali soluble solids;ND- not determined

The percentage yield obtained when phosphate buffers (subsequently followed by alcohol precipitation) were used for extracting okra polysaccharides comprised of 13.3 % and 15.7 % at pHs 2.0 and 6.0 respectively (Alba *et al*., 2015). However the protein content of polysaccharide extracted at pH 6.0 was higher (6.3 %) than extraction at a buffer pH 2.0 (4.3 %) (Alba *et al*., 2015). High pectin yields are usually obtained from hot acid extraction of cellular material and the approach is also less laborious for industrial extraction of pectin (Alba *et al*., 2015). Since there is the need to isolate and investigate polysaccharides from different genotypes of okra and study their unique characteristics, the genotypes must be subjected to the same experimental procedure in order to ascertain the uniqueness of their pectin composition for specific food and non-food applications. The suggested convenient

protocol for isolating okra polysaccharides using hot acid buffer extractions (Alba *et al*., 2015) was adopted to isolate pectins from six different okra genotypes cultivated in the same environment under the same conditions.

In the previous investigation (chapter 3), it was shown that the geographical source of the okra plant significantly influenced the physicochemical characteristics of the pectin isolates obtained. However, the impact of genetic differences on their physicochemical characteristics were not evaluated because the raw material (different okra types) used for the study were purchased from local farmers in different geographical areas. Furthermore, okra pectins were not purified using exhaustive dialysis hence isolates obtained had high molecular weight attributed to the presence of excess cellulose as co-extracts. The objective of the present study, therefore, was to isolate purified pectins from six different okra genotypes cultivated under the same environmental conditions in order to evaluate their structural and macromolecular characteristics.

4.2 Material and Methods

4.2.1 Cultivation of Okra Genotypes under Controlled Environmental Conditions

The experiment was conducted at Akrofu, a town associated with okra farming in the Volta Region of Ghana from October, 2015 to January, 2016. The land was slashed and sprayed with herbicide and harrowed. An acre land was demarcated into six portions with demarcating tape, sowing was done by direct seeding with a garden line and cutlass. Seeds of six different okra genotypes were grown on each plot. Seed sowing on the field was done at the rate of 2-3 seeds per hole. All cultural practices including thinning, weed control and watering were carried out. Weeding was done with hoe at 4-5 weeks after emergence and at early flowering state respectively and when necessary. The soil at the experimental

site was sandy-loam. Okra genotypes cultivated were *Asha, Agbagoma, Asontem, Balabi, Sengavi and Penkruma*.

4.2.2 Determination of Morphological and Genotypic Characteristics of Okra Genotypes

A standardized crop descriptor for okra (IBPGR, 1991), was used to measure the various phenotypic characteristics of the 6 different okra genotypes. Genetic diversity was determined using Fragment Length Analysis (FLA) of ten simple sequence repeat (SSR) markers. Fragment length analysis (FLA) was performed by Ecogenics GmbH (Switzerland) using singleplex PCR and FAM-labelled oligonucleotides. Forward and reverse primer sequences targeting 10 simple sequence repeats (SSRs) were based on known loci with high information content (Schafleitner *et al.*, 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 and Jacquemoud-Collet, 2006) using Jaccard's coefficient $dij = b + c / (a + b + c)$, where a is the number of alleles that are common to both genotypes i and j. b are 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 and Jacquemoud-Collet, 2006)

4.2.3 Isolation of Okra Pectin

The Okra fruits were separated into three parts: calyx, pod and seed. The okra pods were oven dried and then subjected to further analysis. The okra pectin isolation procedure described by Alba *et al*. (2015) was employed in this study. Pectin was extracted from the

dried okra pods using phosphate buffer at pH 6.0. Following extraction, the polysaccharides were precipitated using ethanol (96% v/v). Purified pectins were obtained by extensive dialysis of the solubilized crude pectin (5% w/v) for 3 days with frequent replacement of deionized water. The resultant extract was concentrated by rotary evaporation and then freeze dried.

4.2.4 Chemical Characterization of Okra Pectins

4.2.4.1 Protein Content Determination (Bradford Protein Assay)

The Bradford protein assay was used in the determination of the protein content of the purified okra pectins. The method involves the binding of Coomassie Brilliant Blue G250 dye to proteins (Bradford, 1976). The dye exists in three forms: cationic (red), neutral (green, and anionic (blue). Under acidic conditions, the dye is predominantly in the doubly protonated red cationic form (A_{max} = 470 nm). However, when the dye binds to a protein, it is converted to a stable unprotonated blue form which is detected using a spectrophotometer (A_{max} = 595nm) (Shimazu UV-VIS 160A).

A standard calibration curve was generated with Bovine Serum Albumin (BSA) standards and used to estimate the protein content of samples. The blank (0 µg ml^{-1}) was made using distilled water and dye reagent. A stock solution (2 mg ml^{-1}) was prepared using the BSA. Dilutions with concentrations 40, 80, 100, 200, 400 and 800 ug ml^{-1} were made by transferring respective amounts of calibration protein from the stock solutions and adjusting the volume to a total of 100 µl by adding distilled water (Appendix 4.2). The dilution of the protein standard is shown in appendix. Each standard, blank and unknown samples (100 µl) were pipetted into separate clean test tubes. The dye reagent (5 ml) was

then added to each tube and vortexed. The resulting solution was incubated at room temperature for 10 min (samples should be incubated for at least 5 min and not longer than 1 h). Absorbance readings were then taken at 595 nm using a spectrophotometer (Shimazu UV-VIS 160A).

4.2.4.2 Preparation of Okra Sample for Protein Assay

Freeze dried pure okra polysaccharide powder (1 mg) was weighed and dissolved in 10 ml of distilled water (0.1 mg ml^{-1}). The pectin was dissolved by stirring on a magnetic stirrer. Each sample (100 µL) was pipetted into separate clean test tubes. Bradford reagent (5 ml) was added to each tube, vortexed and incubated for 10 min at room temperature. Absorbance readings were then taken at 595 nm using a spectrophotometer (Shimazu UV-VIS 160A).

4.2.4.3 Total Carbohydrate Determination

Total carbohydrate was estimated using the phenol-sulphuric acid assay (Dubois *et al.*, 1956). A standard calibration curve was generated with galactose (10 - 500 µg ml^{-1}) and used to estimate the total carbohydrate content of samples. The blank (0 µg ml^{-1}) was made using distilled water. The stock solution was prepared with calibration sugar-galactose (1 mg ml^{-1}). The dilution of the galactose standard is shown in Appendix 4.3. Each standard and blank (500 µl) were pipetted into separate clean test tubes. To all test tubes 500 µl of 5 % phenol in 0.1 M hydrochloric acid was added followed by 2.5 ml concentrated sulphuric acid. The resulting solution was vortexed and allowed to cool to room temperature. Absorbance readings were then taken at 490 nm using a UV-VI spectrophotometer (Shimazu UV-VIS 160A).

4.2.4.4 Preparation of Okra Sample for Total Carbohydrate Analysis

Freeze dried okra polysaccharide powder (1 mg) was weighed and dissolved in 10 ml of distilled water. The polymer was dissolved by stirring on a magnetic stirrer. Each sample (500 µl) was pipetted into separate clean test tubes. To all test tubes 500 µl of 5 % phenol in 0.1 M hydrochloric acid was added followed by 2.5 ml concentrated sulphuric acid. The resulting solution was vortexed and allowed to cool to room temperature. Absorbance readings were then taken at 490 nm using a UV-VI spectrophotometer (Shimazu UV-VIS 160A).

4.2.5 Determination of the Degree of Esterification and Methoxyl Content of Pectin Using Potentiometric Titration

Methoxyl content was determined by titration (Schultz, 1965). Freeze dried okra pectin (0.2 g) was moistened with 1 ml ethanol and dissolved in distilled water (20 ml) heated at 40 °C. The mixture was stirred for 2 h and the resulting solution titrated against 0.1 N NaOH using phenolphthalein as indicator. At the end point of titration when pectin sample is neutralized, the number of free carboxy groups (K_f) was calculated according to the following equation:

$$K_f = \frac{N_{NaOH} V_{NaOH}\, x\, 0.045}{a} x\, 100$$

Where a- is the weighted portion of pectin (g), N_{NaOH} and V_{NaOH} is the normality and volume (ml) of the alkali solution spent for titration.

In order to determine the number of esterified carboxy groups (Ke), 10 ml of 0.1 N NaOH solution was added to the neutralized pectin sample after determination of the free carboxy groups. The flask was plugged with a stopper and content was stirred at room temperature

for 2 h to saponify the esterified carboxy groups of the polymer, then 10 ml of 0.1 N HCl was added to the solution. Excess of HCl was titrated with 0.1 N NaOH and the number of the esterified carboxy groups was calculated from the volume of 0.1 N NaOH solution spent for titration using the equation:

$$K_e = \frac{N_{NaOH} V_{NaOH}\, x\, 0.045}{a} x\, 100$$

The total number of the COOH groups (%) is equal to the sum of the free and esterified groups and is given as:

$$K_t = K_f + K_e$$

The degree of esterification (%) is expressed as the number of esterified carboxy groups per total number of carboxy groups:

$$DE = \frac{K_e}{K_t} x\, 100$$

The number of methoxy groups (%) in pectin sample can be calculated from the DE by the equation:

$$K_{MeO} = \frac{100DE\ x\ 31}{176 + DE\ x\ 14}$$

Where 31 is the molecular weight of MeO and 176 is the molecular weight of anhydrouronic acid.

4.2.6 Determination of the D-Galacturonic Acid Content

Galacturonic acid content of pectins was determined using *m*-hydroxydiphenyl method (Filisetti-Cozzi and Carpita, 1991). D-galacturonic acid was determined using

calorimetric method. A standard calibration curve was generated with D-galacturonic acid (20 - 500 µg ml^{-1}) from a 1 mg ml^{-1} stock solution. The dilutions of the D-galacturonic acid standard solutions is as shown in Appendix 4.4.

4.2.6.1 Sample Preparation: Hydrolysis of Cell Wall and Cell Fractions

Concentrated sulphuric acid (1 ml) was added to 500 µl of all samples in tubes and capped. A reagent control tube containing only 1 ml of concentrated sulphuric acid was prepared and carried through all the procedures. The tubes were placed in an ice bath and the contents stirred for 5 min. Another 1 ml of concentrated sulphuric acid was added to all the tubes and stirred on ice for 5 min. This was subsequently followed by the addition of 0.5 ml of water with stirring for 5 min on ice and the addition of another 0.5 ml water, also stirred for 5 min. Contents of each tube were diluted with water to 10 ml and transferred and centrifuged for 10 min at 2000 g at room temperature, to pellet any unhydrolyzed material. The hydrolysate was collected and analyzed in the next step.

4.2.6.2 Colorimetric Assay of D-Galacturonic Acid Content

For each hydrolysate three (3) preparations were made and 500 µl from each hydrolysate supernatant and reagent control were placed in respective tubes. Sulfamic acid/potassium sulfamate solution (40 µl of 4 M; pH 1.6) was added to all the tubes and vortexed. 75 mM sodium tetraborate in sulphuric acid solution (2.4 ml) was added and the mixture vortexed vigorously. The tubes were placed in a 100 °C water bath for 20 min, and then cooled by plunging tubes into an ice bath for 10 min. Glass marbles were put on top of the tubes to prevent contamination of the samples by condensation. After heating the contents of the tube, 80 µl of m-hydroxydiphenyl solution was added to 2 tubes of each sample and reagent control tubes whereas to the third tube of each sample 80 µl of 0.5 %

w/v NaOH instead of m-hydroxydiphenyl was added and marked as a sample control. The contents of the tubes were vortexed to ensure they were mixed well. Neutral sugars present in the sample are capable of producing some colour (browning) after boiling even in the presence of sulfamate. Hence to eliminate this variable, a sample control was prepared and subtracted from the final samples absorption values. A pink colour develops within 5 to 10 min and is stable for 1 h after which it fades. Absorbance readings were then taken at 525 nm using a UV-VI spectrophotometer (Shimazu UV-VIS 160A).

4.2.7 Determination of Degree of Methylation

The degree of methylation of okra pectins was determined from the methoxyl and galactouronic acid contents using the expression as described by Alba *et al*. (2015):

$$\text{DM (\%)} = \frac{176 \text{ x methoxyl content [\%(w/w)]}}{31 \text{ x GA content [\%(w/w)]}} \text{x } 100$$

Where 176 and 31 are the molecular weights of anhydrous galacturonic acid and methoxyl respectively.

4.2.8 Determination of Acetyl Content of Pectins

4.2.8.1 Preparation of Calibration Curve

Acetyl content was determined with the hydroxamic acid method (McComb and McCready, 1957). Freshly prepared (2 ml of 1:1) mixture of sodium hydroxide (94 g L^{-1}) and hydroxylamine hydrochloride (37.5 g L^{-1}) was pipetted into each of four 25 mL volumetric flasks. Dilute standard glucose pentaacetate solutions containing 120, 240, 300 and 420 ug of acetyl were respectively added to each solution in the volumetric flask while stirring on a magnetic plate. Solutions were then allowed to stand for 30 minutes and 5 ml of acid-methanol solution was added and mixed thoroughly. The volume of the solutions

were diluted to volume with ferric perchlorate solution by adding in small increment with thorough mixing. The colour was allowed to develop for five minutes and the absorbance was determined at 510 nm.

4.2.8.2 Sample Analysis for Determination of Acetyl Content

Freeze dried okra pectin (0.1 g) was weighed into a 150 ml conical flask and 25 ml of hydroxylamine solution added while stirring on a magnetic stirrer over a period of 1015 minutes. Sodium hydroxide solution (25 ml) was subsequently added and the flask was covered with a watch glass with continued stirring until sample dissolved. The solution (2 ml) was then pipetted into a 25 ml volumetric flask with the addition of water (5 ml) and acid-methanol (5 ml). The volume was diluted with ferric perchlorate solution by addition in small increments with mixing. Absorbance was determined at 510 nm and acetyl content calculated with the formula:

$$\text{Acetyl}(\%) = \frac{\text{Acetyl µg (From Graph) x 25 x 100}}{\text{Sample Weight}(\mu g)}$$

The degree of acetylation was calculated from the galacturonic acid and acetyl content values using the equation as described by Alba *et al*. (2015):

$$\text{DA}\ (\%) = \frac{176 \text{ x acetyl content } [\%(w/w)]}{43 \text{ x GA content } [\%(w/w)]} \text{x } 100$$

Where 176 and 43 are the molecular weights of anhydrous galacturonic acid (GA) and acetyl, respectively.

4.2.9 Determination of Neutral Sugars Using Methanolysis and Analysis with GCMS

Neutral sugars were determined using methanolysis conducted with 1 M methanolic HCl at 85 °C for 24 h, as described previously (Bleton *et al.*, 1996). Freeze dried polysaccharide (2 mg) and myo-Inositol/internal standard (0.5 mg) were transferred into a 13 x 100 mm screw-cap tube and 5 ml of 1 M HCl in methanol was added. Samples were left at 85 ^{o}C for 24 h to hydrolyse and the solution was evaporated to dryness with a stream of nitrogen. MeOH (5 ml) was added in smaller sequence to wash of the HCl and then evaporated. Samples were acetylated by adding 1 ml Ac_2O and 1 mL of pyridine at 100 ^{o}C for 60 minutes, and evaporated to dryness with a stream of nitrogen. The samples were subsequently dissolved in EtOAc. Sugar derivatives were analysed using an Agilent 7890A GC system (Santa Clara, CA, USA) coupled to an Agilent 5675C quadruple MS. The samples were eluted from an HP-5 column (30 m x 0.25 mm, 0.25 um film) using helium as carrier at a flow rate of 1 mL min^{-1} by applying the following temperature setting: start temperature 140 ^{o}C hold time 1 minute and final column temperature 220 ^{o}C with 25 ^{o}C min^{-1} gradient. Sugar composition was calculated using molar ratios for pectins. Molar percentage of homogalacturonan (HG) and rhamnogalacturon-1 (RG-1) were also determined using the formula:

$$\mathrm{HG\ (mol\ \%)} = \mathrm{GalA(mol\ \%)} - \mathrm{Rha\ (mol\ \%)}$$

$$\mathrm{RG-1(mol\ \%)} = 2\mathrm{Rha(mol\ \%)} + \mathrm{Ara\ (mol\ \%)} + \mathrm{Gal\ (mol\ \%)}$$

4.2.10 Zeta Potential

Zeta potential was determined for the pectins from the different okra genotypes using a ZetaSizer Nano Series ZEN2600 (Malvern Instruments, Malvern, UK) at 25 ^{o}C with a refractive index of 4.6 was used. Diluted okra pectins were prepared with a buffer solution (0.1 %) to avoid multiple scattering effects. Measurement of Zeta potential of samples provide information on the surface charge of the biopolymer. The parameter is an indicator of interactions that occur at the surface of colloidal systems. Electrostatic repulsions in emulsions is attributed to the presence of electrically charged surfaces.

4.2.11 Structural Analysis

4.2.11.1 Fourier-Transform Infrared (FT-IR) Spectroscopy

FTIR spectra were obtained between 500 and 4000 cm^{-1} for all okra samples in Attenuated Total Reflection (ATR) mode at a resolution of 4 cm^{-1} using 128 scans (Nicolet 380, Thermo Scientific, UK). Spectral smoothing was applied using instrument software (OMNIC 3.1).

4.2.11.2 ^{1}H Nuclear Magnetic Resonance Analysis

NMR spectroscopy is a non-destructive technique used to provide information on the chemical structure of high molecular weight polymers such as polysaccharides. 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). ^{13}C proton decoupled spectra were recorded at 70 ^{o}C using 12, 800 scans with a relaxation delay of 2s and a 30^{o} pulse angle. ^{1}H spectra were recorded with 64 scans at the same temperature.

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

High Performance liquid chromatography is a chromatographic technique used for analysing carbohydrates. A high pump pressure is used to move the mobile phase and the analyte through a column coated with a stationary phase. Size exclusion chromatography separates molecules on the basis of their molecular size. HPSEC is used for characterizing molecular weight. The molecular weight (MW) of the okra polysaccharides from different genotypes was estimated using Size Exclusion Chromatography Coupled to Multi – Angle Light Scattering (SEC-MALS) 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. The pectins were eluted with 0.1 M $NaNO_3$ solution at a flow rate of 0.7 mL min^{-1} (Morris *et al*., 2000).

4.2.12 Data Analysis

Data obtained was analysed using Minitab version 17 (Minitab Inc., Philadelphia, U.S.A.). Comparisons between the different okra genotypes were done using analysis of variance (ANOVA) with a probability, $p<0.05$. Fisher's least significant difference (LSD) was used to determine differences among means when ANOVA indicated significant F values. All treatments were conducted in triplicate and mean (± standard deviation) values reported.

4.3 Results and Discussion

4.3.1 Morphological and Genotypic Variations among Okra Genotypes

Morphological variations among okra genotypes were scored using the standard international crop descriptor for okra (IBPGR, 1991). The okra genotypes 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 of the genotypes were predominantly yellow. Fruits were green to dark green, smooth and rough. The result showed that the fruit colour displayed different variations that ranged from green, with hairs to dark green. Tables 4.2, 4.3 and 4.4 show the stem, petiole, leaf, flowering and fruit characteristics.

Table 4.2: Stem, petiole and leaf characteristics

Genotype	StC	PtC	MLC	LSh	LRC	IPL
Asha	Green + Purple tinge	Purple	Green+Red Veins	3	Green	Red
Agbagoma	Green	Green	Green	3	Green	Green
Asontem	Green	Green	Green	3	Green	Green
Balabi	Green	Green	Green	3	Green	Green
Sengavi	Green	Green + Red Veins	Green	6	Green	Red

Penkruma	Green+ Purple tinge	Purple	Green+Red Veins	3	Green	Red

StC-stem colour; PtC-petiole colour; MLC-mature leaf our; LSh-leaf s ; LRC-leaf col rib colour; IPL; Intersection between petiole and lea

Table 4.3: Flowering characteristics

Genotype	PC	FSp	DF	DFF
Asha	Yellow	Single flowering	Flowering up	47
Agbagoma	Yellow	Single flowering	Flowering up	54
Asontem	Yellow	Single flowering	Flowering up	64
Balabi	Yellow	Single flowering	Flowering up	47
Sengavi	Yellow	Single flowering	Flowering up	44
Penkruma	Golden yellow	Single flowering	Flowering down	70

PC-petal colour; FSp-flowering span; DF-description of flowering; DFF-days to first flowering

Table 4.4: Fruit characteristics

Genotype	FC	FP	FSh	PFMS
Asha	Green	Little rough	12	Erect
Agbagoma	Green	Little rough	2	Erect
Asontem	Green	Smooth	2	Erect
Balabi	Green	Smooth	2	Erect
Sengavi	Green	Little rough	12	Erect
Penkruma	Green+purple veins	Smooth	-	Erect

FC-fruit colour; FP-fruit pubescene; FSh-fruit shape; PFMS-position of fruit from the main stem

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 polyploidy 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 and 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. 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-AsontemPenkruma* as a group, *Agbagoma Balabi* as a second group and *Sengavi* as an outlier (Figure 4.1). 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.

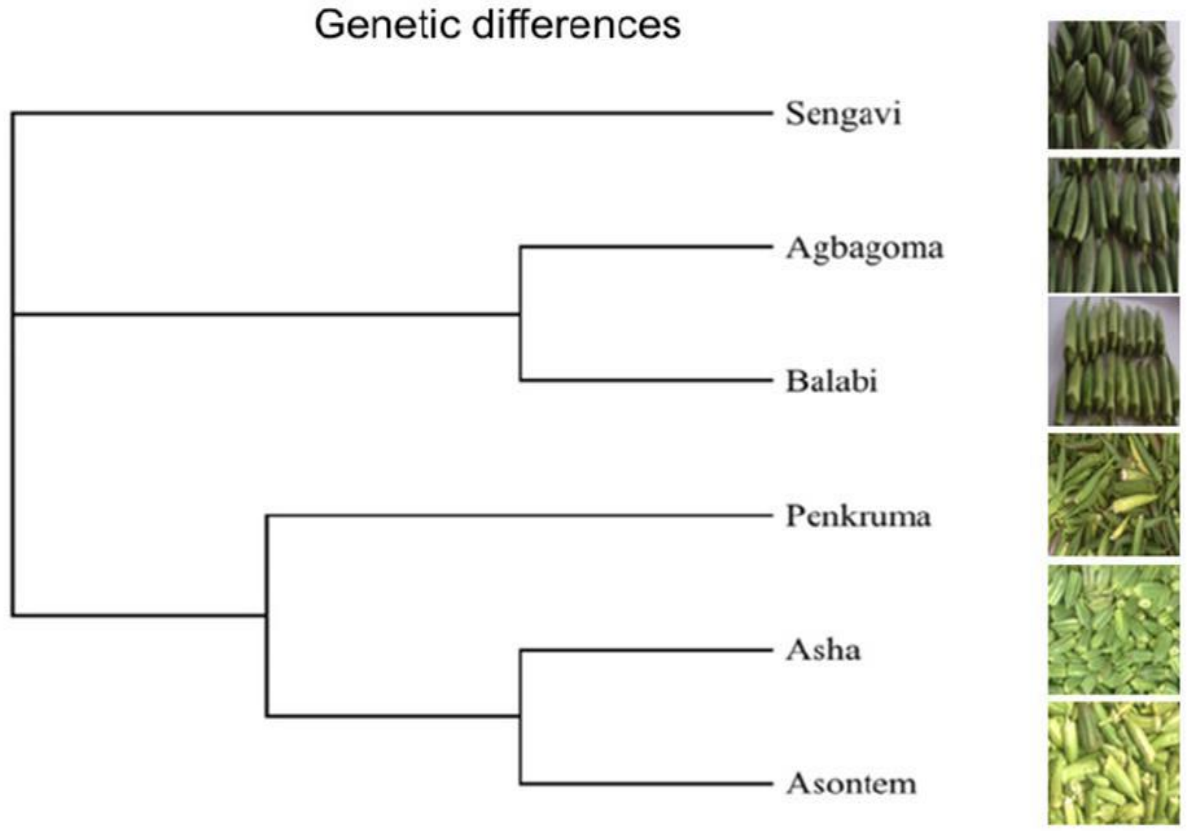


Figure 4.1 Phylogenetic tree of okra genotypes

4.3.2 Pectin Yield and Purity of Okra Pectins

Pectins extracted from different okra genotypes using hot buffer extraction varied in yield and purity levels (Table 4.5). The pectin yields ranged approximately between 11.4 to 14.6 %, and were comparable with yields (8.8 % to 15.7 %) obtained by Alba *et al*. (2015) and Samavati (2013). The genotypes *Asha, Penkruma* and *Agbagoma* recorded high and comparable pectin yields (14.2 – 14.6 %) whiles the genotypes *Sengavi* and *Balabi* had the lowest pectin yields (11.3 – 11.4 %). Pectin yields obtained from all genotypes were higher than reported values for extracts using acetone (8.6 %), methanol (0.28 %) and distilled water containing 1 % sodium metabisulphite (1.46 %) (Noorlaila *et al.*, 2015; Archana *et al.*, 2013).

Genotypic differences had significant effect on the protein and total carbohydrate composition of the okra extracts (Table 4.5). There were differences in the protein and total carbohydrate content of the varying genotypes studied. Purified pectins from the genotype *Balabi* recorded the least protein and highest total carbohydrate content but *Sengavi* recorded high protein and low total carbohydrate values. Prior to dialysis, the crude pectins relatively had higher protein (5.7 – 7.8 %) and lower total carbohydrate content (57.7 – 77.5 %). However after purification, okra pectins had lower protein content (3.3 - 7.1 %) and high total carbohydrate content (66.2 - 87.5 %). Generally protein and total carbohydrate content of the pure extracts were comparable to results obtained by Alba *et al*. (2015) and higher than values obtained using the sequential extraction protocol.

Table 4.5: Purity of okra pectins

Okra	Pectin	Before Dialysis		After Dialysis	
Genotype	Yield	% Protein	% Total Carbohydrate	% Protein	% Total Carbohydrate
Asha	14.6 (1.2)[a]	5.7 (2.2)[a]	65.2 (5.5)[a]	5.5 (3.1)[a]	86.3 (2.0)[a]
Penkruma	14.4 (0.1)[a]	5.8 (2.6)[a]	77.5 (0.2)[b]	4.4 (1.4)[a]	87.4 (1.0)[a]
Asontem	13.2 (0.6)[a]	7.8 (1.0)[a]	62.6 (1.6)[a]	3.8 (1.8)[a]	72.5 (2.5)[b]
Agbagoma	14.2 (0.1)[a]	7.3 (1.3)[a]	61.8 (5.9)[a]	5.4 (2.8)[a]	66.2 (1.0)[b]
Sengavi	11.3 (0.1)[b]	7.3 (1.0)[a]	65.4 (0.2)[a]	7.1 (2.4)[a]	66.2 (4.3)[b]
Balabi	11.4 (1.3)[b]	5.9 (1.9)[a]	57.7 (1.0)[a]	3.3 (1.0)[a]	87.5 (3.5)[a]

Means sharing the same letters in a column are non-significant (P>0.05)

4.3.3 Degree of Methylation and Acetylation of Okra Pectins

Application of low methoxyl pectin as functional ingredients in food and pharmaceutical products have been of paramount interest in recent times and hence the focus of most researchers. Pectin from different genotypes had varied chemical characteristics. The degree of methylation of okra pectin was shown to be 24 % (Sengkhamparn *et al.*, 2009) and 24 – 40 % (Alba *et al*., 2015). In this study okra pectin from different genotypes had varied but comparable degree of methylation values which ranged between 17.0 and 25.5 % (Table 4.6). Low methoxyl pectin from other plant sources such as sunflower and sugar beet have degree of methylation of 27 % and 44 % respectively (Guo *et al.*, 2016; Hua *et al.*, 2014; Kang *et al.*, 2015). The GalA content of the different okra pectin varied from 42.8 to 63.4 % (Table 4.6) and values were comparable to that recorded for pectin from previous studies (Alba *et al*., 2015) and sugar beet (Guo *et al.*, 2016). The acetyl content of okra pectin were between 3.0 and 6.1 %. Degree of acetylation varied from 19.9 to 40.1 % with the genotype *Penkruma* recording the lowest value. Degree of acetylation of okra pectins were generally higher than values obtained for sugar beet pectins (21.0 to 28.0 %) extracted under different conditions (Guo *et al.*, 2016; Chen *et al*., 2016). Values were however comparable to DA obtained by Alba *et al*. (2015) for okra pectins extracted at pH 6 (37.6 %).

Table 4.6: Chemical composition of okra pectins

Okra Genotype	Methoxyl (%)	D-GalA (%)	Acetyl (%)	DM %	DA %
Asha	1.92 (0.01)^{b}	63.4 (1.1)^{a}	6.1 (0.1)^{a}	17.2 (1.4)^{a}	39.3 (4.3)^{a}
Penkruma	1.87 (0.01)^{a}	62.4 (4.7)^{a}	3.0 (0.2)^{d}	17.0 (0.1)^{a}	19.9 (0.5)^{c}
Asontem	1.94 (0.01)^{c}	54.2 (4.6)^{b}	5.3 (0.3)^{b}	20.4 (1.8)^{bc}	40.1 (5.7)^{a}

Agbagoma	1.91 (0.01)^{b}	51.9 (3.4)^{b}	4.0 (0.2)^{c}	20.9 (1.8)^{c}	31.7 (5.5)^{b}
Sengavi	1.92 (0.01)^{b}	59.2 (1.0)^{ab}	3.2 (0.1)^{d}	18.4 (1.4)^{ab}	22.4 (3.3)^{c}
Balabi	1.92 (0.01)^{b}	42.8 (1.3)^{c}	3.9 (0.1)^{c}	25.5 (1.8)^{d}	37.9 (5.8)^{ab}

Means sharing the same letters in a column are non-significant (P>0.05)

The neutral sugar composition of okra pectin from the different genotypes showed that the main neutral sugar was Galactose followed by Rhamnose and Arabinose (Table 4.7). The galactose content of samples varied from 5.9 (*Penkruma*) to 16.6 % (*Asha*). Moreover it has been demonstrated that the genotype *Asha* had the highest L-Rhamnose content (8.9 %). Generally the arabinose content of all samples studied were low and varied from 1.1 to 1.7 %. The low glucose values subsequently recorded for all pectin is indicative of their purity level.

The sugar ratios for the different pectins were calculated based on the sugar content determined experimentally. The larger R_1 values of pectins from *Penkruma*, *Agbagoma* and *Sengavi* is indicative that these pectins are more linear than pectins from *Asha* and *Balabi* which demonstrated a more branched chain. The presence of side chains in pectin extracts from *Asha* and *Balabi* is confirmed by the high Rha:GalA ratio (R_2) when compared to other pectins. The molar ratios R_3 and R_4 described in Table 2 compare the amount of side chains to rhamnose as an estimate of the length of RG-I branching (Denman and Morris, 2015). The genotype *Penkruma* showed the lowest ratios (R_3-1.6, R_4-1.2) indicating a smaller average size of the branching side chains. The HG fraction of the okra pectins studied ranged from 53.7 to 72.1 % whereas the RG-1 recorded values between

23.6 to 42.7 %. Again the genotype *Penkruma* had the highest HG (72.1 %) and lowest RG-1 (23.6 %) segments whereas *Asha* and *Balabi* had the lowest HG segment (54 %) and highest RG-1 (41 %). Results confirms that okra pectins from the different genotypes demonstrated structural variations in terms of linearity and branching with the ratio of HG:RG-1 varying from 1.3 to 3.1.

Table 4.7 Percentage neutral sugar composition and molar ratios of okra pectins

Genotype	D-Gal	L-Rha	L-Ara	D-Glc	R_1	R_2	R_3	R_4	HG	RG-1	HG/RG-1
Asha	16.6(0.3)	8.9(1.5)	1.7(0.4)	0.8(0.1)	2.0	0.17	1.9	1.7	53.7	42.7	1.3
Penkruma	5.9(1.4)	4.3(1.5)	1.5(0.5)	0.7(0.1)	4.6	0.08	1.6	1.2	72.1	23.6	3.1
Asontem	12.0(4.9)	3.5(1.0)	1.1(0.1)	1.3(0.4)	2.9	0.08	3.4	3.1	64.9	29.8	2.2
Agbagoma	10.4(2.3)	2.7(6.9)	1.1(0.1)	0.4(0.1)	3.2	0.06	3.9	3.5	68.5	27.2	2.5
Sengavi	11.2(0.5)	4.0(6.7)	1.2(2.3)	1.7(0.5)	3.2	0.08	2.8	2.5	66.0	28.5	2.3
Balabi	12.0(2.2)	5.2(1.2)	1.6(0.4)	0.5(0.1)	2.0	0.15	2.4	2.1	54.2	41.1	1.3

R_1 = GalA/ (Rha + Ara + Gal); R_2 = Rha/GalA; R_3 = (Ara + Gal)/Rha; R_4 = Gal/Rha; HG = GalA – Rha; RG-1 = 2Rha + Ara + Gal (Denman and Morris, 2015).

4.3.4 Zeta Potential

Zeta potential was determined for the pectins from the different okra genotypes using a ZetaSizer. The zeta potential value of the okra pectin from the different genotypes

ranged from -15.83 mV to -22.06 mV and were negative (Table 4.8). The negative zeta potential values were due to the presence of anionic polysaccharides in the okra isolates (Alba *et al.*, 2013). The genotype *Balabi* gave a highly negative potential value whereas *Asontem* had the lowest charge. Although potential values obtained in this study were comparable with values obtained by (Georgiadis *et al.*, 2011) for hot buffer soluble solids (-21.5 mV) and chelating agent soluble solids (-14.3 mV) sequential extraction extracts, zeta values were highly negative when compared with values (-6.9 to -4.6) obtained by Alba *et al*. (2013).

Table 4.8: Zeta potential value of okra pectins from different genotypes

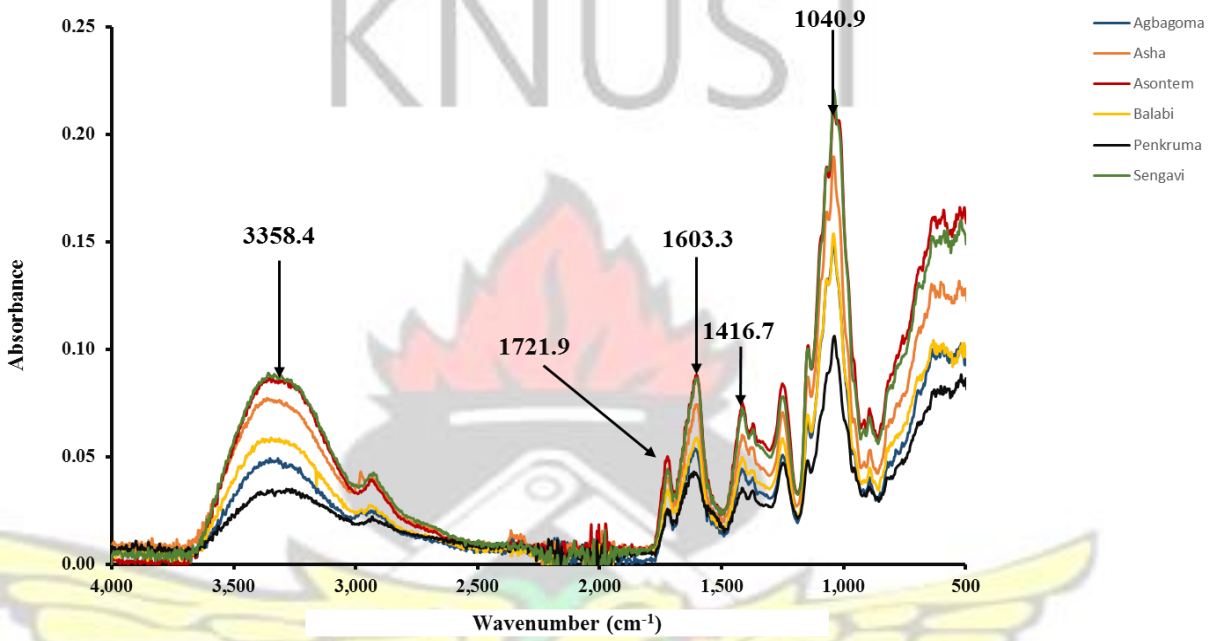
Okra Genotype	Zeta Potential (mV)
Asha	-17.30 (1.3)
Penkruma	-21.00 (2.2)
Asontem	-15.83 (2.1)
Agbagoma	-16.66 (3.2)
Sengavi	-15.96 (0.9)
Balabi	-22.06 (1.0)

4.3.5 Structural Analysis

4.3.5.1 Fourier-Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectra of pectins from different okra genotypes cultivated under controlled environmental condition are shown in Figure 4.1. A broad stretching characteristic peak was observed in the range of 3200 – 3600 cm^{-1} for all pectins from the genotypes which signifies the presence of –OH group which is due to inter- and

intramolecular hydrogen bonding of the GalA backbone. A relatively small peak was observed in the region of 3000 – 2800 cm^{-1} for the C-H stretching band showing the presence of methyl and methylene groups in the okra pectin. Peaks in the region of 1600 –



1800 cm^{-1} were critical for the identification and quantification of the different pectins. Peaks between 1630-1600 cm^{-1} corresponds with non-esterified carboxyl groups whereas those in the region of 1730-1720 cm^{-1} are related to the esterified carboxyl groups of the pectins. The region from 900 to 1200 cm^{-1} is typically referred to as the carbohydrate finger print region because most polysaccharides show peaks at this region. FTIR spectra of all the purified pectins from the different okra genotypes showed this carbohydrate finger print.

Figure 4.2: FTIR of okra pectin

4.3.5.2 ^{1}H and ^{13}C Nuclear Magnetic Resonance Analysis

A comparison of ^{1}H-NMR spectra of okra isolated (Figure 4.2) shows a high similarity between most spectra. The spectra of *Penkruma* however showed some variation in terms of absence of peaks in the region of 5.5 ppm. Okra pectins from all genotypes showed a broad peak at 4.20 ppm which indicated the presence of methyl groups connecting to carboxyl groups of GalA (Wang *et al.*, 2016; Alba *et al.*, 2015). The intensity of the peak at 4.20 ppm varied among different genotypes. The signal at 2.50 ppm was indicative of the presence of *O*-acetyl substituent and was similar to that reported in previous study for okra pods (2.10 ppm). A similar signal had been reported for the acetyl group in pectin from grape fruit peel (Wang *et al.*, 2016). The presence of unbranched α1, 2-linked rhamnose of pectins for the different okra genotypes varied from 1.30 to 1.60 ppm whereas the branched α- 1, 2, 4-linked rhamnose varied from 1.60 to 1.70 ppm depending on the type of genotype. For grape fruit pectin, these signals occurred at 1.14 and 1.22 ppm respectively (Wang *et al.*, 2016). Proton signals occurring around 5.0 ppm (5.5 and 5.7 ppm) can be assigned to non-esterified galacturonic acid residues as observed for other pectins (Wang *et al.*, 2016). These signals were however absent in pectin obtained from the genotype- *Penkruma* (d).

The ^{13}C NMR spectra of pectin from the different okra genotypes showed peaks around 173 to 173.64 ppm indicative of the carboxyl group of galaturonic acid. This C=O peak was also observed for grewia gum at 173 ppm (Nep *et al.*, 2014). The signal at 175 ppm which was obvious in the genotype *Asha* (a), signified the C-6 of the esterified group

of galaturonic acid. C-6 signal around 174.6 was also observed in polysaccharides from prunes and cacao pod (Cantu-Jungles *et al.*, 2014; Vriesmann *et al*., 2011). The signal occurring around 50 to 53.04 ppm 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 *et al*., 2011), cupuassu (Vriesmann and Petkowicz, 2009) and white cabbage (Westereng *et al.*, 2006). The peaks at 20.63 to 20.83 ppm can be assigned to the methyl of the acetyl group and the resonance around 16.91 to 18.39 ppm corresponds with the methyl of the rhamnose group (Alba *et al*., 2015).

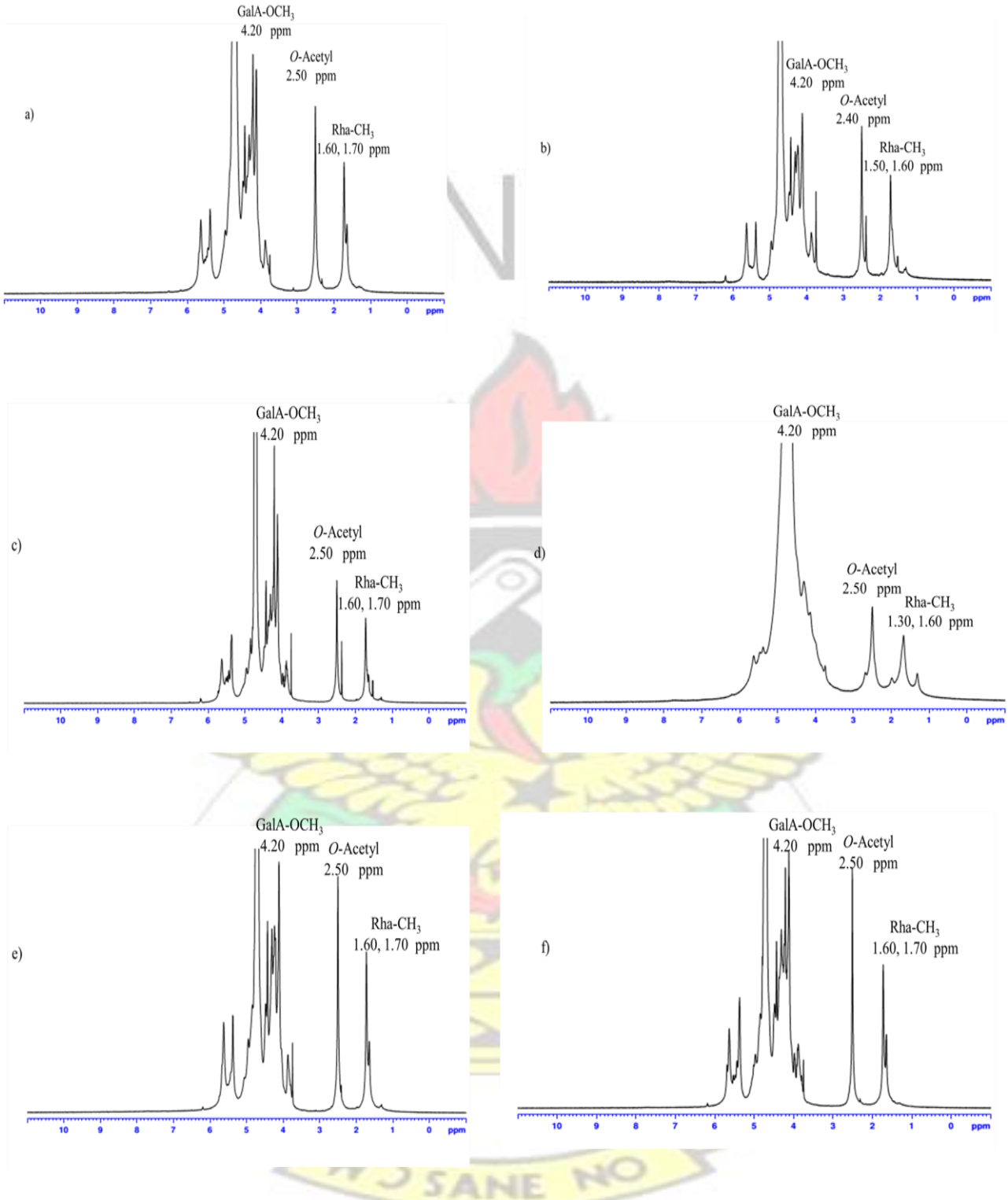


Figure 4.3: ^{1}H NMR spectra of *Asha* (a), *Agbagoma* (b), *Asontem* (c), *Penkruma* (d), *Balabi* (e) and *Sengavi* samples (f)

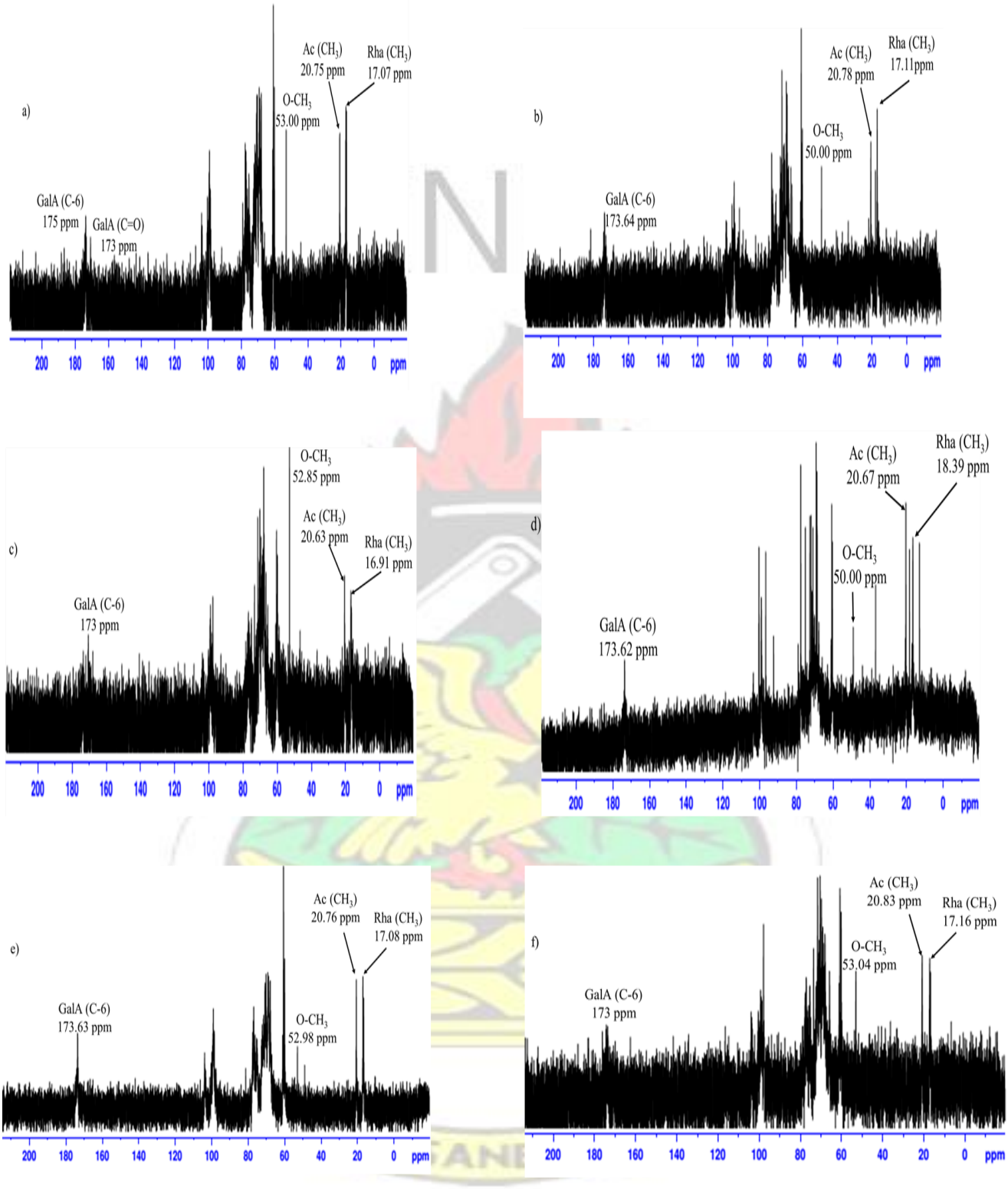


Figure 4.4: ^{13}C NMR spectra of *Asha* (a), *Agbagoma* (b), *Asontem* (c), *Penkruma* (d), *Balabi* (e) and *Sengavi* samples (f)

4.3.5.3 Polymer Molecular Weight Distribution of Okra Pectins from Different Genotypes

Okra pectin polymers consist of sugar monomer units chemically connected into long chains by glycosidic bonds. The size of the polymer chain is expressed in molecular weight and this is dependent on the relative molecular mass and number of the sugar units in the polymer chain. Most carbohydrate polymers contain more than one chain length (Polydisperse) and the average distribution of the chain masses can be expressed in different forms. The molecular weight of polymers from different okra genotypes were determined using size exclusion chromatography (SEC) coupled to multiangle laser light scattering. Molecular weight averages were expressed as Mn (number-average molecular weight) and Mw (weight-average molecular weight) and the polydispersity of the chains expressed as the ratio of the weight-average molecular weight to the number-average molecular weight (Table 4.9). The weight-average molecular weight (Mw) of the major peak of polymers from the different okra genotypes ranged from 791.5 to 1693.7 x 10^3 g mol^{-1}. Mw of the polymer from the genotype *Sengavi* (1693.7 x 10^3 g mol^{-1}) was relatively higher than the other genotypes and this value corresponded with a high intrinsic viscosity (5.10 dL g^{-1}) whereas the genotype with the lowest Mw value- *Balabi* (791.5 g mol^{-1}) likewise recorded the lowest intrinsic viscosity (2.91 dL g^{-1}) in rheological studies. Differences in molecular weight of the polymers can be attributed to differences in genotype. The molecular weight of the pectins of the genotypes *Balabi* and *Penkruma* were comparable to values obtained by Alba *et al*. (2015) which ranged from 640 x 10^3 to 767 x 10^3 g mol^{-1}. However Mw values of pectins from *Asontem, Asha, Agbagoma* and *Sengavi* were higher than okra pectins from other studies (Alba *et al*., 2015; Kontogiorgos *et al.*, 2012; Sengkhamparn, *et al.*, 2009) and sugar beet- 184 x 10^3 g mol^{-1} (Guo *et al.*, 2016).

Table 4.9: Molecular weight characteristics of pectin from the different okra genotypes

Genotypes	(Parameters) Peak 1			(Parameters) Peak 2		
	Mw (g mol^{-1})	Mn (g mol^{-1})	Mw/Mn	Mw (g mol^{-1})	Mn (g mol^{-1})	Mw/Mn
Asha	1202.9	897.1	1.34	100.7	78.4	1.28
Penkruma	893.8	742.7	1.20	113.3	88.0	1.28
Asontem	1233.5	910.7	1.35	94.7	75.1	1.26
Agbagoma	1419.7	1197.8	1.18	120.8	89.7	1.34
Sengavi	1693.7	1202.7	1.41	135.5	110	1.23
Balabi	791.5	526.8	1.50	112.3	104.8	1.07

Mn is number-average molecular weight and Mw is weight-average molecular weight

The number average molecular weight of polymers from the okra genotypes studied was between 526.8 to 1202.7 x 10^3 g mol^{-1}. The refractive index profile of the different okra genotypes generally grouped polymer chains of the genotypes into three different populations based on elution times- around 25, 40 and 50 min (Figures 4.5 and 4.6). Natural polysaccharide have usually shown a polydispersity between 1.5 and 2.0 (Fissore *et al.*, 2013), nonetheless the polydispersity of the major portion of okra pectin genotypes studied in this case was in the range of 1.2 to 1.5.

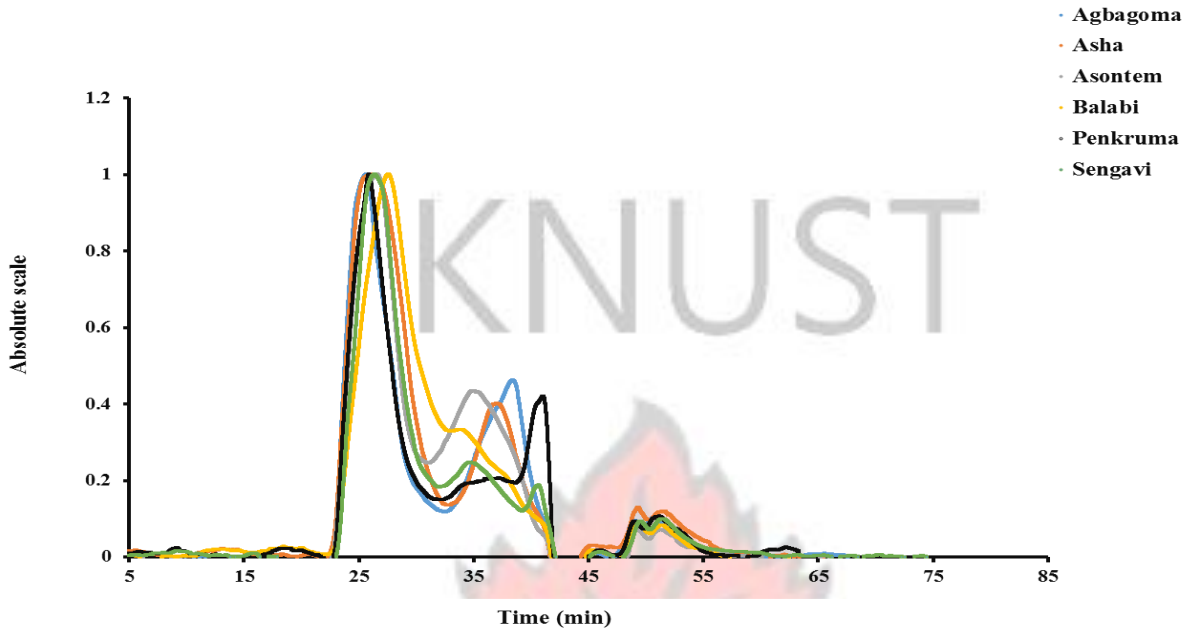


Figure 4.5: Refractive index (RI) of different okra genotypes

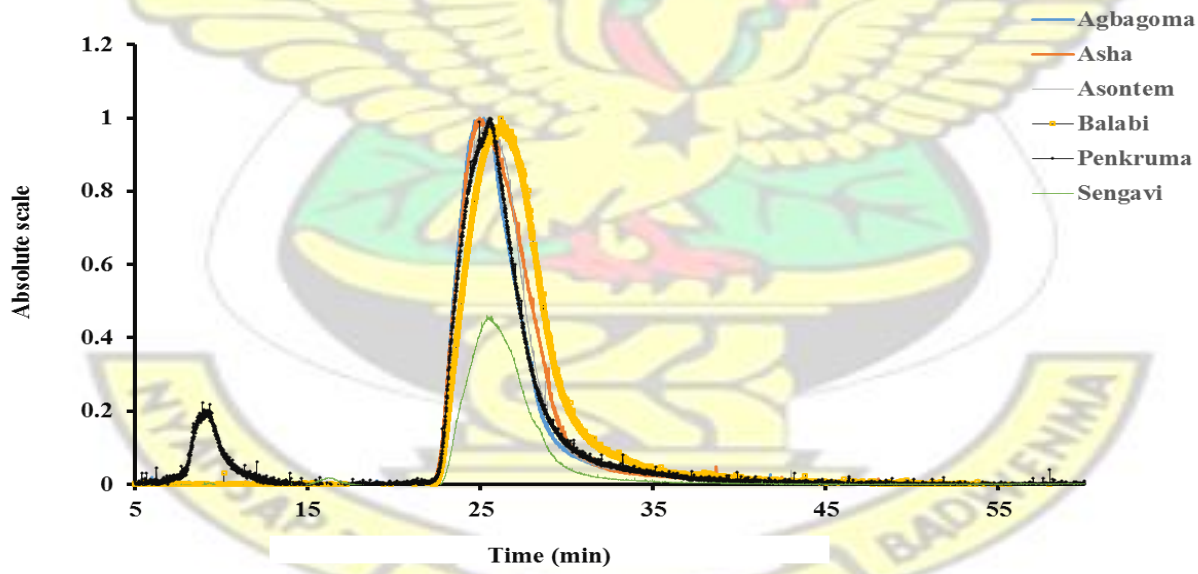


Figure 4.6: MALS traces (LS) of size exclusion chromatograms of different okra genotypes

4.4 Conclusion

The results indicated that local okra genotypes had considerable pectin yield suggesting that okra can be used as a sustainable source of pectin for potential applications. Genotypic differences among okra plants considerably influenced the physicochemical properties of the isolated pectins. It has been shown that okra pectins from different genotypes had different chemical composition, macromolecular and physical properties. Pectins from the genotype *Asha* had high neutral sugars composition, acetyl and galatouronic acid content whereas pectins from the genotype *Balabi* were of low molecular weight. Pectins from the genotype *Penkruma* were more linear and demonstrated a smaller average size of branching side chains whereas *Asha* and *Balabi* pectins were more branched. From this study, okra pectins from different genotypes have been systematically characterized and the differences between genetic variations have the potential to be exploited/engineered in a number of potential applications as emulsifying agents, thickeners, stabilizers and binders in food and pharmaceutical products.

CHAPTER 5

RHEOLOGICAL BEHAVIOUR OF PECTINS FROM DIFFERENT OKRA GENOTYPES

5.1 Introduction

Food rheology is the study of the flow or deformation of a food material when subjected to an applied force (Barbosa-Canovas *et al*., 1996). Rheological investigations are relevant from a food perspective in the design of flow processing, quality control, storage and processing stability measurements, predicting texture and elucidating the molecular and structural changes in food during processing (Heldman and Lund, 2007; Barbosa-Canovas *et al*., 1996). Determination of the rheological properties of foodstuffs also provide useful information that guides ingredient selection, product optimization and packaging design strategies (Heldman and Lund, 2007).

Pectins from *Abelmoschus esculentus*, a plant of the Malvacae family is of major technological interest in recent times with current studies indicating that it can function as thickeners, emulsifiers, stabilizers, and as fat replacers (Li and Nie, 2016; Ghori *et al.*, 2014; Alba *et al*., 2015; Shittu and Olaitan, 2011; Romanchik-Cerpovicz *et al*., 2006). Non starch polysaccharides from plants have been used to modify rheological properties and improve the stability of food products (Alamri *et al.*, 2013). Xu *et al*. (2006) noted that dispersions of polysaccharides in water improved the gelation and thickening properties of aqueous food systems. The inter- and intra-reactive forces between polymers and solvent systems significantly influence hydration and the hydrodynamic volume of polysaccharides in solution. Polysaccharides from different genotypes of the same plant species are thus likely to have different molecular hydrodynamic volumes, conformations and interactions due to differences in their chemical make-up which consequently influence

polymer rheology (Behrouzian *et al*., 2014). Okra pectin is known to contain a substantial amount of galacturonic acid residues which influence the rheological behaviour of the polymer in solution (Alba *et al*., 2015). The presence of galacturonic acid residues and counterions in okra pectin solutions influence inter and intra-molecular interactions. Okra gum contains a substantial proportion of charged galacturonate residues which also affects its viscosity in a salt solution (Ndjouenkeu *et al.*, 1996; Kontogiorgos *et al.*, 2012). The larger the amount of galacturonic acid residues on a pectin chain the greater the repulsive forces between the residues, hence polymers tend to expand and occupy a larger hydrodynamic volume. However polymers that contain a less amount of the galacturonic acid residue coil up via hydrophobic interactions and occupy less hydrodynamic volume in a solution with reduced intrinsic viscosity (Harding, 1997; Ndjouenkeu *et al.*, 1996; Kontogiorgos *et al*., 2012). Intrinsic viscosity is a measure of the hydrodynamic volume of macromolecules in dilute solutions (Xu *et al*., 2006). It usually reflects the hydrodynamic volume of individual polymer coils, and when multiplied by concentration gives an index of the total degree of space occupancy (Ndjouenkeu *et al.*, 1996). Intrinsic viscosity measurements are of importance in biopolymer characterization and hence are used to characterize the volume occupied by individual polymer molecules in isolation (Kontogiorgos *et al.*, 2012). Viscosity of polysaccharide solutions is influenced by factors such as molecular weight, flexibility or stiffness, charge and charge density, polymer conformation/structure and pH (Phillips and Williams, 2009; Alba *et al.*, 2013; Kontogiorgos *et al.*, 2012; Xu *et al*., 2006; Alistar *et al*., 2006). Depending on the plant source, genotype, stage of ripening and extraction method, pectins exhibit heterogeinity in molecular weight which subsequently affect their dilute solution rheology. The pH of the extraction medium has been reported to also influence the viscosity of polysaccharides in

solutions. Alba *et al.* (2013) indicated that okra pectins extracted at higher pH have greater degree of methylation and this contribute to stronger intermolecular interactions.

Different genotypes of okra have different mucilage yield and composition (Chapter 4), and this can affect the rheological properties of specific polymers. Studies have been done to elucidate the structure, functional and physicochemical properties of okra pectins (Alba *et al*., 2015; Kontogiorgos *et al.*, 2012; Sengkhamparn *et al.*, 2009; Georgiadis *et al.*, 2011; Ndjouenkeu *et al.*, 1996), however there is limited information on how genotypic differences of the plant species could influence these properties. Understanding how different genotypes of okra pectin- their concentration, shape, size, polydispersity affect their functionality is of great industrial importance. This study therefore seeks to unveil the structural features of pectins from different genotypes of okra and their specific role in influencing rheological behavior.

5.2 Material and Methods

5.2.1 Isolation of Okra Pectins

Oven dried okra pulp from six different genotypes were subjected to extraction procedure as described by Alba *et al*. (2015). The extracted pectins were freeze dried after precipitation in ethanol, solubilized (5 % w/v), and extensively dialyzed for 3 days with frequent replacement of deionized water. The purified okra pectins were then concentrated by rotary evaporation and then freeze dried.

5.2.2 Rheological Analysis

5.2.2.1 Intrinsic Viscosity Measurement

Okra pectins were dispersed at 0.01 – 5 % g dL^{-1} at pH 7.0 in Sorensen's phosphate buffer in the presence of 0.1 M NaCl with 0.02 g dL^{-1} NaN_3 as a preservative. Isolates were placed in sealed glass-vials and left overnight under continuous stirring using a magnetic stirrer to ensure complete solubilization of the pectin. Intrinsic viscosity measurements of okra isolates were performed using a Ubbelohde capillary viscometer (Psl rheotek OB. C 80705) placed in a water bath at 20 ± 0.1 ^{o}C. Determination of the intrinsic viscosity of the different polymer solutions was obtained by extrapolation of viscometric data to zero concentration according to the twin Huggins and Kraemer expressions (Li *et al.*, 2013; Kontogiorgos *et al.*, 2012).

Huggins' Equation (Huggins, 1942):

$$\frac{\eta_{SP}}{\mathrm{C}} = [\eta] + \mathrm{k}_1[\eta]^2\mathrm{C}$$

Kraemer's Equation (Kraemer, 1938):

$$\frac{\mathrm{In}\ \eta_{\mathrm{rel}}}{\mathrm{C}} = [\eta] + \mathrm{k}_2[\eta]^2\mathrm{C}$$

Where k_1, k_2 and C are Huggins constant, Kraemer constant and solute concentration respectively.

The Huggins and Kraemer constants were obtained using the following equations;

$$\mathrm{k}_1 = \frac{\mathrm{d}_{\mathrm{Huggins}}}{[\eta]^2}$$

$$k_2 = \frac{d_{Kraemer}}{[\eta]^2}$$

The Huggins and Kraemer constants were also related using the equation: $k_1 = k_2 + 0.5$

5.2.2.2 Steady Shear Measurement

Steady shear measurements were carried out at 20 ^oC using a Bohlin Gemini 200HR nano rotational rheometer equipped with a double gap geometry and then a cone-and-plate geometry (55 mm diameter,cone angle 2). Flow curves were determined in the range of 0.01-1000 s^{-1} at 20 ^oC. Generalized flow curves were produced using a modified Cross model with GraphPad Prism v.5 (Graph-Pad Software, San Diego, USA) (Kontogiorgos *et al.*, 2012).

5.2.3 Data Analysis

Data obtained was analysed using Minitab version 17 (Minitab Inc., Philadelphia, U.S.A.). Comparisons of means between the different okra genotypes were done using analysis of variance (ANOVA) with a probability, $p<0.05$. Fisher's least significant difference (LSD) was used to discriminate among means when ANOVA indicated significant F values. All treatments were conducted in triplicate and mean values reported.

5.3 Results and Discussion

5.3.1 Intrinsic Viscosity of Pectins from Different Okra Genotypes

Intrinsic viscosity, Huggins and Kraemer constants calculated from the HugginsKraemer plots were determined for the okra pectin from the different genotypes (Table 5.1). According to the results the Huggins plots better estimated intrinsic viscosity for the pectin solutions since it showed higher determination coefficients ($R^2>0.95$) than

the Kraemer plot. The Huggins model thus provided a more reliable measure for the intrinsic viscosity than the Kraemer Model. Intrinsic viscosity of the purified okra pectins from the different genotypes ranged from 2.91 to 5.10 dL g^{-1} for pectins for the Huggins plot.

Table 5.1: Intrinsic viscosity, Huggins and Kraemer constants for purified okra pectins

Okra Genotype	Huggins Model			Kraemer Model			
	$[\eta]$ (dL g^{-1})	K_H	R^2	$[\eta]$ (dL g^{-1})	K_K	R^2	$K_H - K_K$
Asha	4.35	0.66	0.99	4.49	0.07	0.61	0.59
Penkruma	4.35	0.73	0.95	4.72	0.09	0.59	0.65
Asontem	3.55	0.69	0.99	3.72	0.06	0.92	0.62
Agbagoma	3.56	0.69	0.99	3.72	0.06	0.92	0.62
Sengavi	5.10	0.55	0.99	5.26	0.08	0.96	0.47
Balabi	2.91	0.37	0.93	2.94	0.14	0.75	0.23

Intrinsic viscosity-η; Huggins constants K_H; Kraemer constants- K_K

The genotype *Sengavi* had the highest intrinsic viscosity value whereas *Balabi* had the lowest value. Intrinsic viscosity values of pectin extracts from the genotypes *Asha* and *Penkruma* were consistent with values (4.1 and 4.4 dL g^{-1}) reported by Alba *et al*. (2015). However [η] values for all the okra pectins studied were higher than those obtained using the sequential extraction methods (Kontogiorgos *et al.*, 2012; Sengkhamparn *et al.*, 2009;

Georgiadis *et al.*, 2011).

5.3.2 Huggins Constant (K_H)

The Huggins constant has been used to indicate polymer-solvent interactions in dilute solution regimes. Polymer molecular architecture and the extent of coil expansion have been known to greatly depend on K_H (Hesarinejad *et al*., 2015; Irani *et al.*, 2016). The Huggins constant usually has values between 0.3 – 0.5 for a polymer in good solvents, 0.5 – 0.8 for polymers in theta solvents and values greater than 1 for poor solvents (Curvale *et al*., 2008; Pamies *et al.*, 2008; Ma and Pawlik, 2007; Hesarinejad *et al*., 2015; Irani *et al.*, 2016). K_H values > 1 indicate the formation of aggregates in biopolymers (Irani *et al.*, 2016). K_H values have also been used to predict the flexibility of macromolecules and the state of interactions among different chains; for flexible chains, values range from 0.2 to 0.8 (Kontogiorgos *et al.*, 2012; Pamies *et al*., 2008) . K_H values calculated for okra pectins in this study ranged from 0.3 to 0.7 and were close to 0.43 and 0.58 indicated by Kontogiorgos *et al*. (2012). The result in this study indicates that the okra pectins from the different genotypes had flexible polymer chains. Theoretically the expression: $K_H + Kk = 0.5$ has been predicted as valid for Huggins and Kraemer constants for random coil conformation, although the validity of this expression is not conditioned by the nature of the polymer or solvent system but a mathematical consequence (Pamies *et al*., 2008). At 20 ^oC in a phosphate buffer of pH 7 in the presence of 0.1 N NaCl salt, the sum of Huggins and Kraemer constants for okra isolates extracted using a hot acid buffer (pH 6) ranged from 0.2 to 0.6 and most values were generally closer to 0.5.

5.3.3 Steady Shear Measurement

The transition from dilute to concentrated regime of okra pectin from different genotypes was studied by plotting a double logarithm graph of η_{sp} against $c[\eta]$. The double logarithm plot is important

because it is used to determine the critical concentration (coil overlap parameter) of the pectin solutions in different dilute and concentrated regimes (Hesarinejad *et al*., 2015; Behrouzian, *et al*., 2014; Morris *et al.*, 1981). For most disordered polymers the double logarithm plots superimpose and fall into two dilute and concentrated regions with a change of slopes from approximately 1.4 to 3.3 (Morris *et al.*, 1981). Nonetheless deviations from this behavior have been observed in other polymers such as β glucan, locust bean and guar gum (Agbenorhevi *et al.*, 2011). Polymer extracts from different okra genotypes in this study were in three different regimes of concentration (dilute, semi-concentrated and highly concentrated) and correspondingly recorded three different slope values in the range of 0.03 to 0.55 (slope 1); slope 2- 1.38 to 2.54; and slope 3- 3.35 to 6.14 (Table 5.2).

Table 5.2: Slopes, intrinsic viscosity, critical concentration and coil overlap parameter of pectin solution from different okra genotypes

Genotypes	Slope 1	Slope 2	Slope 3	[η] (dL g^{-1})	c* (g dL^{-1})	c** (g dL^{-1})	c*[η]	c**[η]
Asha	0.46	2.54	4.55	4.35	0.18	1.34	0.80	5.83
Penkruma	0.55	2.07	5.52	4.35	0.17	1.37	0.72	5.95
Asontem	0.03	1.43	4.55	3.55	0.07	1.51	0.24	5.37
Agbagoma	0.10	1.38	6.14	3.56	0.06	1.04	0.21	3.72
Sengavi	0.46	2.32	5.65	5.10	0.18	1.65	0.90	8.39
Balabi	0.48	1.45	3.35	2.91	0.15	1.94	0.44	5.65

Intrinsic viscosity is η; c* is concentration that demarcates transition from dilute to semi dilute whereas c** from semi-dilute to concentrated regime of the biopolymers

Double logarithm plots of the polysaccharide extracts from the different okra genotypes superimposed closely. From the coil overlap plots (Figure 5.1) there is an obvious curvature around the breakpoint of the two extreme concentration regimes (dilute and highly concentrated). As observed by Ndjouenkeu *et al*. (1996) in a rheological study

of okra gum, three regimes of concentration-dependence was observed for polymers from the different okra genotypes. The transition at c* can be associated with the initial interaction between the individual coils of each polymer in the dilute regime and has been noted to usually occur when $c^*[\eta] \approx 1$ (Ndjouenkeu *et al*., 1996). In the case of polymers from the different okra genotypes studied, results obtained ranged from 0.21 to 0.90. Values obtained for $c^*[\eta]$ for polymers from the genotypes- *Sengavi* (0.90), *Asha* (0.80) and *Penkruma* (0.72) agreed with the above generalization ($c^*[\eta] \approx 1$) although *Asontem* (0.24) and *Agbagoma* (0.21) exhibited somewhat lower $c^*[\eta]$ values (Table 5.2).

The upper two regimes of higher concentration dependence have also been attributed to the initial compression and inter penetration of polymer coil and the point of intersection designated c**. This occurrence is as a result of increasing space occupancy and greater interaction between polymer chains. For most polymers the product of c** and intrinsic viscosity is known to vary from 2 to 10. Again results obtained in this study agreed with this generalization in that $c^{**}[\eta]$ ranged from 3.72 to 8.39 with the polymer from the okra genotype- *Sengavi* recording the highest value (Table 5.2).

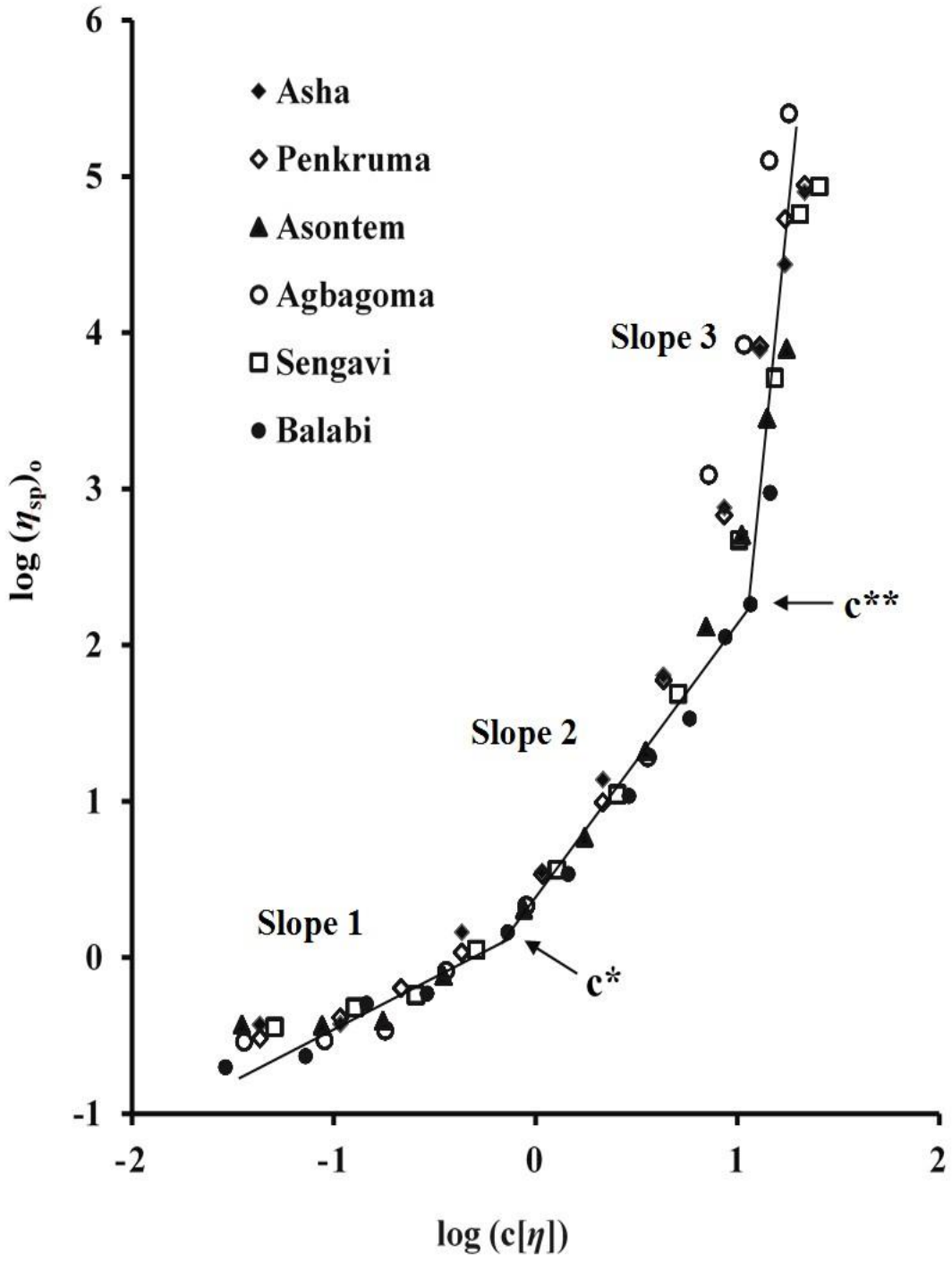


Figure 5.1: Double logarithmic plots of zero shear specific viscosity ($(\eta_{sp})_o$) against reduced concentration (c[η]) for all samples. c* is the concentration that demarcates the transition from dilute to semi-dilute whereas c** from semi-dilute to concentrated regime of the biopolymers. The lines serve solely as a guide to the eye.

5.3.4 Viscosity Dependence on Shear Rate for Pectin Solution of Different Concentrations

The shear rate sweeps (0.01 to 1000 s^{-1}) reveals the viscosity dependence of okra pectin on shear rate at 4 % (w/v) and 1 % (w/v) for the six (6) genotypes studied (Figure 5.2). Pectin solutions exhibited pseudoplastic behavior at higher concentrations and Newtonian behavior at lower concentrations (Figure 5.3). From the results pectins from the okra genotypes *Agbagoma* and *Sengavi* had the highest viscosities whereas pectins from *Balabi* and *Asontem* recorded low viscosity values. A critical observation of the viscosityshear rate plot for pectin at 4 % uniquely separate the genotypes (6) studied into three distinct regions- *Agbagoma, Sengavi, Penkruma* and *Asha* with high viscosities clustering at one point; pectin from the genotype *Asontem* having an intermediary viscosity; and *Balabi* pectin at 4 % demonstrated extremely low viscosity. The viscosity of

polysaccharide depends on factors such as molecular weight, flexibility or stiffness, charge and charge density, polymer conformation/structure and pH (Phillips and Williams, 2009; Alba *et al.*, 2013; Kontogiorgos *et al.*, 2012; Xu *et al*., 2006; Sengkhamparn *et al.*, 2010; Alistar *et al*., 2006).

From physicochemical studies, the galacturonic acid content of the highly viscous genotypes (*Asha, Penkruma* and *Sengavi*) were comparatively high and this probably increased repulsive forces between the residues and explains the larger hydrodynamic volume of these genotypes. The genotype *Balabi* with the lowest viscosity value also had the least galauronic acid content (42.8 %) explaining an increase in hydrophobic interactions in the polymer, resulting in chain contraction and hence a reduced intrinsic viscosity. The results from this study shows that genotypic differences can influence the

viscosity of polymer solutions. Differences in the galacturonic acid content of the pectins from the different genotypes accounts for these differences in viscosities.

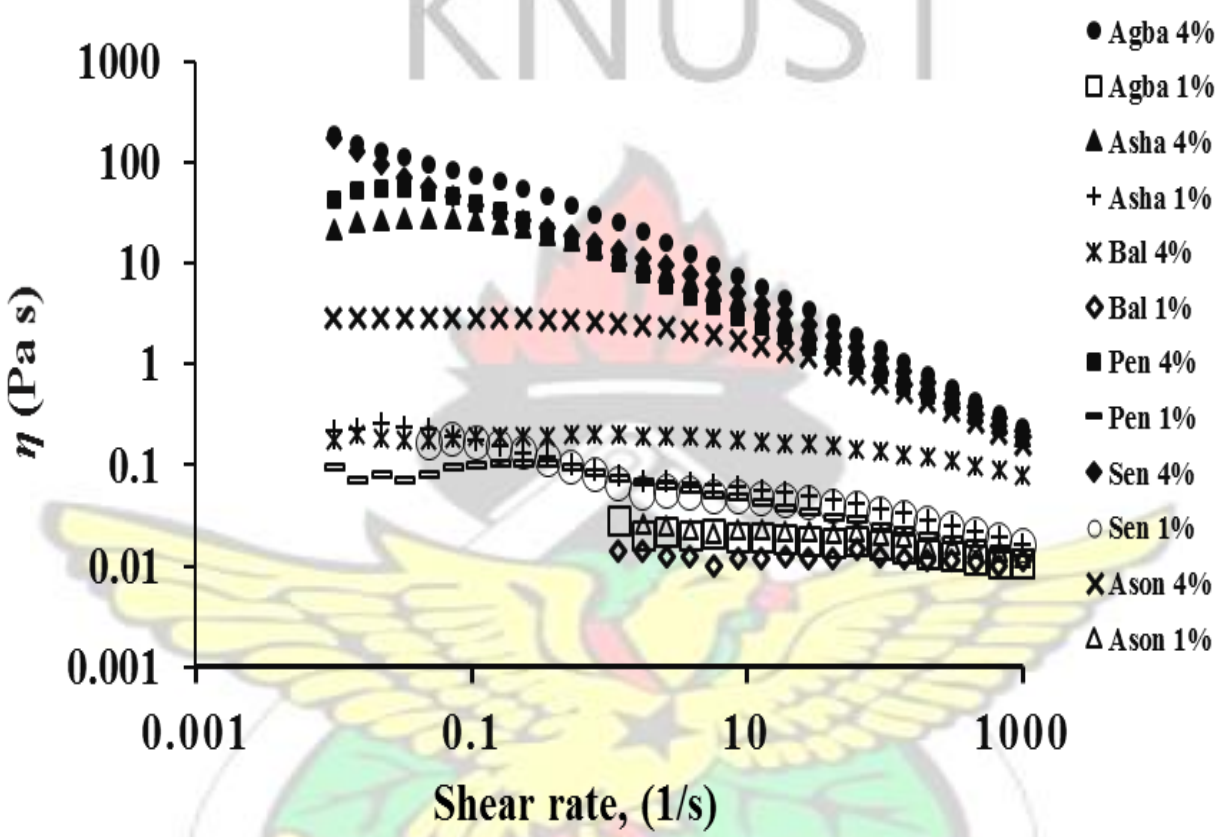


Figure 5.2: Viscosity dependence on shear rate of pectins at 1% (w/v) and 4% (w/v)

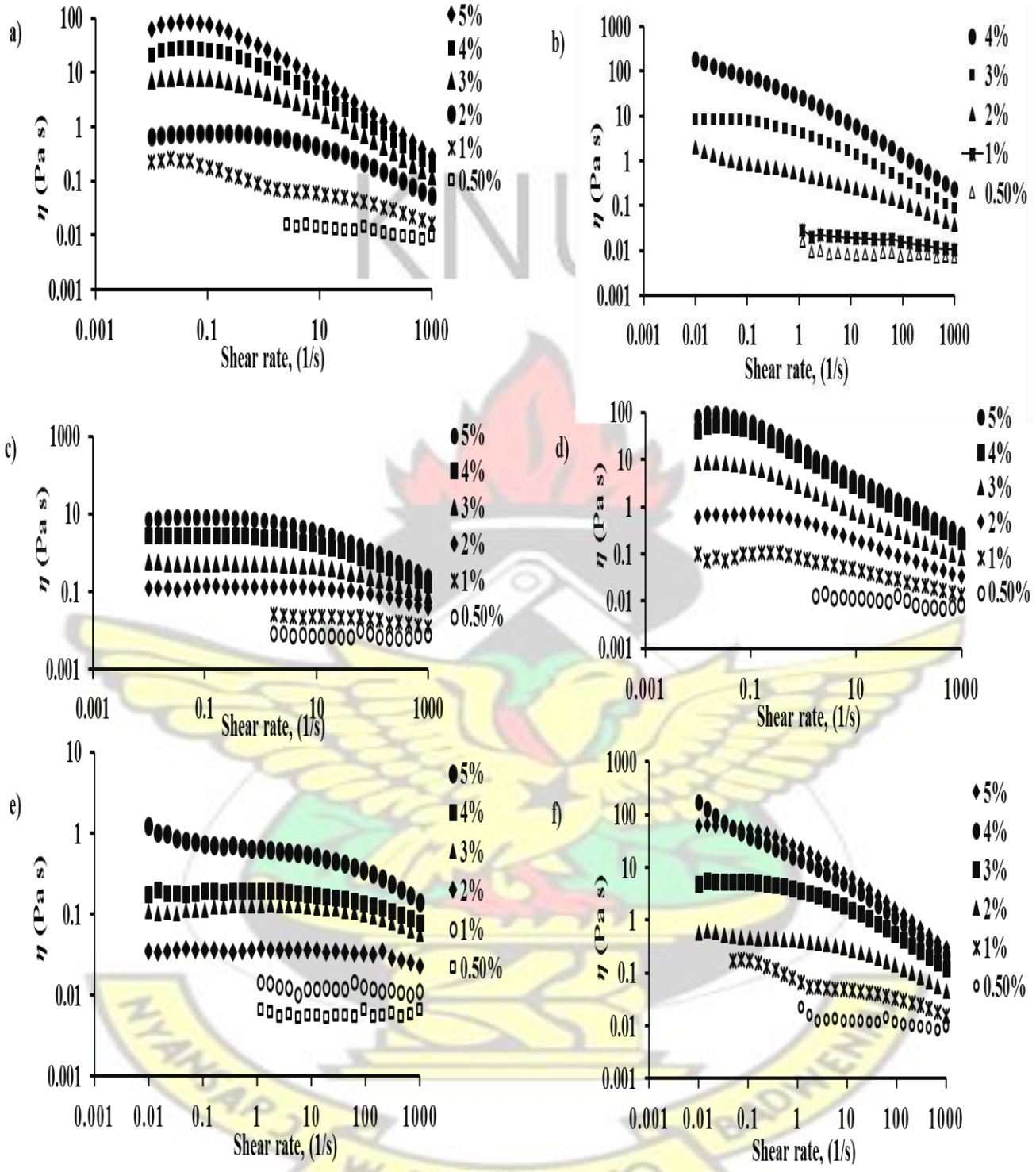


Figure 5.3: Viscosity dependence on shear rate for specific pectin solution of different concentrations: *Asha* (a), *Agbagoma* (b), *Asontem* (c), *Penkruma* (d), *Balabi* (e) and *Sengavi* samples (f)

5.3.5 PCA Plot of Intrinsic Viscosity Data

Principal component analysis (PCA) of the data emphasized differences (in the intrinsic viscosity, Huggins constant and rheological data pulled together) between the okra pectins by separately grouping pectin from the different genotypes (Figure 5.4). Genotypic variations of samples might have accounted for the differences in rheological characteristics. Pectin from the genotype *Balabi* negatively correlated with the first component due to its low viscosity and hence molecular weight. Pectin from the genotype *Sengavi* is separated from the others probably due to its high viscosity and molecular weight.

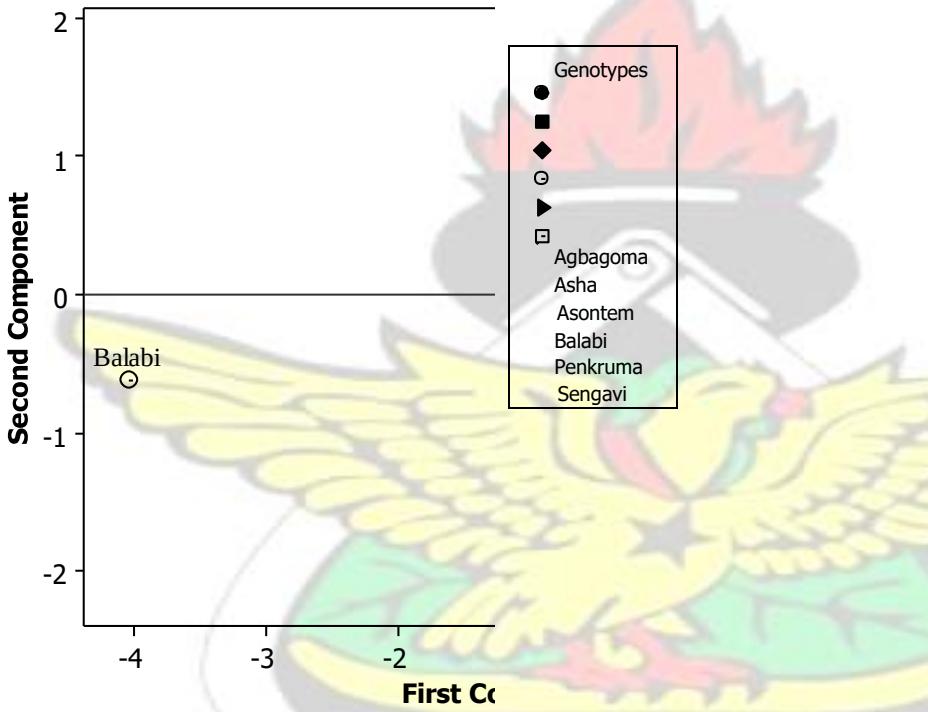


Figure 5.4: A principal component analysis (PCA) plot for okra pectins of different genetic make-up

5.4 Conclusion

Polymers determine the rheological behaviour of aqueous solutions. Viscosity measurements in dilute solutions where individual polymers are well separated provides important information on the structure, properties and the behaviour of macromolecules in solution. Genotypic differences among okra pectin studied significantly influenced the

rheological behaviour of the polymers both in dilute and concentrated regimes. Results from the study revealed that okra pectin from different genotypes had different intrinsic viscosities, flexibility, critical concentrations and demonstrated different shear behavior in dilute and concentrated solutions. The differences in rheological behavior will greatly affect the functionality of each specific pectin studied in different food systems and thus is of great industrial importance. Using the Huggins plot, intrinsic viscosity of the purified okra pectins from the different genotypes ranged from 2.91 to 5.10 dL g^{-1}, with pectins from *Balabi* and *Sengavi* having the lowest and highest intrinsic viscosity values respectively. K_H values calculated for okra pectins in this study ranged from 0.3 to 0.7 revealing flexible chain conformation. Polysaccharide extracts from different okra genotypes in this study recorded values indicative of disordered polymers. Coil overlap plots revealed an obvious curvature around the breakpoint of the two concentration regimes indicative of two critical concentrations. Viscosity-shear rate plot for pectin at 4% separated the genotypes (6) studied into three distinct zones- *Agbagoma, Sengavi, Penkruma* and *Asha* with high viscosity values, *Asontem* having an intermediary viscosity; and *Balabi* pectin at 4 % showing extremely low viscosity. Principal component analysis (PCA) of the data emphasized these rheological differences by separately grouping the pectins from different okra genotypes with a negative correlation from *Balabi*.

CHAPTER 6

EMULSIFYING CHARACTERISTICS OF PECTINS FROM DIFFERENT OKRA GENOTYPES

6.1 Introduction

Consumer demand for natural ingredients has increased in recent times and propelled the need to explore properties of pectins from different sources for new technological functionality in emulsions (Cuevas-Bernardino *et al.*, 2016). Most food products such as milk, ice cream, beverages, dressings, sauces, batters, desserts and mayonnaise require emulsifiers to stabilize the small lipid/water droplets dispersed in a continuous water/oil phase (Guo *et al.*, 2014). The stability of an emulsion is defined by no change in the size distribution of droplets and the state of dispersion over time (Gharsallaoui *et al.*, 2010). Emulsifiers are chemical agents that enhance emulsion formation and reduce the interfacial tension between two phases. Conventionally, proteins have been used as excellent emulsifiers because they possess flexible molecular conformation which can adsorb and realign at the interphase between oil-water phases and stabilize emulsion systems (Li and Nie, 2016). Polysaccharides, however, because of their rigidity and high molecular weight are usually not utilized as emulsifying agents. However, pectins that can perform this emulsion functionality in food must have hydrophobic zones in their structure (Tamnak *et al.*, 2016). The emulsifying properties of pectins have been demonstrated in sugar beet pectin (Chen *et al.*, 2016; Ma *et al.*, 2013; Schmidt *et al.*, 2015) pumpkin pectin (Cui and Chang, 2014), citrus pectin (Leroux *et al.*, 2003; Schmidt *et al.*, 2017), hawthorn pectin (Cuevas-Bernardino *et al.*, 2016) and okra pectin (Alba *et al.*, 2013). The emulsifying properties of sugar beet and okra pectins have generally been

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attributed to high acetyl content and covalently bound proteins (Schmidt *et al.*, 2015; Alba *et al.*, 2013). In contrast pectins from citrus and apple have not been considered as useful emulsifiers due to the presence of low protein and acetyl groups (Schmidt *et al.*, 2015). Coupled to high acetyl groups and presence of protein moieties in pectin emulsifiers, pectins also increase the viscosity of the continuous aqueous phase of oil-water emulsion and reduce the movement of oil droplets which suppress sedimentation and have significant stabilizing effect on colloidal solutions and emulsions (Guo *et al.*, 2014). The aim of this study was to compare the emulsifying properties of pectins from different okra genotypes with varied macromolecular and physicochemical properties.

In previous investigations, it was shown that okra pectin can be useful for emulsification in acidic emulsions (Alba *et al.*, 2013). Furthermore, pectin isolated from six different okra genotypes were characterized indicating diversity in structure, composition and viscosity, and thus present new sources of pectin that can be utilized in food products depending on the desired functionality (Kpodo *et al*., 2017). The objective of the present study, therefore, was to investigate the emulsifying properties of pectin from different okra genotypes with varied macromolecular and physicochemical properties.

6.2 Material and Methods

6.2.1 Preparation of Emulsions

The capacity of the six okra pectin extracts to act as emulsifiers was tested by means of emulsifying sunflower oil into an aqueous medium buffered at pH 2.0 (100Mm

KCl/HCl) containing 1.67 % w/v of pectin so as to yield emulsions of φ=0.1 of a nominal pectin concentration in the entire emulsion volume of 1.5 % w/v. Emulsions were fabricated at room temperature in two stages first by obtaining pre-emulsions with a high speed homogenizer for 2 min (IKA T18 basic, Ultra-Turrax, Staufen, Germany) followed by further emulsification using a Model UP 100H ultrasound device (Hielscher Ultrasonics, Teltow, Germany) with a MS7 tip at 30 kHz for 40 s with pulsed ultrasound (30% per second) at 100% amplitude.

6.2.2 Determination of Cream and Serum Layers

Cream and serum layers of the emulsions were determined by centrifugation of the okra pectin stabilized emulsions at 3000 x g for 5 min (Centrifuge 5702, Eppendorf, Hamburg, Germany) in order to separate the dispersed phase (oil droplets) from the continuous phase (serum). The cream and serum layers were recorded.

6.2.3 Determination of Particle Droplet Distribution

In order to quantify the capacity of these emulsifiers towards long-term emulsion stability, the droplet size distribution and the average droplet sizes were measured at set time intervals (Alba *et al.*, 2013; Ghori *et al.*, 2014). Droplet size distribution was measured immediately after the emulsion preparation and after 0, 5 and 15 days of storage using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) laser diffraction particle size analyzer with a` small volume sample dispersion unit Hydro 2000SM (Malvern Ltd., UK) (Alba *et al.*, 2013; Ghori *et al.*, 2014). Refractive index of sunflower oil and dispersion medium (HCl/KCl buffer, pH 2) was set to 1.435 and 1.333, respectively. Consequently, droplet size was described using the surface-weighted mean diameter ($d_{3,2}$) and volume-weighted mean diameter ($d_{4,3}$) (Alba *et al.*, 2013; Ghori *et al.*,

2014).

6.2.4 Interfacial Composition Analysis

The interfacial composition of formulated emulsions was characterized in terms of adsorbed protein and pectin at the oil-water interface. First, okra pectin stabilized emulsions were centrifuged at 3000 x g for 5 min (Centrifuge 5702, Eppendorf, Hamburg, Germany) in order to separate the dispersed phase (oil droplets) from the continuous phase (serum). The serums were then carefully collected using a syringe. Concentrations of protein and pectin (expressed as total carbohydrate) were measured in pectin solutions (i.e., aqueous phase before emulsification) and in serums according to Bradford method (1976) or phenol-sulphuric acid assay (Dubois *et al*., 1956). Interfacial protein and pectin concentrations (Γ, mg m^{-2}) were calculated as protein or pectin concentration difference between the pectin solution and serum divided by the specific surface area (SSA, m^2 mL^{-1}) of the oil droplets that was obtained from the instrument.

6.2.5 Determination of ζ- (Zeta) Potential

All ζ-potential measurements were performed using a ZetaSizer Nano Series ZEN2600 (Malvern Instruments, Malvern, UK) at 25 °C. Emulsions were diluted 1000 times in buffer solutions in order to avoid multiple scattering effects. All measurements were performed in triplicates immediately after emulsion preparation and after 0, 5 and 15 days of storage (Alba *et al.*, 2013).

6.2.6 Emulsion Morphology

Fluorescent microscopy was performed using Imager Z1 AxioCam MRm camara equipped with ZEN 2011 software (Carl Zeiss Microscopy, GmbH, Germany). The fluorescent dye (Rhodamine B, 0.02%) was placed in the extract solutions prior to

emulsification. Emulsions were placed on a glass slide and consequently covered with a coverslip prior to imaging (Alba *et al.,* 2013).

6.2.7 Rheological Measurement

Rheological properties of the samples (stock okra pectin solution and emulsions) were measured using a Bohlin Gemini 200HR nano rotational rheometer equipped with cone-and-plate geometry (40 mm diameter, cone angle 4°). Viscosity measurements were performed on the fresh pectin solutions and emulsions, and during storage (after 5 and 15 days). All measurements were completed in a steady shear mode in the range of 0.01-1000 s^{-1} at 25 °C (Alba *et al.*, 2013).

6.3 Results and Discussion

6.3.1 Creaming of Oil-Water Emulsions

Droplets in an oil-in-water emulsion tend to cream because most liquid oils have a lower density than water and hence there is the tendency for oil to accumulate at the top of the emulsion whereas water accumulates at the bottom (Kasapis *et al*., 2009). Creaming of emulsion droplets is an undesirable process for food manufacturers. Emulsions prepared using okra pectin extract from different genotypes showed varied cream layers when they were subjected to centrifugation at 3000 x g for 5 min. Creaming was highest in emulsions that contained *Penkruma* (16.9 %) and *Agbagoma* (15.4 %) pectin extracts whereas emulsions containing pectin extracts from *Sengavi*, *Asha*, *Asontem* and *Balabi* had creaming index ranging from 0.8 to 3.8 % (Table 6.1). From the results emulsions prepared with pectin extracts from okra genotypes *Sengavi*, *Asha*, *Asontem* and *Balabi* demonstrated higher stability towards creaming than emulsions containing okra pectin extracts from

Penkruma and *Agbagoma*. Emulsions containing pectin extracts from *Sengavi* had the least creaming index (0.8 %) and hence was more stable towards creaming.

Table 6.1: Serum and cream layers of okra pectin stabilized emulsions after centrifugation

Sample	Serum layer (ml)	Serum layer (%)	Cream layer (%)
Asha	12.8	98.5	1.5
Penkruma	10.8	83.1	16.9
Asontem	12.5	96.2	3.8
Agbagoma	11.0	84.6	15.4
Sengavi	12.9	99.2	0.8
Balabi	12.5	96.2	3.8

6.3.2 Interfacial Activity of Okra Extracts from Different Genotypes

Biopolymers acting as emulsifiers in oil-water emulsions will usually accumulate at a higher concentration in a phase where the polymers have better interaction with the solvent system (Coupland, 2014). An effective emulsifier operates by rapidly adsorbing to an oil-water interface, reduce interfacial tension between two immiscible phases and protect emulsion droplets from aggregation (Kasapis *et al*., 2009). To better understand the emulsifying action of biopolymers of different composition, the surface activity of extracts from different okra genotypes with varied amounts of pectins (9.4 – 16.6 %) and proteins (2.1 – 3.5 %) were studied and results presented in Table 6.2.

Table 6.2: Interfacial pectin and protein concentration

Sample	Adsorbed Pectin (%)	ΓPectin (mg m^{-2})	Adsorbed Protein (%)	ΓProtein (mg m^{-2})

Asha	47.7±1.3^{b}	1.9±0.1^{c}	78.2±0.1^{ab}	0.7±0.0^{b}
Penkruma	55.4±0.5^{a}	0.9±0.0^{e}	82.2±5.3^{ab}	0.3±0.0^{c}
Asontem	49.0±1.5^{b}	3.6±0.1^{a}	89.9±8.2^{a}	1.0±0.1^{a}
Agbagoma	41.5±1.3^{c}	0.6±0.0^{f}	74.8±6.4^{b}	0.4±0.0^{c}
Sengavi	59.5±2.5^{a}	1.5±0.1^{d}	46.0±3.2^{c}	0.3±0.1^{c}
Balabi	50.1±3.3^{b}	2.3±0.2^{b}	56.2±7.1^{c}	0.3±0.0^{c}

Values indicate the mean ± SD of triplicates. Mean values with different letters in a column are significantly different ($p < 0.05$); Γ- Interfacial

The results showed that although a larger proportion of proteins (46.0 – 82.2 %) were rapidly adsorbed at the interface than pectins (41.5 – 59.5 %), the surface coverage of the pectins (0.6 – 3.6 mg m^{-2}) were relatively higher than that of the proteins (0.3 – 1.0 mg m^{-2}) in all emulsions studied (Table 6.2). Alba *et al*. (2016) observed similar trends for okra pectins extracted at pH 6 (Adsorbed protein- 49.5 %, Γprotein – 1.6 mg m^{-2}; Adsorbed pectin- 16.3 %, Γpectin – 9.4 mg m^{-2}). Proteins because of their flexible molecular conformation can better adsorb at an oil-water interface than polysaccharides. Proteins can attach through multiple anchor points to a surface but form relatively thin interfacial coating (Kasapis *et al*., 2009). In an oil-water emulsion a protein at the interface can unfold and find a new conformation with hydrophobic amino acid residues interacting with the oil phase- whereas hydrophilic amino acids interact with the aqueous phase of an oil-water emulsion (Coupland, 2014). Alternatively the presence of hydrophobic groups such as acetyl and methyl in some pectin extracts contribute to their ability to adsorb to lipid

surfaces while the hydrophilic chains extend into the aqueous phase and provides stability against droplet aggregation through steric hindrance (Kasapis *et al*., 2009; Alba *et al*., 2016).

The surface coverage of pectins in this study were lower than values previously reported for okra pectin- 9.4 mg m^{-2} (Alba *et al*., 2016), sugar beet pectin-7.5 mg m^{-2} (Siew and Williams, 2008) and citrus pectin-9.8 mg m^{-2} (Akhtar *et al.*, 2002). However among the genotypes studied, pectin extracts from *Asontem* demonstrated the highest surface coverage in terms of pectins (3.6 mg m^{-2}) and protein (1.0 mg m^{-2}) whereas *Penkruma* (0.9 mg m^{-2}) and *Agbagoma* (0.6 mg m^{-2}) showed the least pectin surface coverage. Pectins from *Pe*nkruma and *Agbagoma* therefore did not provide effective steric barrier in the emulsions studied due to their low pectin surface coverage and this concurrently confirm the high index for creaming observed in emulsions containing *Penkruma* and *Agbagoma* pectin extracts.

Results from this study confirm that, the capability of pectin-protein biopolymers to confer emulsion stability at the interface is a consequence of both proteinaceous and pectin components of the biopolymer complex. Figure 6.1 shows typical microscopic morphology of the o/w emulsions stabilized with okra pectin. The protein components of the samples are shown in white indicating their presence at the oil-water interface to stabilize the emulsions. The images clearly revealed that the droplet sizes are less polydisperse/heterogeneous in the fresh emulsion but more heterogeneous with increasing sizes as the emulsion ages. Thus the average droplet size/diameter of the emulsions at day 0 < day 5 < day 15 attributable to emulsion destabilization mechanisms (coalescence) (Alba *et al*., 2013). The microscopic observations are also in agreement with the results of the

particle size distribution studies (Table 6.3; Figure 6.2) as discussed in the following section.

6.3.3 Emulsifying Ability of Different Okra Pectin

The ability of the okra isolate from different genotypes to stabilize long-term emulsions was determined using the average droplet sizes at set time intervals for a period of 15 days. Significant differences were observed for the average particle diameter of the different okra pectin emulsions (Table 6.3, Figure 6.1). The initial surface weighted mean diameter ($d_{3,2}$) for the different okra pectin emulsions ranged from 1.3 to 3.7 μm. The okra pectins thus demonstrated good emulsifying capacity in the o/w emulsions studied.

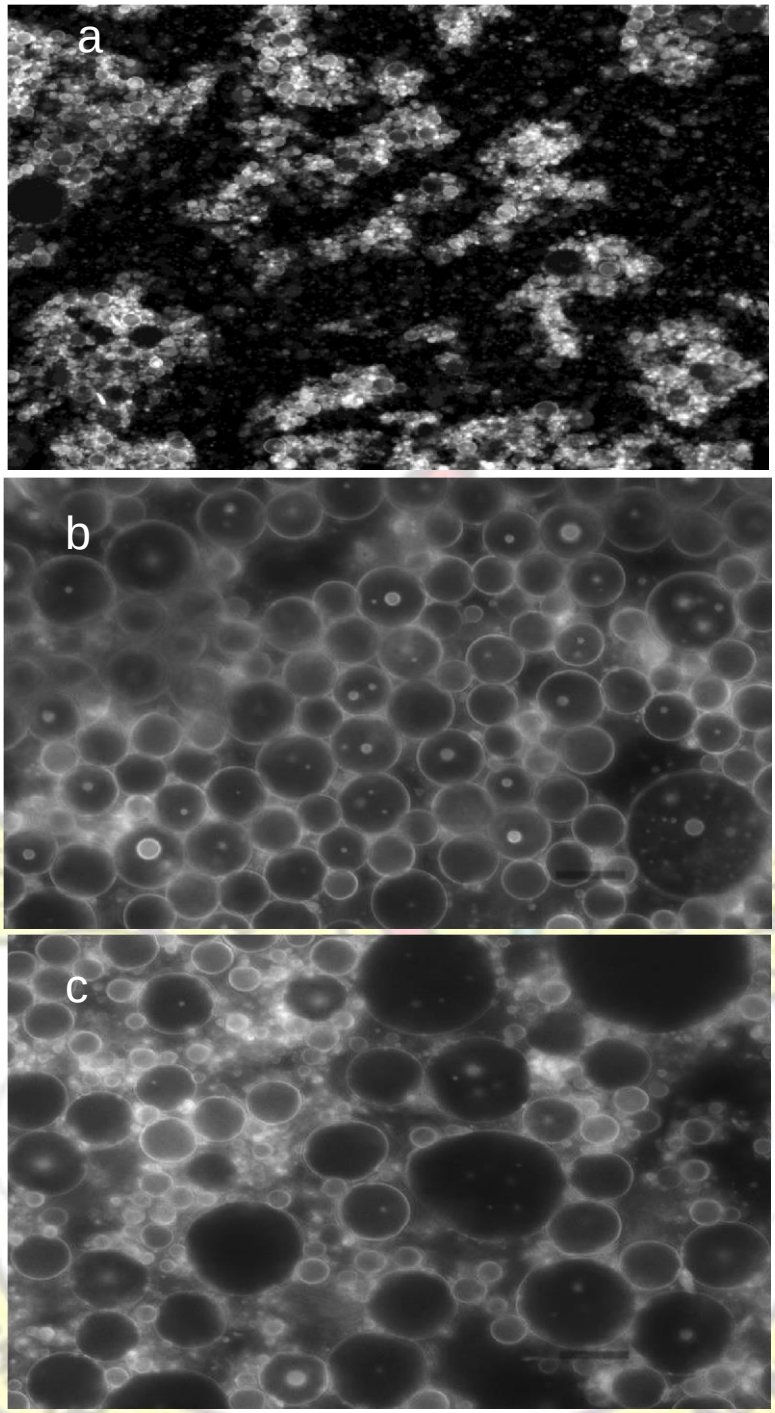


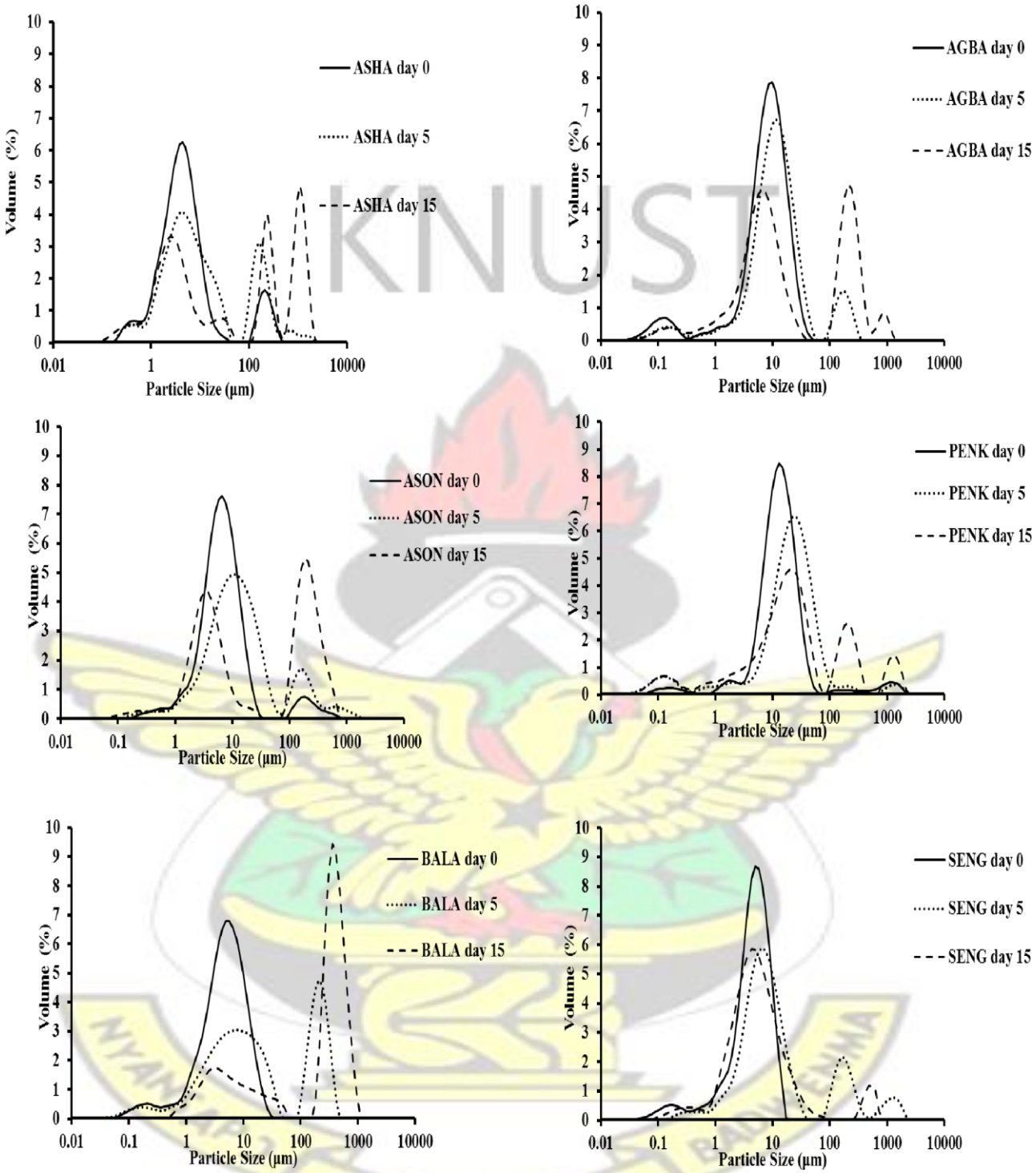
Figure 6.1: Typical fluorescent microscopic image of okra pectin stabilized emulsion at (a) day 0, (b) day 5 and (c) day 15. Scale bars depict 100 μm

Table 6.3: Effect of storage time on droplet diameters

Sample	Time (days)	$d_{3,2}$ (µm)	$d_{4,3}$(µm)	Span	ζ-Potential (mV)
Asha	0	2.2 (0.0)	25.3 (0.7)	28.9 (0.2)	-2.7 (0.1)
	5	2.6 (0.4)	60.9 (9.6)	32.8 (8.1)	-3.2 (0.2)
	15	1.8 (0.8)	161.3 (0.3)	75.8 (7.7)	-1.8 (0.5)
Penkruma	0	3.5 (0.0)	51.7 (0.0)	1.9 (0.0)	-4.6 (0.2)
	5	1.3 (0.0)	51.8 (0.0)	2.7 (0.0)	-5.5 (0.7)
	15	1.2 (0.1)	105.0 (4.8)	13.1 (1.7)	-2.7 (0.5)
Asontem	0	3.7 (0.0)	20.5 (0.0)	2.4 (0.0)	-3.0 (0.0)
	5	4.6 (0.3)	53.9 (0.5)	14.5 (1.9)	-3.2 (0.1)
	15	2.8 (0.1)	112.1 (4.6)	18.9 (8.1)	-1.0 (0.2)
Agbagoma	0	1.3 (0.1)	10.8 (0.4)	2.0 (0.0)	-3.2 (0.0)
	5	1.8 (0.1)	23.5 (3.3)	3.0 (0.6)	-3.3 (0.3)
	15	1.6 (0.3)	103.8 (1.7)	31.3 (3.1)	-2.0 (0.0)
Sengavi	0	1.4 (0.0)	4.8 (0.0)	1.8 (0.0)	-2.4 (0.0)
	5	3.6 (0.4)	38.6 (0.0)	22.5 (3.9)	-2.7 (0.1)
	15	2.2 (0.0)	32.8 (0.0)	4.5 (0.0)	-1.8 (0.4)
Balabi	0	2.0 (0.5)	5.7 (0.2)	2.3 (0.1)	-3.0 (0.7)
	5	1.8 (0.0)	72.5 (0.1)	18.4 (2.4)	-3.1 (0.7)
	15	1.8 (0.2)	115.8 (0.2)	8.3 (1.4)	-1.7 (0.1)

$d_{3,2}$ is the surface weighted mean diameter; $d_{4,3}$ is the volume weighted mean diameter; ζ (Zeta) potential

Figure 6.2: Particle size distribution of emulsion prepared using different pectins; Asha,



AGBA-Agbagoma, ASON-Asontem, PEN- Penkruma, BALA-Balabi, SENG- Sengavi

Emulsions prepared with pectins from the genotypes *Sengavi* (1.4 µm) and *Agbagoma* (1.3 µm) recorded the least initial $d_{3,2}$ value whereas *Penkruma* (3.5 µm) and *Asontem* (3.7 µm) emulsions had the highest $d_{3,2}$ values. Emulsion destabilization occurred after the fifth day

in all samples studied and continued drastically throughout the storage period as demonstrated by the volume weighted mean diameter ($d_{4,3}$). However, *Sengavi* pectins stabilized emulsions showed minimal destabilization as compared to emulsions prepared with the other pectin types. Emulsion samples containing pectins from *Sengavi* exhibited the least average particle diameter (4.8 μm) and the value remained stable (around 30 μm) for the duration of 15 days. Hence the *Sengavi* stabilized pectin emulsions exhibited the highest stability characteristic during storage as compared to emulsions prepared with the other pectin types. The droplet size distribution of the emulsions shows a shift from small size distribution to large particle distribution as the emulsions aged. This is also evidenced by the fluorescent microscopic images obtained for the emulsions as aforementioned (Figure 6.1). This mode of emulsion destablilization is better explained by coalescence which is demonstrated when small particles spontaneously diffuse to large particles due to differences in surface curvature (Coupland, 2014).

Careful examination of the molecular properties of the samples disclose the importance of branching (R_3 ratio- Ara + Gal/Rha; Table 4.7) and RG-I content (Table 4.7) in pectin emulsification. Pectin samples with intermediate length of branching ($2 < R < 3$, Sengavi, Balabi) contributed favourably to the emulsifying capacity with reference to the droplet size of fresh emulsions (small $d_{4,3}$ and $d_{3,2}$ at day zero). In the case of stability of emulsions after fifteen days of storage, samples with lower RG-I content (Penkruma, Agbagoma, Sengavi, Asontem) exhibited greater long-term stability in comparison to those with higher (Balabi, Asha).

Zeta potential was determined for the emulsions prepared with pectins from the different okra genotypes using a ZetaSizer. The zeta potential value of the okra pectin

emulsions were negative and decreased in negativity during the 15 days of storage (Table 6.3). The negative zeta potential values was due to the presence of anionic polysaccharides in the okra isolates (Alba *et al.*, 2013). Emulsions with pectin from the genotype *Penkruma* gave a highly negative potential values (-4.6 to -2.7 Mv) whereas *Sengavi* recorded the lowest charge (-2.4 to -1.8 Mv). Potential values obtained in this study were lower than values obtained by Georgiadis *et al*. (2011) for HBSS- hot buffer soluble solids (-21.5 mV) and CHSS- chelating agent soluble solids (-14.3 mV) sequential extraction extracts and zeta values (-6.9 to -4.6 mV) obtained by Alba *et al.* (2013). CHSS- chelating agent soluble solids

6.3.4 Emulsion Rheology of Different Okra Pectin

Figure 6.3 shows the shear rate sweeps of the fresh solutions prepared with pectins from different okra solutions and subsequently used in preparing emulsion solutions for the study. All fresh pectins solutions exhibited a shear thinning behavior except for pectin solutions prepared with the okra type *Balabi* which assumed a Newtonian flow. This reduced intrinsic viscosity behavior of pectins from the genotype *Balabi* have been noted in earlier studies to be due to its low molecular weight and low galacturonic acid content. The fresh pectin solutions showed decreasing viscosity in the order *Agbagoma>Penkruma>Asha>Sengavi>Asontem>Balabi*.

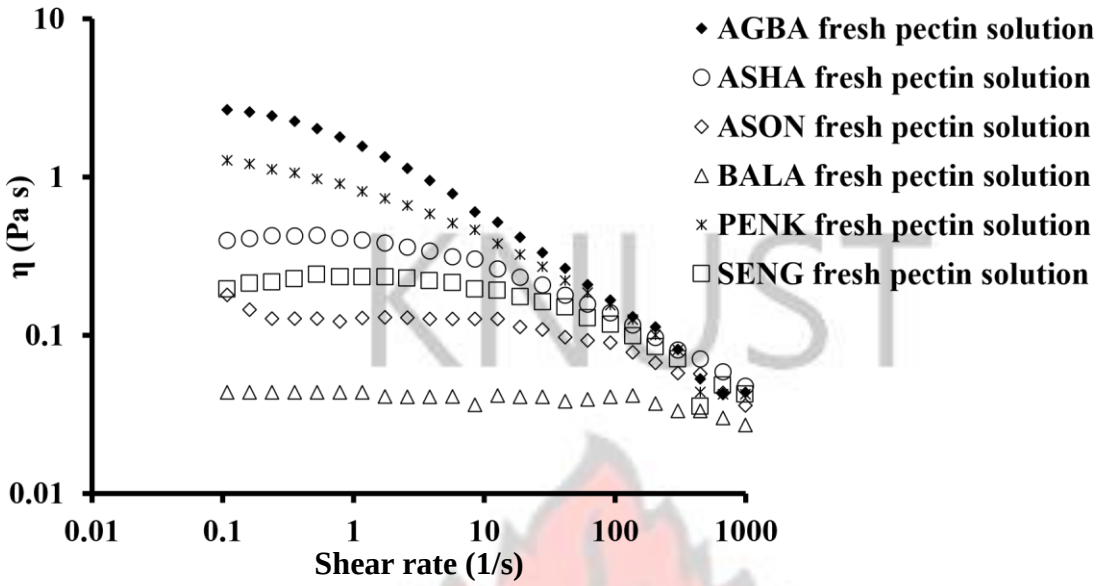


Figure 6.3: Rheological characteristics of fresh okra pectin solutions- Asha, AGBAAgbagoma, ASON-Asontem, PEN- Penkruma, BALA-Balabi, SENG- Sengavi

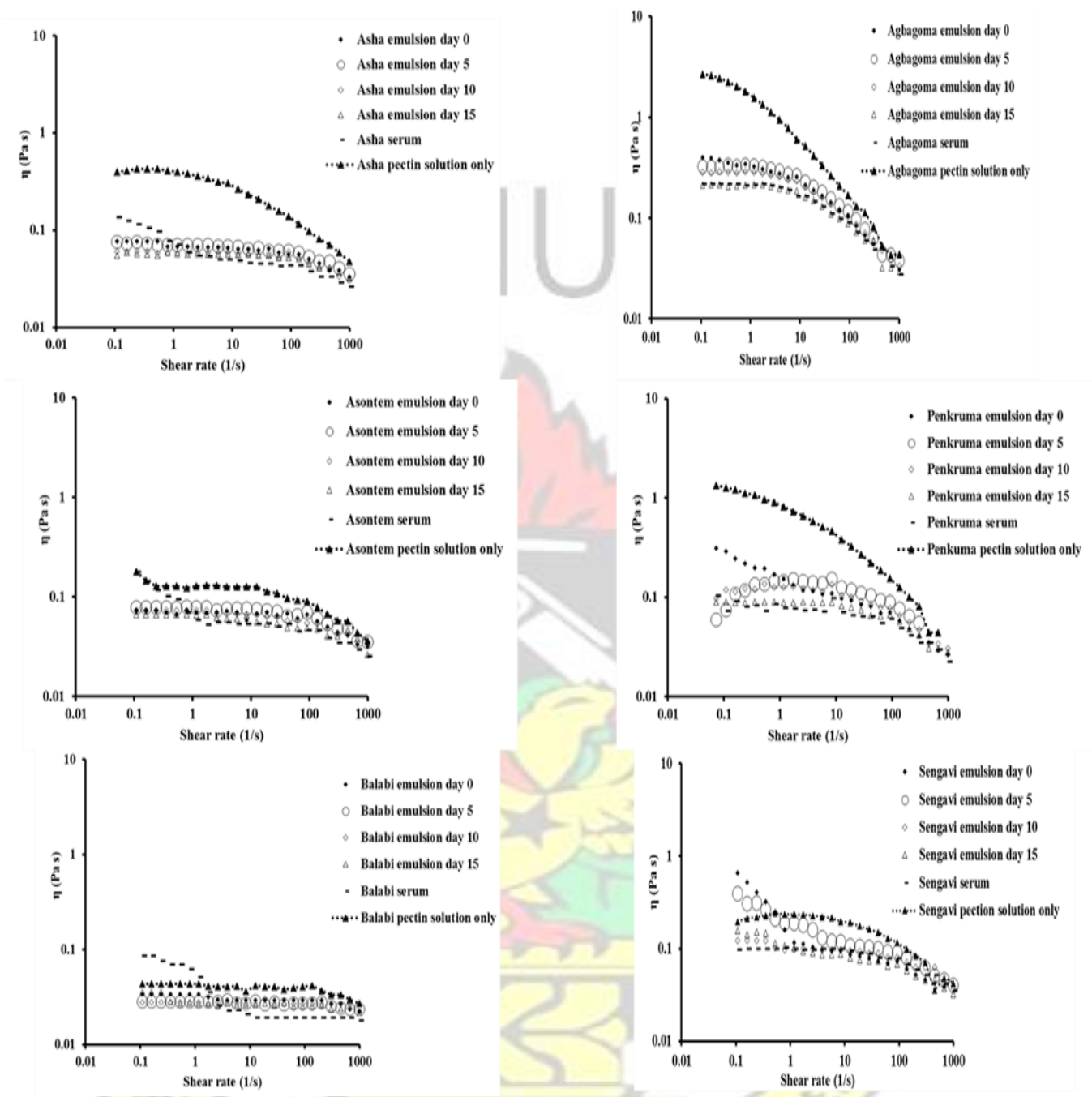


Figure 6.4: Rheological characteristics of okra pectin emulsions Figure 6.4 shows the rheological characteristics of the different okra pectin emulsions during storage. The viscosity of the fresh samples for all pectins except *Balabi* were siginificantly higher and demonstrated a shear thinning behavior, whereas *Balabi* pectin solution assumed a Newtonian flow. A critical

observation of the shear rate sweeps for all the emulsions show that the viscosity of samples did not change as storage days increased. A variety of factors can influence the rheology of emulsions during storage and this includes- the nature of the continuous and dispersed phase, droplet-droplet interactions and the droplet size (Kasapis *et al*., 2009). Constant viscosity throughout the storage period indicates limited weak flocculation. Additionally, increase in viscosity during storage can be caused when pectin desorption from the interface occurs, thus increasing the viscosity of the continuous phase. Results indicate that any possible desorption of pectin during the 15 days of storage did not have any measurable changes in emulsion viscosity, which is in contrast to results reported by Alba *et al*. (2015).

6.4 Conclusion

The present results indicate that okra pectin from different genotypes exhibit variable emulsion properties that can be used as functional ingredients in different food systems. Emulsion containing pectins from *Sengavi* exhibited the least average particle diameter and demonstrated the highest stability characteristic during storage as compared to emulsions prepared with the other pectin types.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusion

A preliminary study indicated variations in physicochemical characteristics of pectins depending on sample source and okra type. Hence okra samples were subsequently cultivated under controlled environmental conditions to investigate the influence of genetic differences on physical and chemical characteristics of the okra pectin isolates. The okra

pectins were isolated by aqueous extraction at pH 6 from the pods of six different okra genotypes- *Asha, Penkruma, Agbagoma, Asontem, Sengavi and Balabi.*

Genotypic diversity studies revealed unique genotypes for each okra type studied. Genotypic differences influenced physicochemical characteristics of the pectin isolates. Extraction yield varied between 11 and 14 %, purity/protein content (3.3 – 7.1 %), galaturonic acid content (43 – 63 %), degree of methylation (17 – 25 %) and degree of acetylation (20 – 40 %). Samples were of high molecular weight and values ranged between 700 – 1700 x 10^3 g mol^{-1}. The main neutral sugars in all samples were galactose, rhamnose and arabinose with HG/RG-1 ratios between 1.3 and 3.1. ^1H and ^{13}C NMR spectra obtained for all samples were similar. The results show that individual okra genotypes provide pectins with different physicochemical characteristics.

Rheological studies on the okra pectins from different genotypes showed varied intrinsic viscosity (2.91 to 5.10 dL g^{-1}), flexibility (0.2 – 0.8), critical concentrations and demonstrated different shear behavior in both dilute and concentrated regimes. Viscosity shear rate plots for pectins at 4 % separated the genotypes into three distinct viscosity regions- High viscosity samples (*Agbagoma, Sengavi, Penkruma* and *Asha*); Intermediary viscosity sample (*Asontem*) and low viscosity sample (*Balabi*). Coil overlap plots also revealed three distinct regions of concentration (dilute, demi-concentrated and concentrated). Genotypic differences thus influenced the rheological properties of the different okra pectin isolates studied.

The emulsifying properties of the pectins from the different okra genotypes was studied by analyzing the interfacial protein and pectin concentrations, emulsion rheology, particle size distribution, zeta potential and the emulsion morphology using fluorescence microscopy. Okra pectins from the different genotypes exhibited varied emulsion

properties. Stability of emulsions to creaming varied from 0.8 to 16.9 % and surface coverage of pectins (0.6–3.6 mg m^{-2}) were relatively higher than that of the proteins (0.3–1.0 mg m^{-2}). The okra pectins demonstrated good emulsifying abilities with initial surface weighted mean diameter ($d_{3,2}$) ranging between 1.3 and 3.7 μm. Emulsion destabilization occurred in all samples after five days of storage and coalescence better explained the destabilization process. *Sengavi*-stabilized emulsions exhibited the highest stability during storage compared to emulsions prepared with the other pectin types. All fresh pectin samples used for emulsion studies showed pseudoplastic behavior and the viscosity of samples remained constant with aging.

The results show that okra pectins from different genotypes demonstrated variations in physicochemical, rheological and emulsifying characteristics and hence these unique attributes of each okra pectin can be engineered for specific applications in both food and pharmaceutical products.

7.2 Recommendation

All genotypes studied had unique and varied physicochemical, functional and rheological characteristics which informs specific applications in food and pharmaceutical products. Hence further work can be done on how pectins from the different okra genotypes studied can be used as functional ingredients by the food and pharmaceutical industry. Although six different okra genotypes were used in this study, investigations can be conducted on the influence of different stages of maturity on the characteristics of pectin isolates. Pectins were extracted with a pH 6 phosphate buffer hence other buffer systems could be used for isolating okra pectins to improve the purity of isolates obtained.

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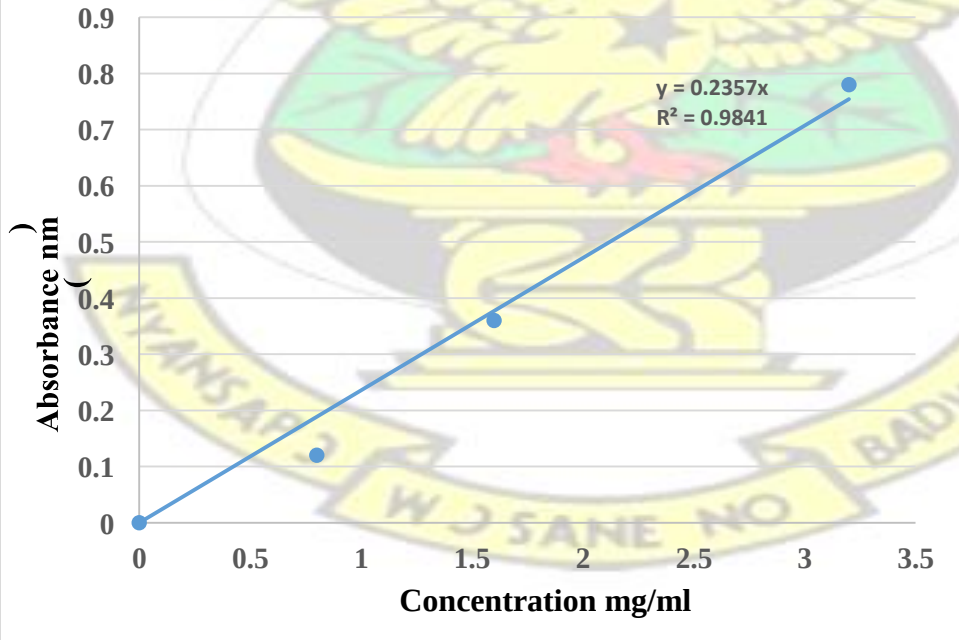
APPENDIX

Appendix 3.1: Preparation of bovine serum albumen

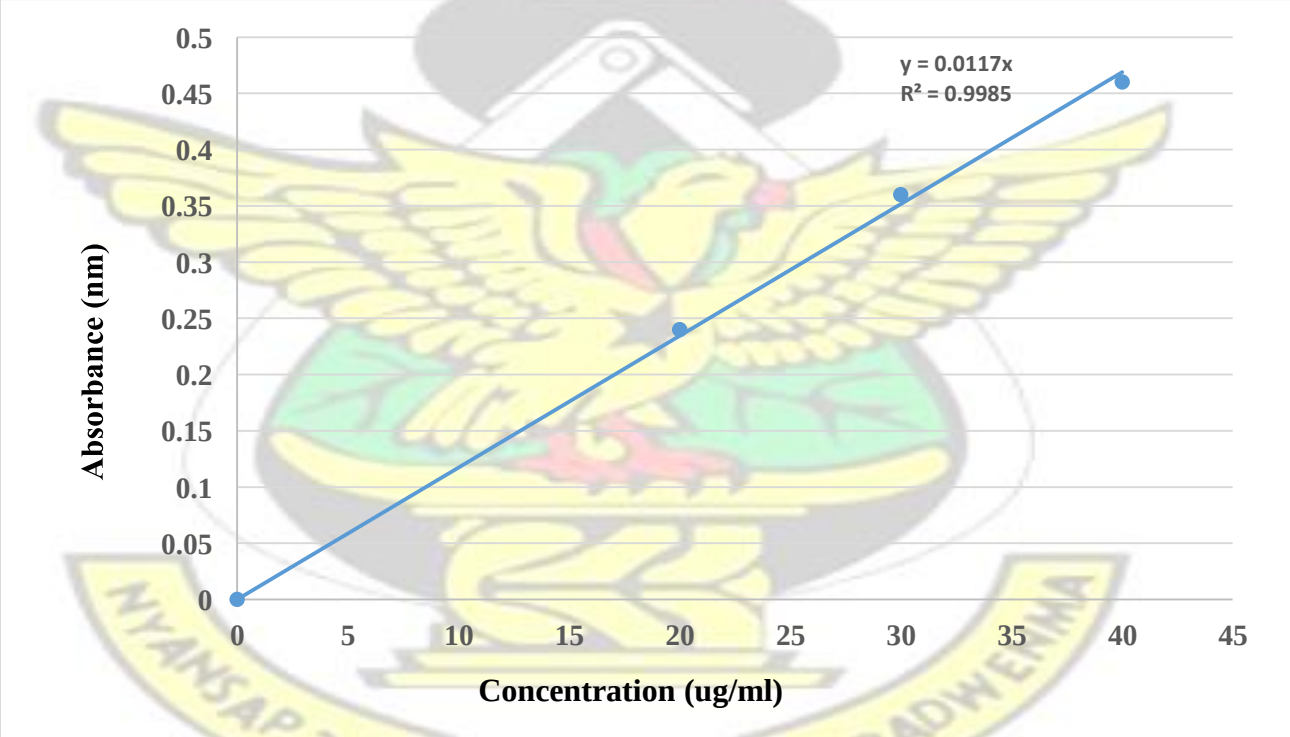
Tube	Source of standard (Original Stock)	Standard BSA [Protein] (□g/ml)	Volume of Stock (□l)	mL 0.85% NaCl (□l)	Volume of standards for spec. analysis (□l)	Volume Lowry Reagent (□l)	Volume Folin's Reagent (□l)
1	-	0	0	100	100	100	50
2	20 mg/ml	200	10	990	100	100	50
3	20 mg/ml	400	20	980	100	100	50
4	20 mg/ml	800	40	960	100	100	50
5	20 mg/ml	1600	80	920	100	100	50
6	20 mg/ml	3600	160	840	100	100	50



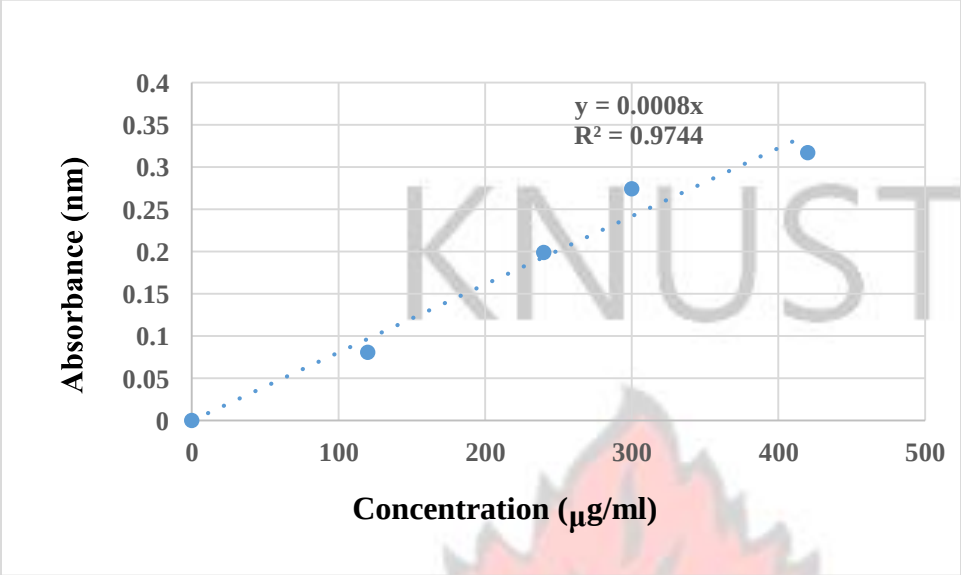
Standard calibration curve of BSA

Appendix 3.2: The mean absorbance readings of standard glucose and mannose solutions from the phenol-sulphuric acid method

Standard Galactose (□g/ml)	mL of Std	mL water	Volume of standards for spec. analysis (□l)	Volume of conc sulphuric acid (□l)	Volume of 5% phenol (□l)
0	0	50	50	150	30
5	25	25	50	150	30
10	25	25	50	150	30
20	33.3	16.7	50	150	30
30	37.5	12.5	50	150	30
40	40	10	50	150	30
50	50	0	50	150	30

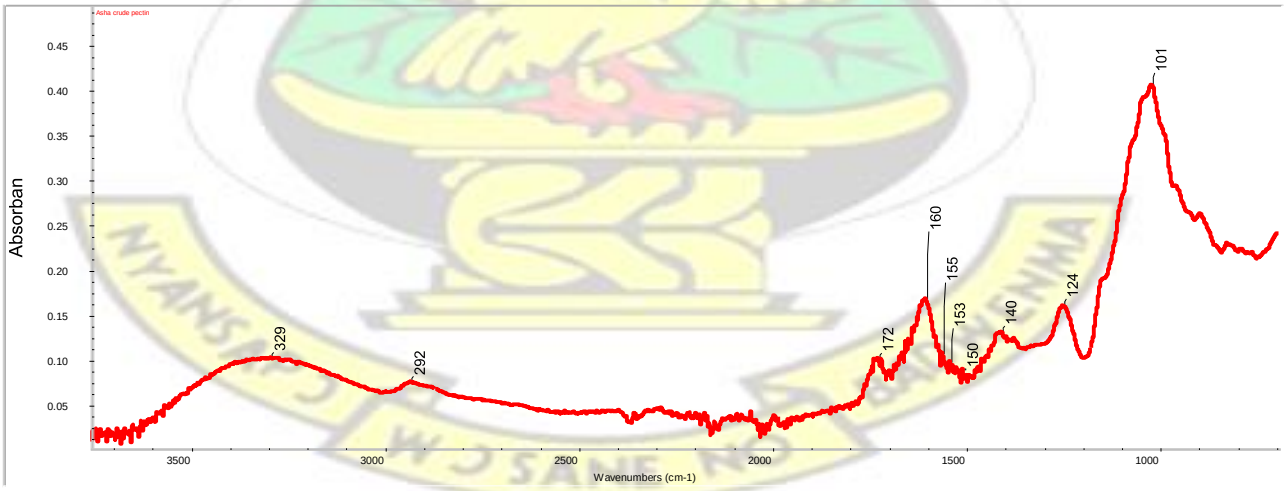


Standard calibration curve of galactose

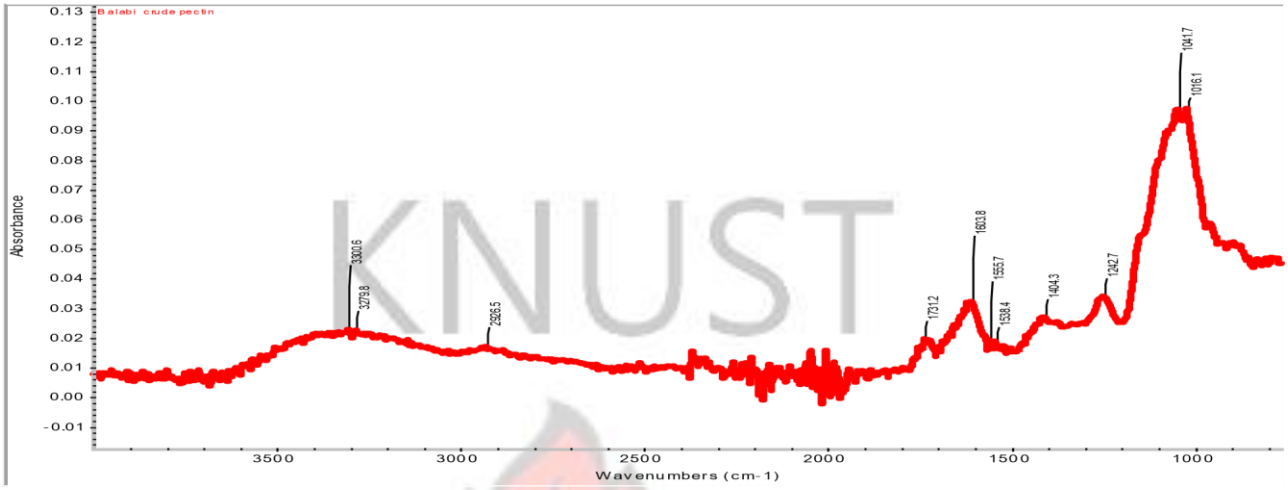


Standard calibration curve of acetyl content of D-glucose pentaacetate

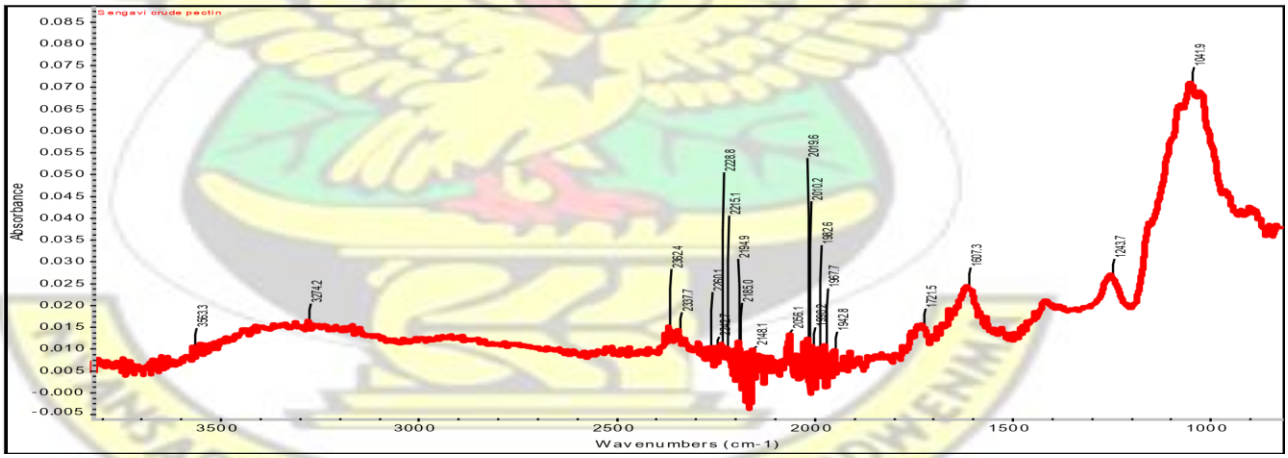
Appendix 3.3: FTIR of crude okra pectins (undialysed)



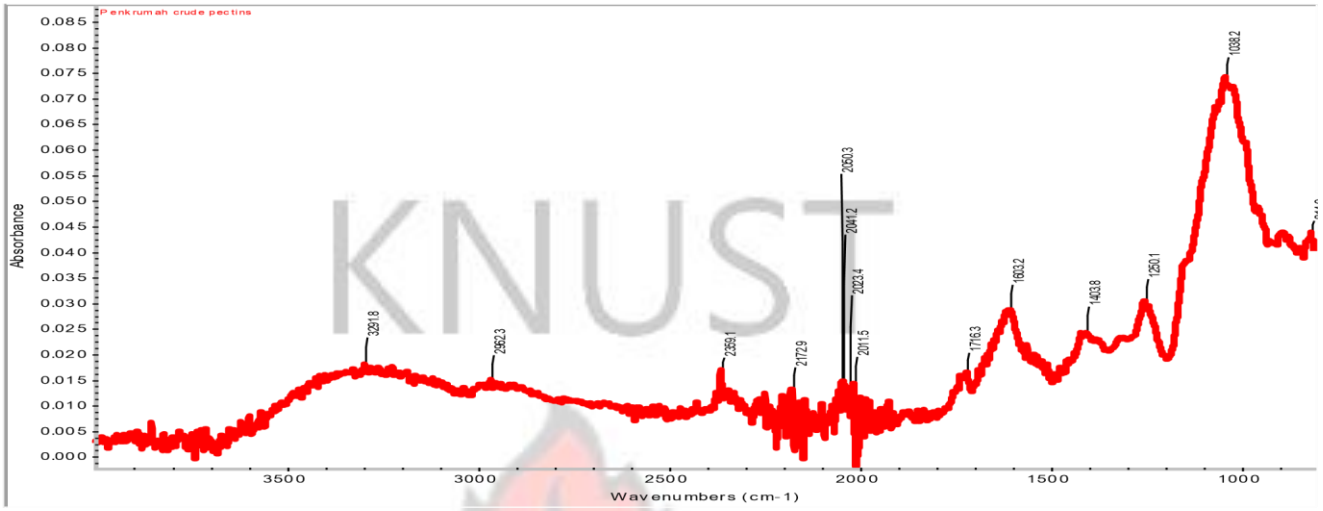
FTIR of Asha okra pectins



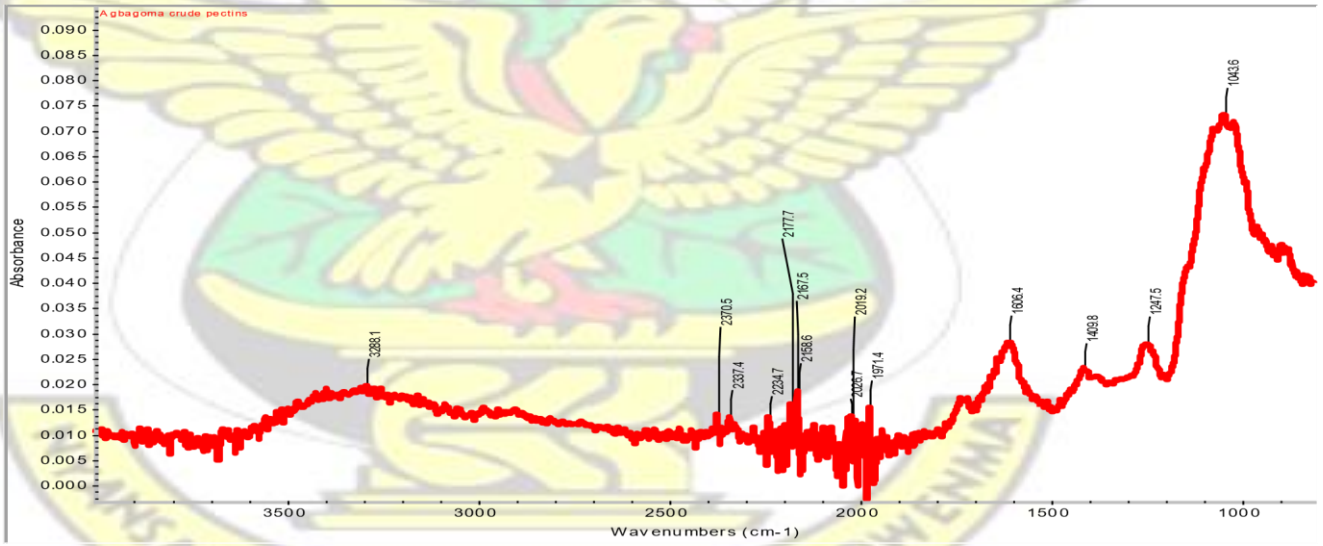
FTIR of Balabi okra pectins



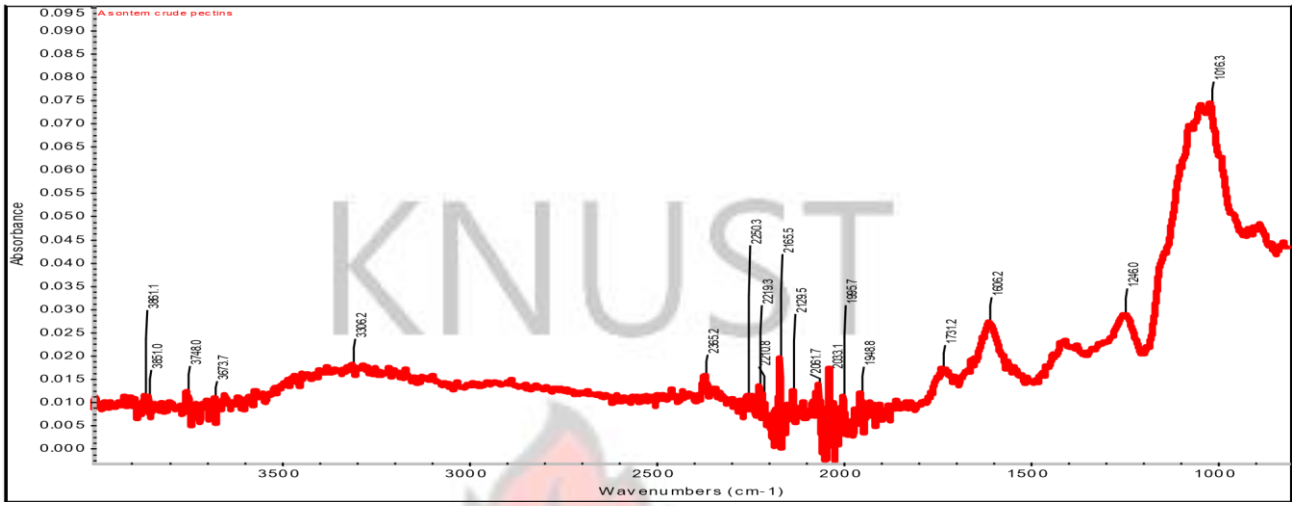
FTIR of sengavi okra pectins



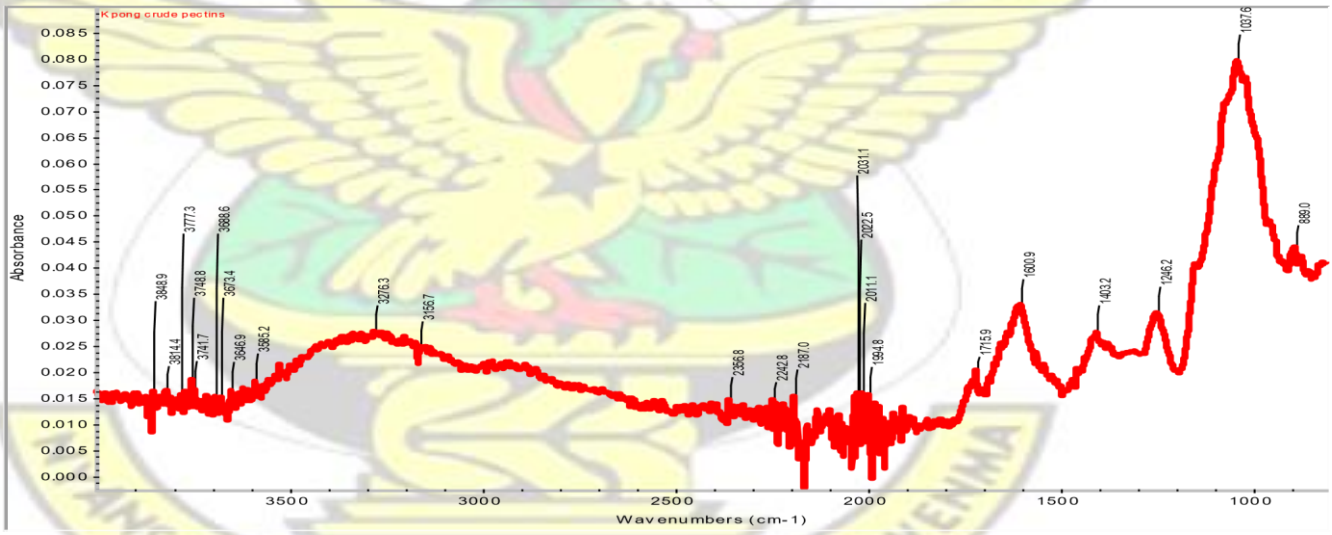
FTIR of penkruma okra pectins



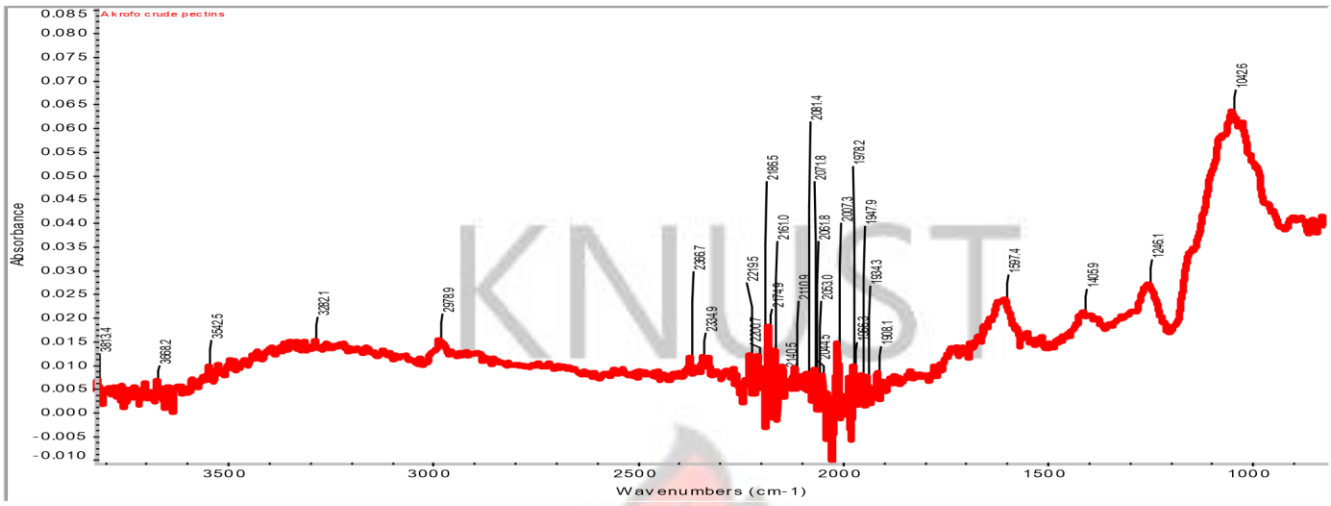
FTIR of Agbagoma okra pectins



FTIR of Asontem okra pectins



FTIR of Kpong okra pectins



FTIR of Akrofo okra pectins **Appendix 4.1**

Standard International Crop Descriptor for Okra (IBPGR, 1991)

Passport Data

1. Name of collector.....

2. Name of farmer.....

3. Vernacular name of Okra
Fruit.....

4. Name of
area.....

5.
Source.....
..

6. Information on pests and diseases of the crop

.....
.
.....
.
.....
.
.....
.
.....

Fruit Morphological Description

1. Fruit
Shape.....

2. Fruit
Size.....

3. Fruit
Colour.....

4. Description of Flowering Flower Dropping ☐ Flower Pointing Up ☐ 5. Petal Colour

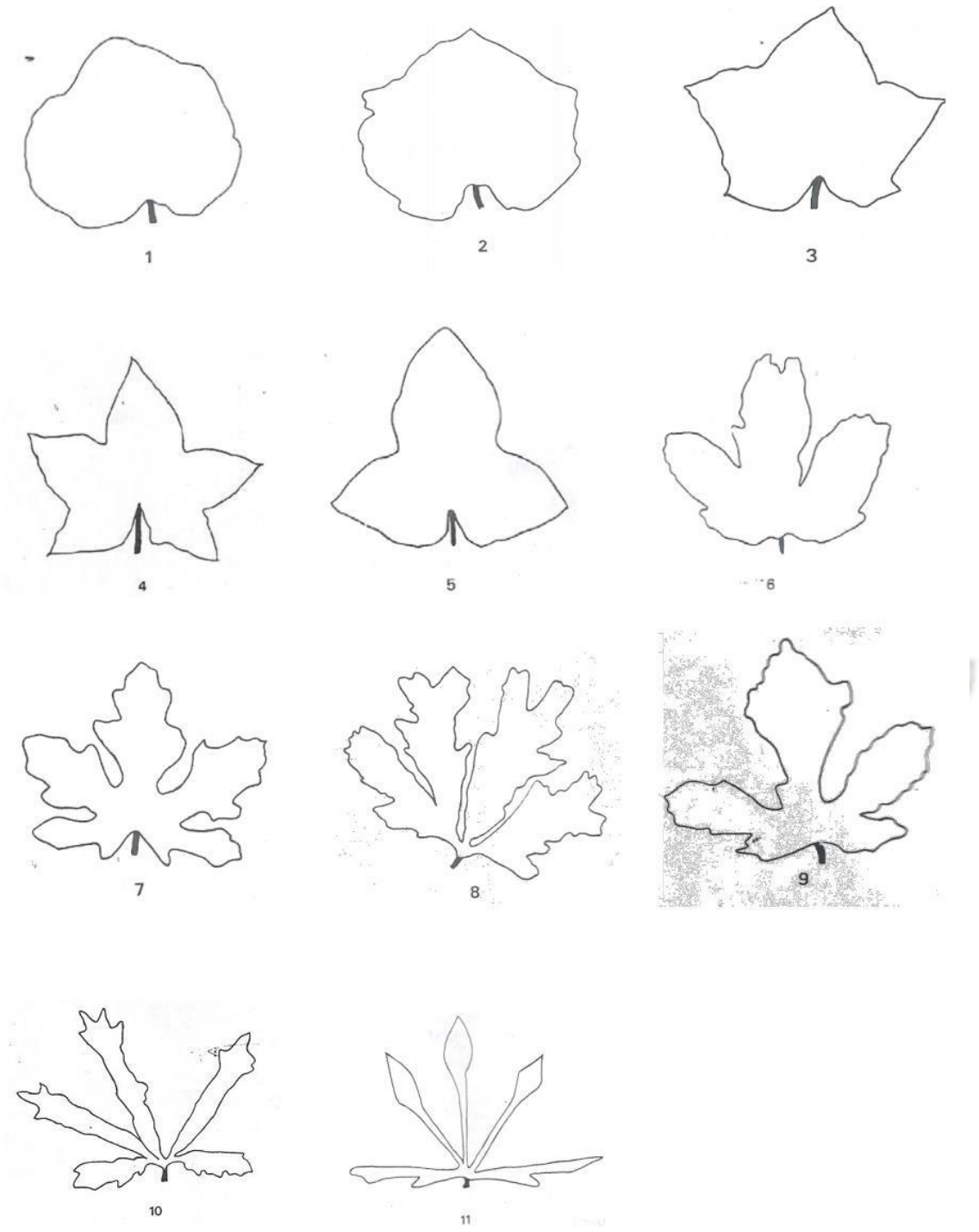


Plate 4.1: Different leaf shapes/forms within okra accessions (IBPGR, 1991)

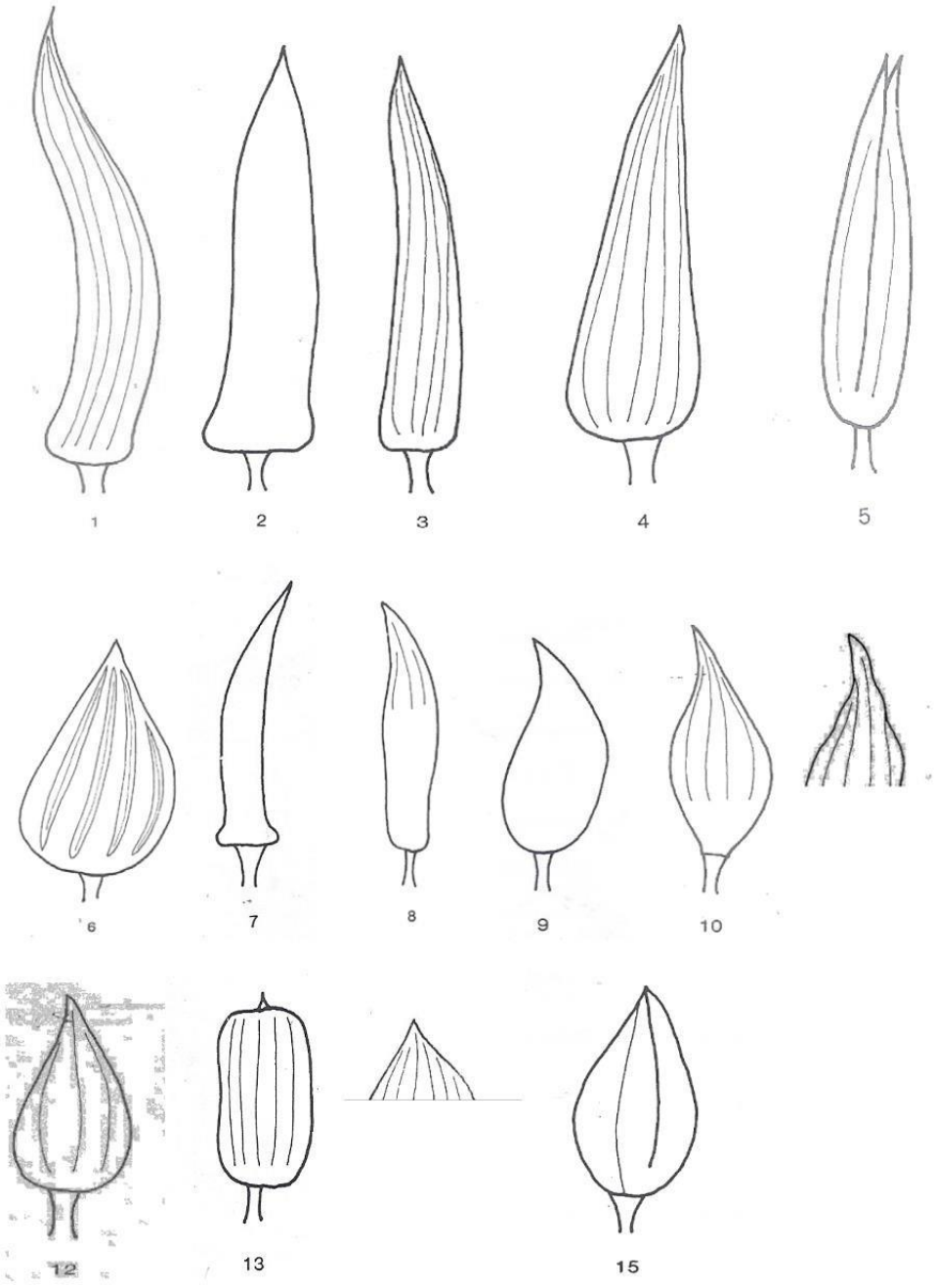


Plate 4.5: Variation in fruit shapes (IBPGR, 1991)

Appendix A

Table 1: Evaluated characteristic of okra collection. Coding of qualitative characters is according to IBPGR, 1991 descriptors for okra.

Code for character Parameter measured Character codes

No. Qualitative

1 SC Seed colour 1=dark, 2=black, 3=whitish to dark, 4=purple to black

2 SSh Seed shape 1=Roundness, 2=Kidney, 3=Spherical

3 SS Seed size 1=Small, 2=medium, 3=large

4 BPMS Branching position at main stem 1=UOA-unique orthotrop axis, 2=DBOdensely branched all over, 3=DBB-densely branched base

5 MLC Mature leaf colour 1=Green, 2=Green+red veins

6 LSh Leaf shape From types 1 to 11

7 LBr (cm) Length of branches 0= no branches, 1= branches rarely > 10cm

8 LRC Leaf rib colour 1=Green, 2=Green+red veins

9 PtC Petiole colour 1=Green, 2=Green+red veins, 3=purple

10 PC Petal colour 1=Golden yellow, 2=yellow

11 CDR Colour of the darkest ridges 1=light, 2=dark, 3=light to dark

12 StC Stem colour 1=green, 2=green+purple tinge, 3=purple

13 NES Number of epicalyx segments 1=8 to10, 2=5 to 7, 3=>10

14 FSp Flowering span 1=Single flowering, 2=grouped flowing

15 NSfS Number of segments from the stigmaFrom 5 to 12 segments 16 FC Fruit colour 1 =Green, 2=green+red spots, 3=dark green to black, 4=green to yellow, 5=purple

17 FP Fruit pubescene 1=Smooth, 2=little rough, 3=downy+hairs

18 FSh Fruit shape From types 1 to 15

19 NR/F Number of ridges per fruit 1=0, 2=b/n 5 and 12, 3=5ridges

20 PFMS Position of fruit from the main stem 1=intermediate, 2=slightly falling, 3=horizontal, 4=Erect, 5=Drooping

21 LFP Length of fruit peduncle 1=1 to 3cm, 2=>3cm

22 SI Susceptibility to insects Scale: 1 to 9; (Podagrica spp, Aphids, Cotton stainer): NS=0-1,WS=1-3,IS=3-5, HS=6-9

23 Sdi Susceptibility to diseases Scale: 1 to 9; (OMV, OLCV): NS=0-1, WS=1-3, IS=3-5, HS=6-9

Quantitative

24 DFF Days to First Flowering

25 FFN First Flowering Node

26 FF-PN First Fruit-Producing Node

27 FFrtWt (g) Fresh Fruit Weight

28 MPH (cm) Maximum Plant Height

29 NS/F Number of Seeds per Fruit

30 NTF/P Number of Total Fruits per Plant

31 SD YLD (kg/plot) Seed Yield

32 SW100 (g) 100 seed weight

33 50%DE 50% Days to emergence

34 CL L (mm) Cotyledon leaf length

35 CLW (mm) Cotyledon leaf width

36 CWR Cotyledon width ratio

90

37 %G Percentage germination

38 NI Number of internodes

39 EL (cm) Epicalyx length

40 EW (cm) Epicalyx width

41 FLM (cm) Fruit length at maturity

42 FW (cm) Fruit width

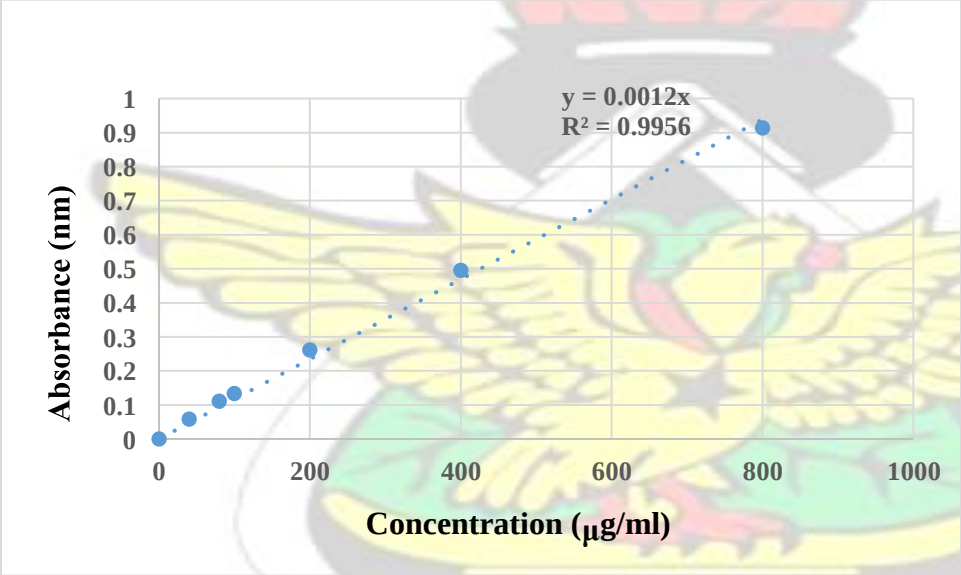
43 LL (cm) Leaf length

44 LB (cm) Leaf breadth

45 StD (mm) Stem diameter

Appendix 4.2: Standard Bradford assay dilutions

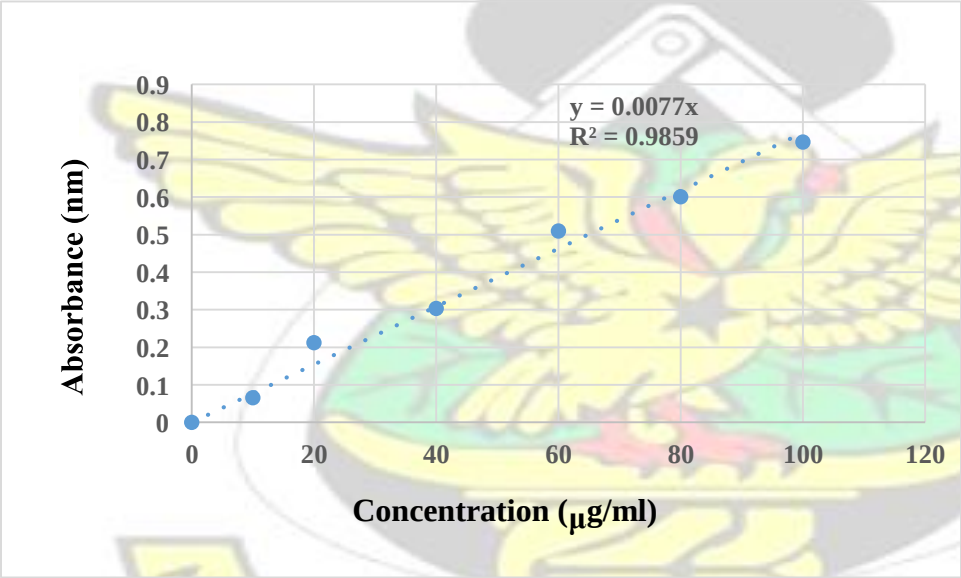
Tube #	Concentration of calibration BSA (µg/ml)	Volume of 2mg/ml of BSA solution (µl)	Volume of Deionized Water	Total Volume of Solution (µl)
1	800	40	60	100
2	400	20	80	100
3	200	10	90	100
4	100	5	95	100
5	80	4	96	100
6	40	2	98	100
7 (Blank)	0	0	100	100



Standard calibration curve of BSA

Appendix 4.3: Standard galactose dilutions for phenol-sulphuric acid assay

Tube #	Concentration of calibration sugar (µg/ml)	Volume of 1 mg/ml of calibration sugar solution (µl)	Volume of Deionized Water	Total Volume of Solution (µl)
1	500	250	250	500
2	200	100	400	500
3	100	50	450	500
4	80	40	460	500
5	60	30	470	500
6	40	20	480	500
7	20	10	490	500
8	10	5	495	500
9 (Blank)	0	0	500	500

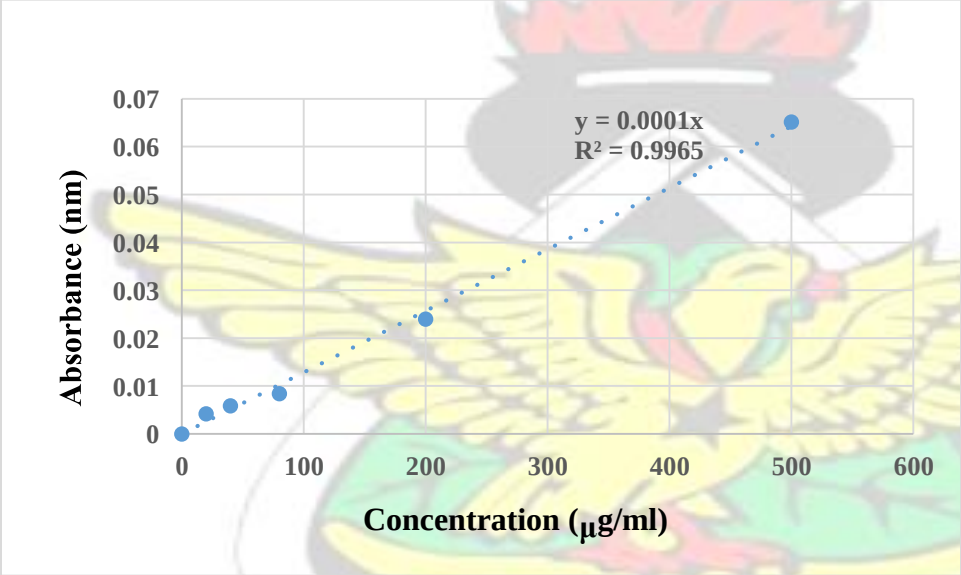


Standard calibration curve of galactose

Appendix 4.4: Standard D-galaturonic acid content using calorimetric assay

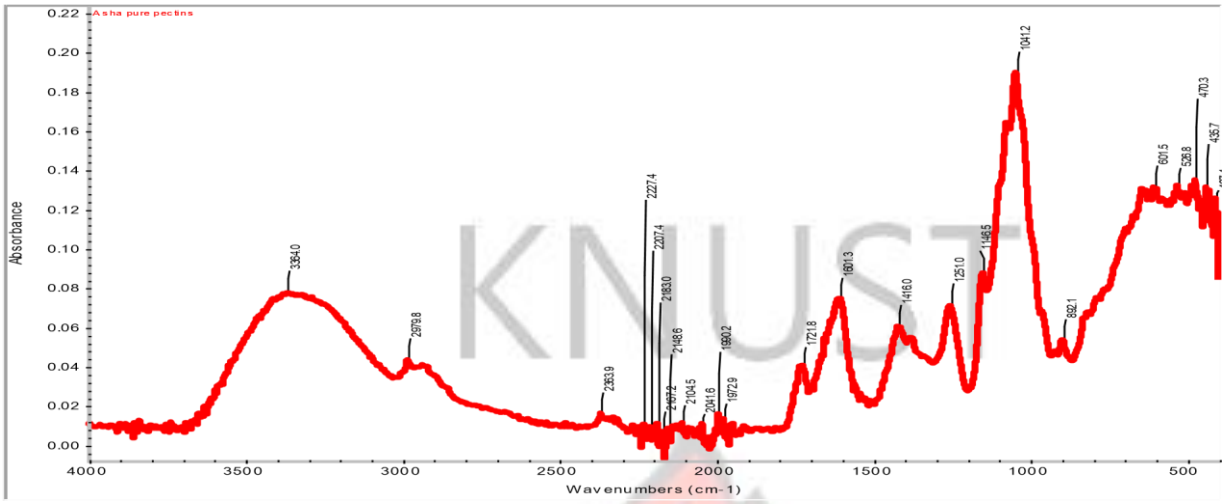
Tube #	Concentration of calibration sugar (µg/ml)	Volume of 1 mg/ml of calibration sugar solution (µl)	Volume of Deionized Water	Total Volume of Solution (µl)

1	500	250	250	500
2	200	100	400	500
3	100	50	450	500
4	80	40	460	500
5	60	30	470	500
6	40	20	480	500
7	20	10	490	500
9 (Blank)	0	0	500	500

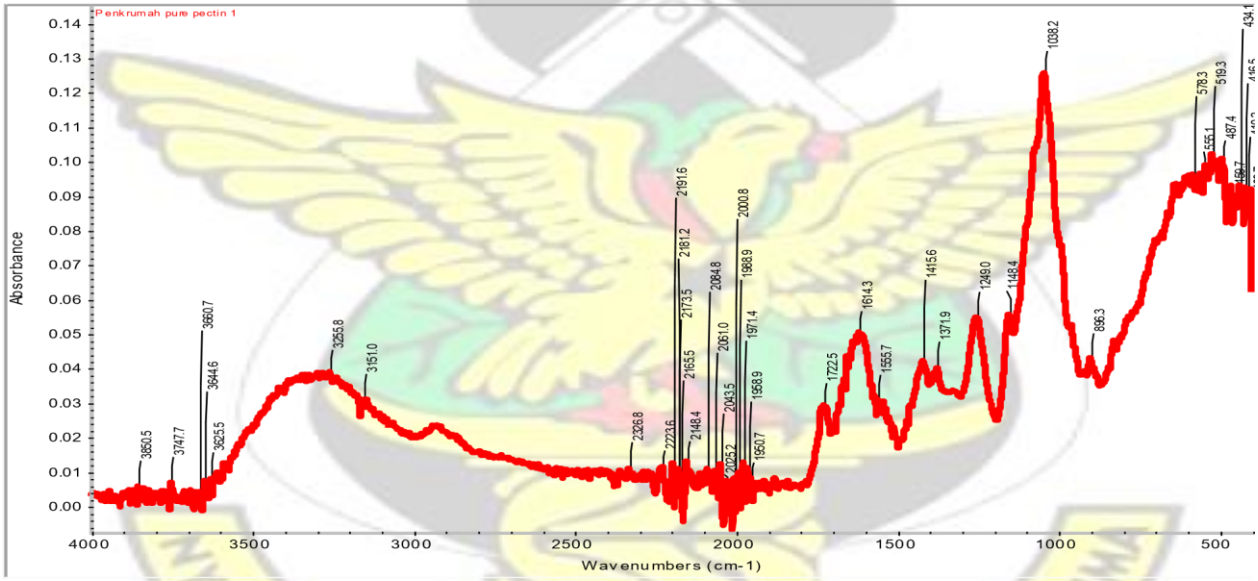


Standard calibration curve of D-galacturonic acid

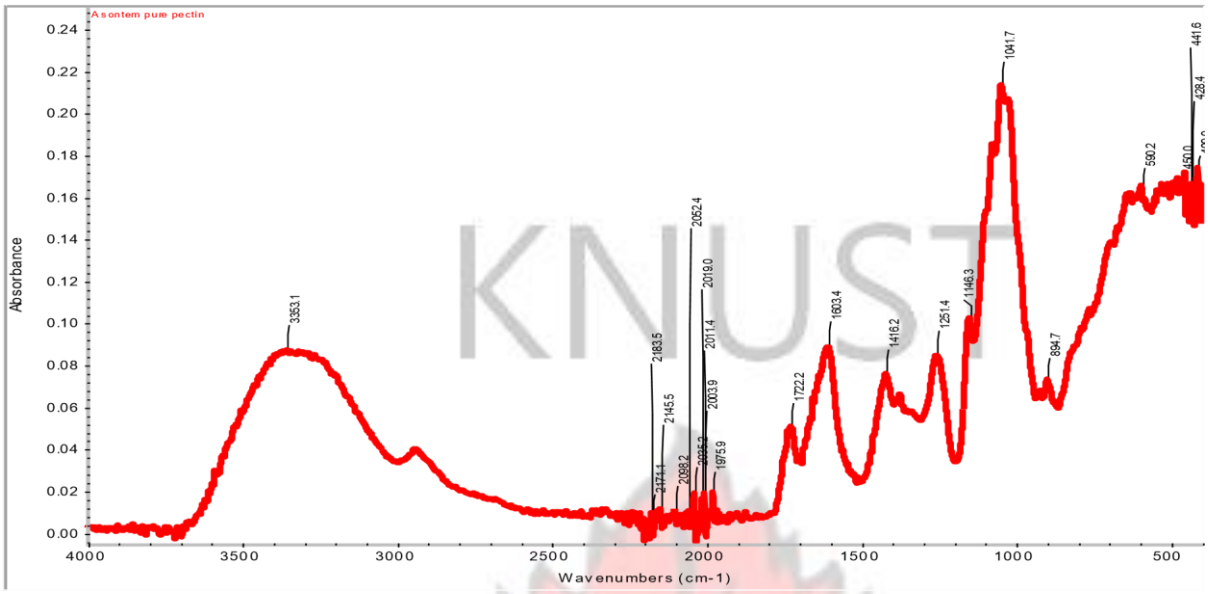
Appendix 4.5: FTIR of crude okra pectins (undialysed)



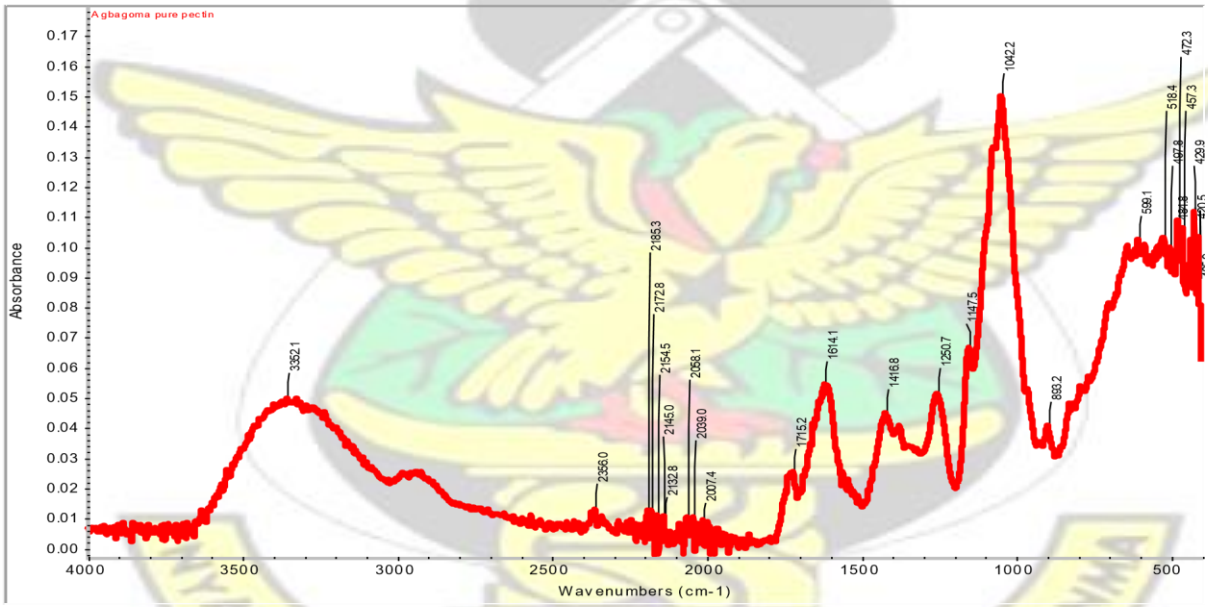
FTIR of Asha pure pectin



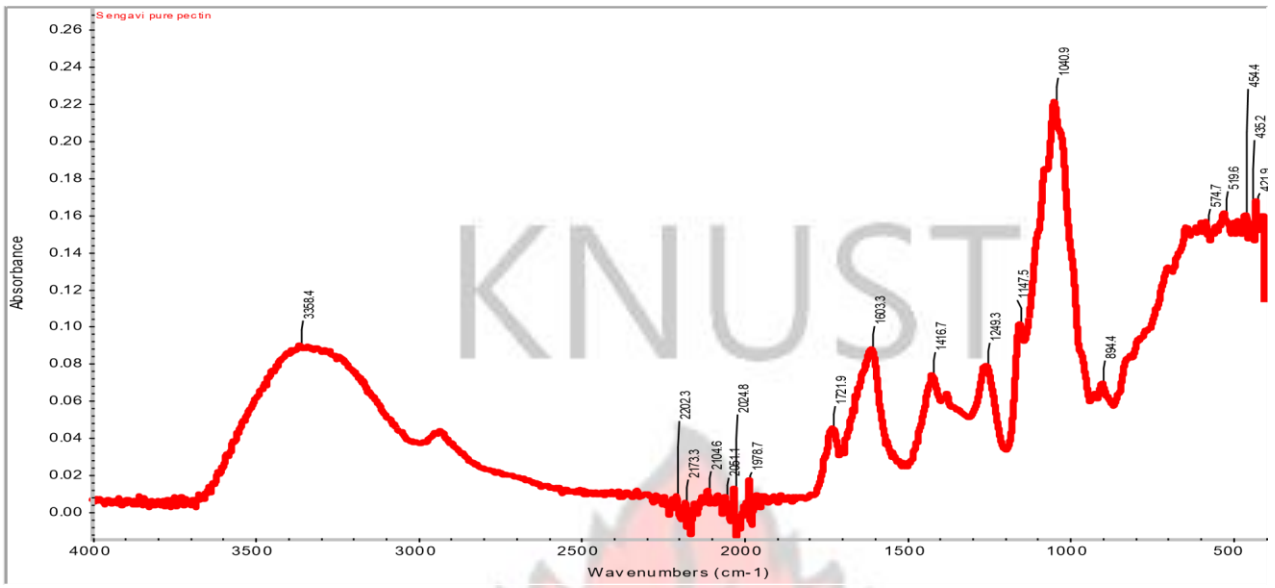
FTIR of Penkrumah pure pectin



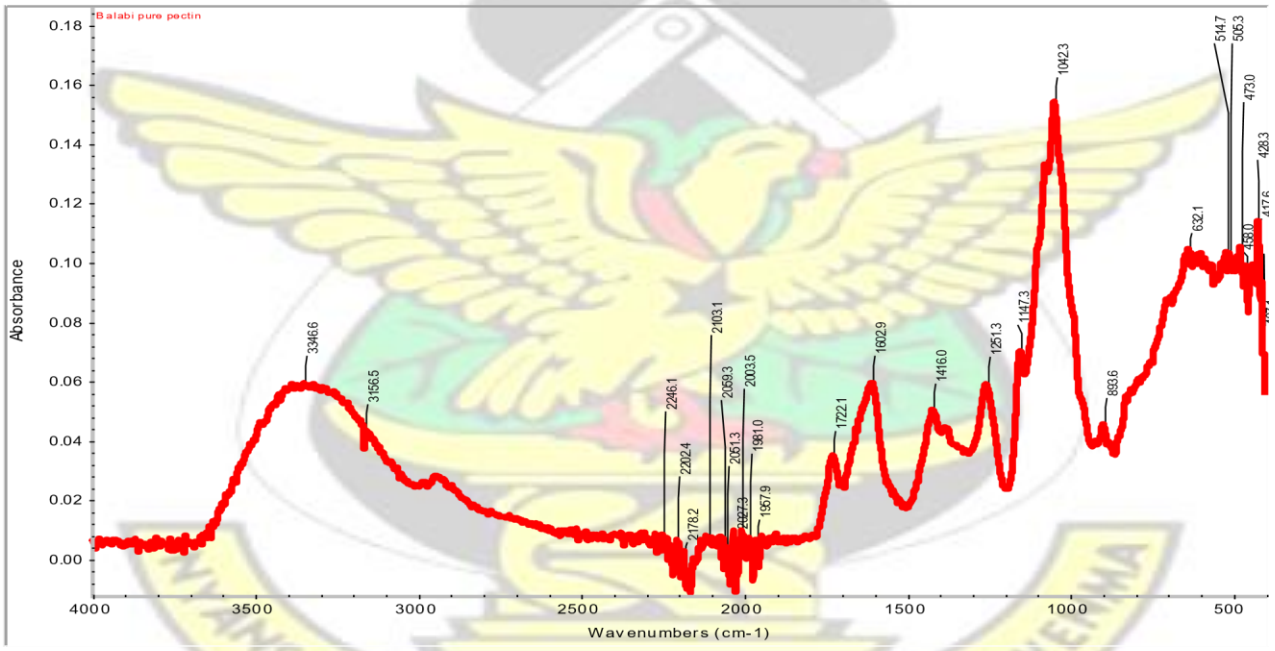
FTIR of Asontem pure pectin



FTIR of Agbagoma pure pectin



FTIR of Sengavi pure pectin



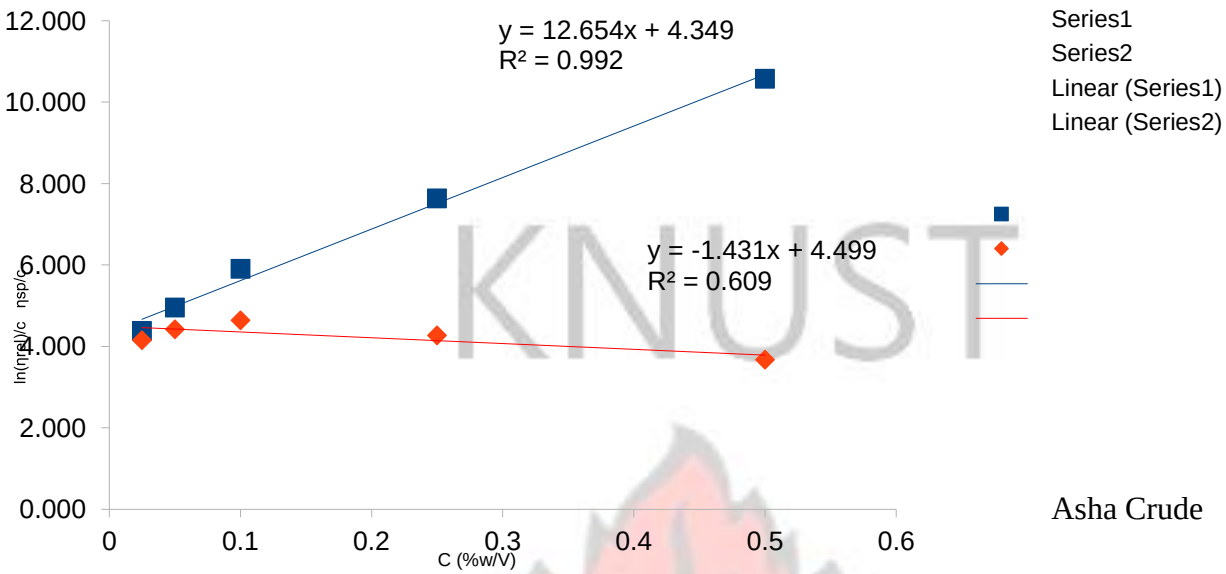
FTIR of Balabi pure pectin **Appendix 5.1:** Intrinsic viscosity, Huggins and Kraemer constants for crude and purified okra pectins

Genotype (Huggins/Kraemer Plot)	**Slope**	**Ƞ**	**Ƞ**	**Ƞ²**	**Slope/Ƞ²**	**$K_H + K_K$**	**R^2**
Asha Pure H	12.654	4.349	4.349	18.9138	0.669035	0.598337	0.992

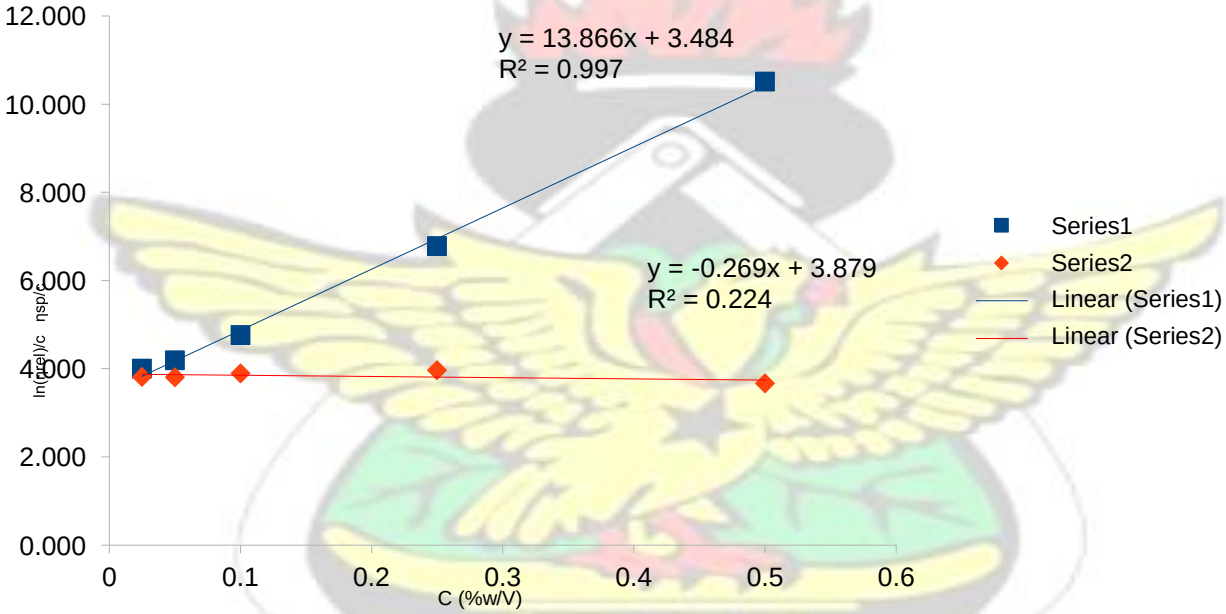
Asha Pure K	-1.431	4.499	4.499	20.241	-0.0707		0.609
Asha Crude H	13.866	3.484	3.484	12.13826	1.142339	1.124461	0.997
Asha Crude K	-0.269	3.879	3.879	15.04664	-0.01788		0.224
Agba Pure H	8.819	3.557	3.557	12.65225	0.69703	0.629139	0.998
Agba Pure K	-0.939	3.719	3.719	13.83096	-0.06789		0.924
Agba Crude H	20.177	4.841	4.841	23.43528	0.860967	0.791491	0.984
Agba Crude K	-1.956	5.306	5.306	28.15364	-0.06948		0.656
Ason Pure H	8.811	3.555	3.555	12.63803	0.697182	0.628965	0.998
Ason Pure K	-0.943	3.718	3.718	13.82352	-0.06822		0.928
Ason Crude H	7.943	3.083	3.083	9.504889	0.835675	0.801603	0.997
Ason Crude K	-0.361	3.255	3.255	10.59503	-0.03407		0.362
Bal Pure H	3.173	2.914	2.914	8.491396	0.373672	0.231408	0.929
Bal Pure K	-1.228	2.938	2.938	8.631844	-0.14226		0.751
Bal Crude H	6.083	2.199	2.199	4.835601	1.257962	1.3657	0.964
Bal Crude K	0.596	2.352	2.352	5.531904	0.107739		0.202
Sen Pure H	14.59	5.108	5.108	26.09166	0.559182	0.477674	0.997
Sen Pure K	-2.262	5.268	5.268	27.75182	-0.08151		0.965
Sen Crude H	8.627	3.084	3.084	9.511056	0.90705	0.893021	0.997
Sen Crude K	-0.149	3.259	3.259	10.62108	-0.01403		0.035
Pen Pure H	13.968	4.349	4.349	18.9138	0.738508	0.651122	0.956
Pen Pure K	-1.946	4.719	4.719	22.26896	-0.08739		0.594
Pen Crude H	41.416	8.552	8.552	73.1367	0.566282	0.477263	0.992
Pen Crude K	-6.867	8.783	8.783	77.14109	-0.08902		0.84

Appendix 5.2: Huggins and Kraemer plots

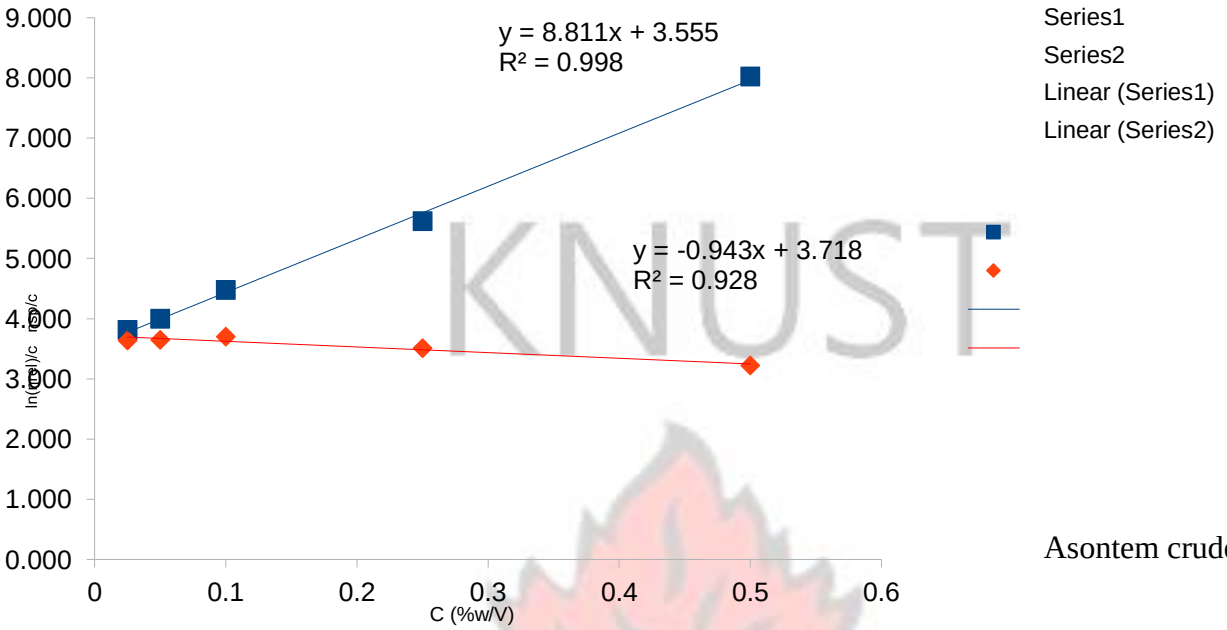
Asha Pure



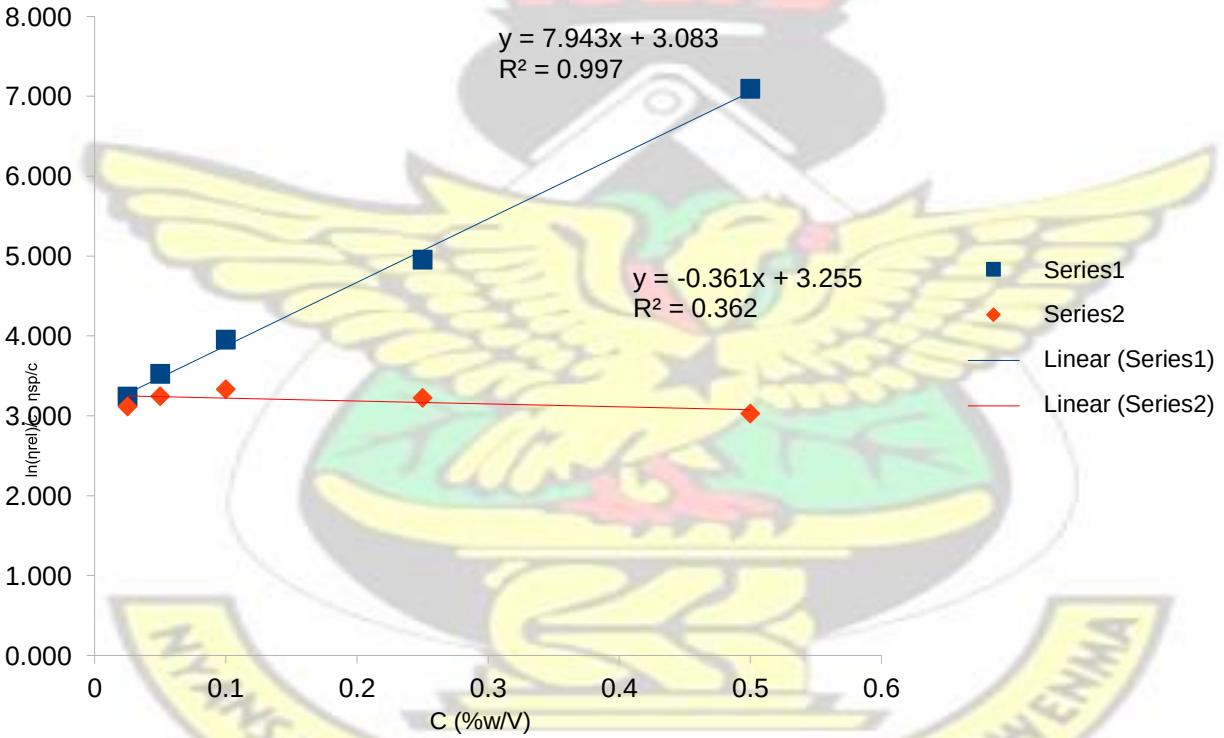
Asha Crude



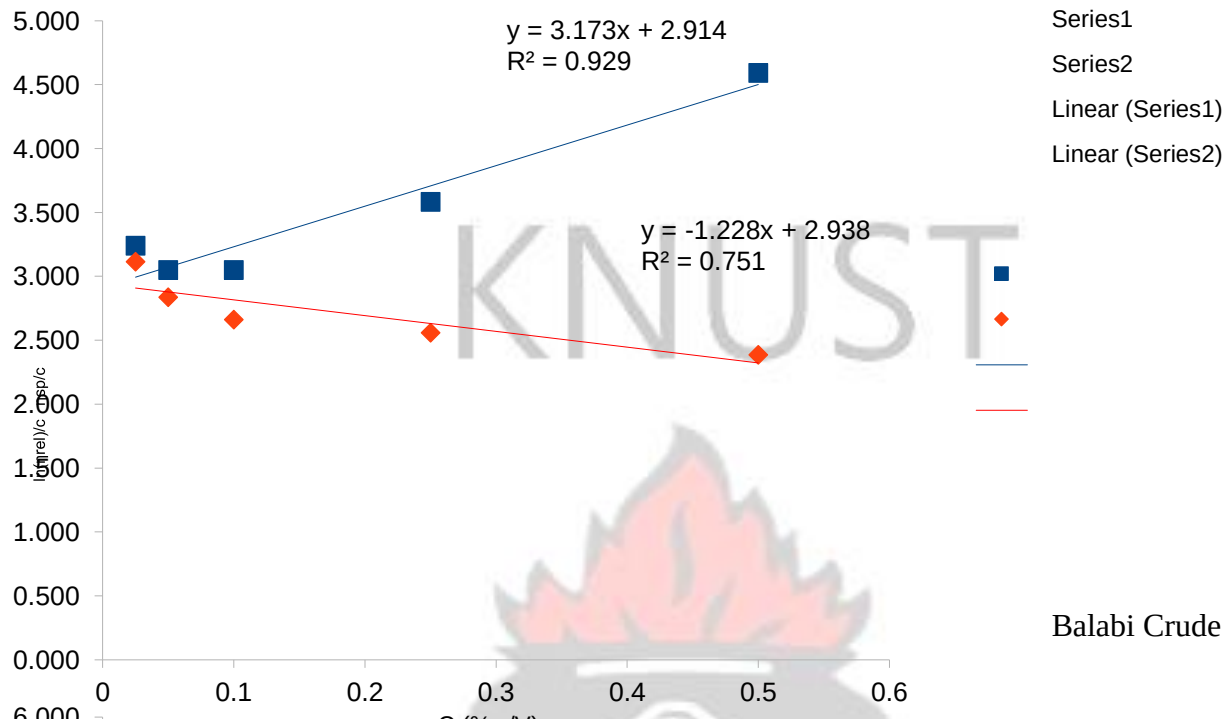
Asontem Pure



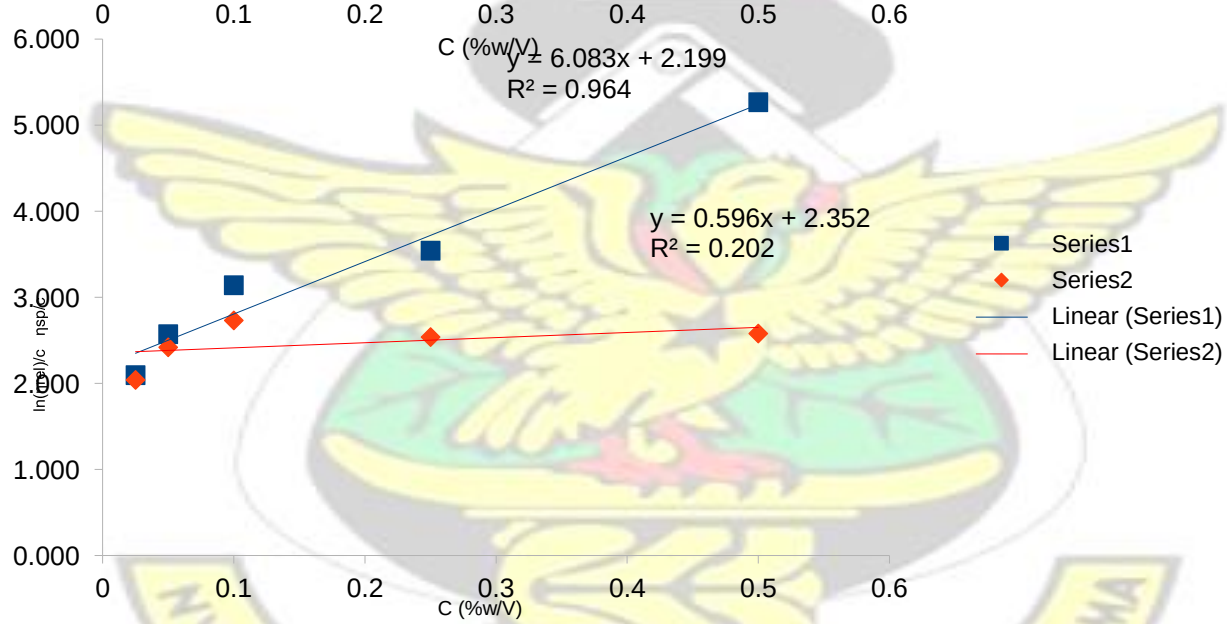
Asontem crude



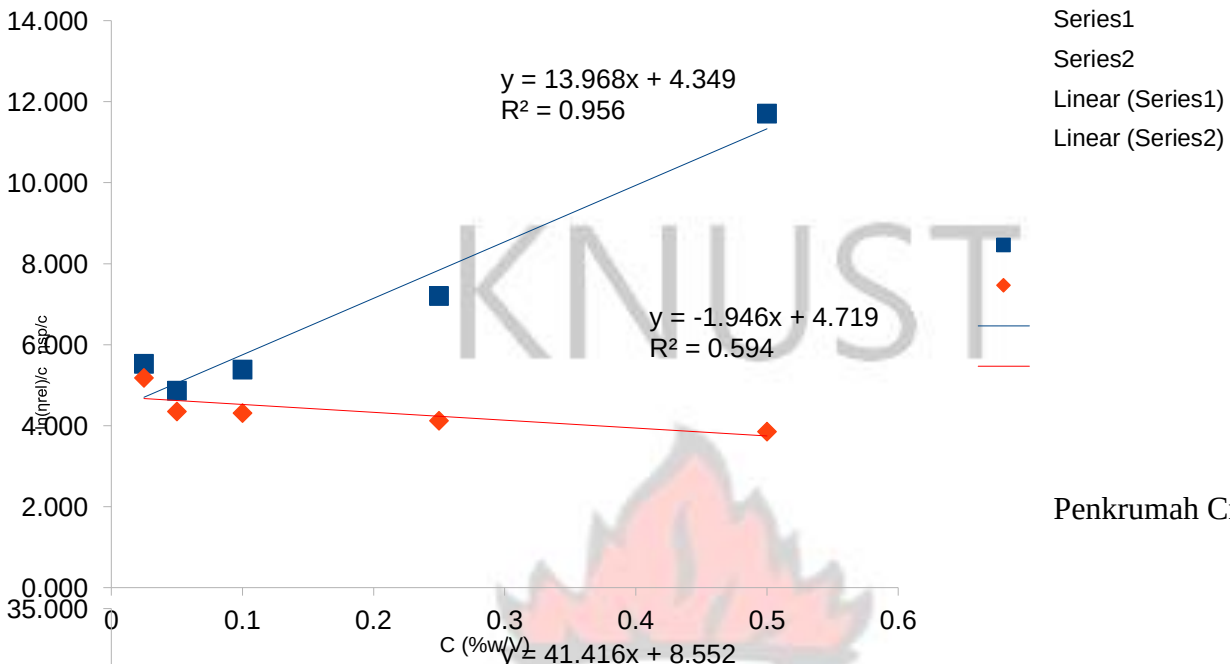
Balabi Pure



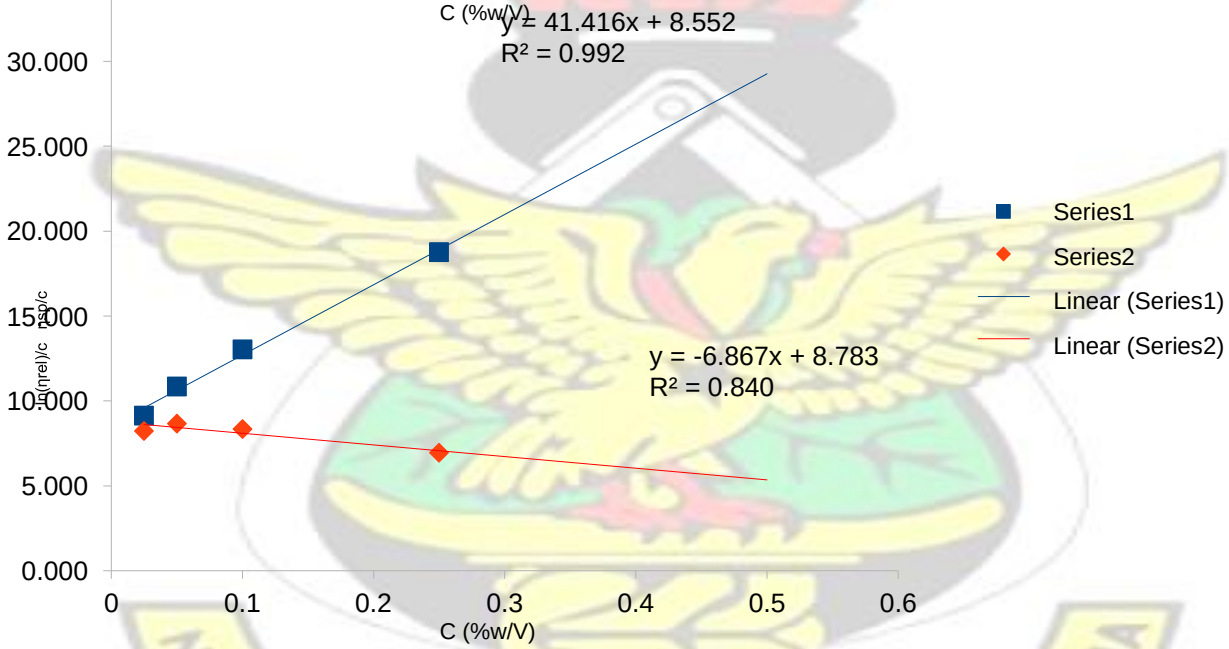
Balabi Crude



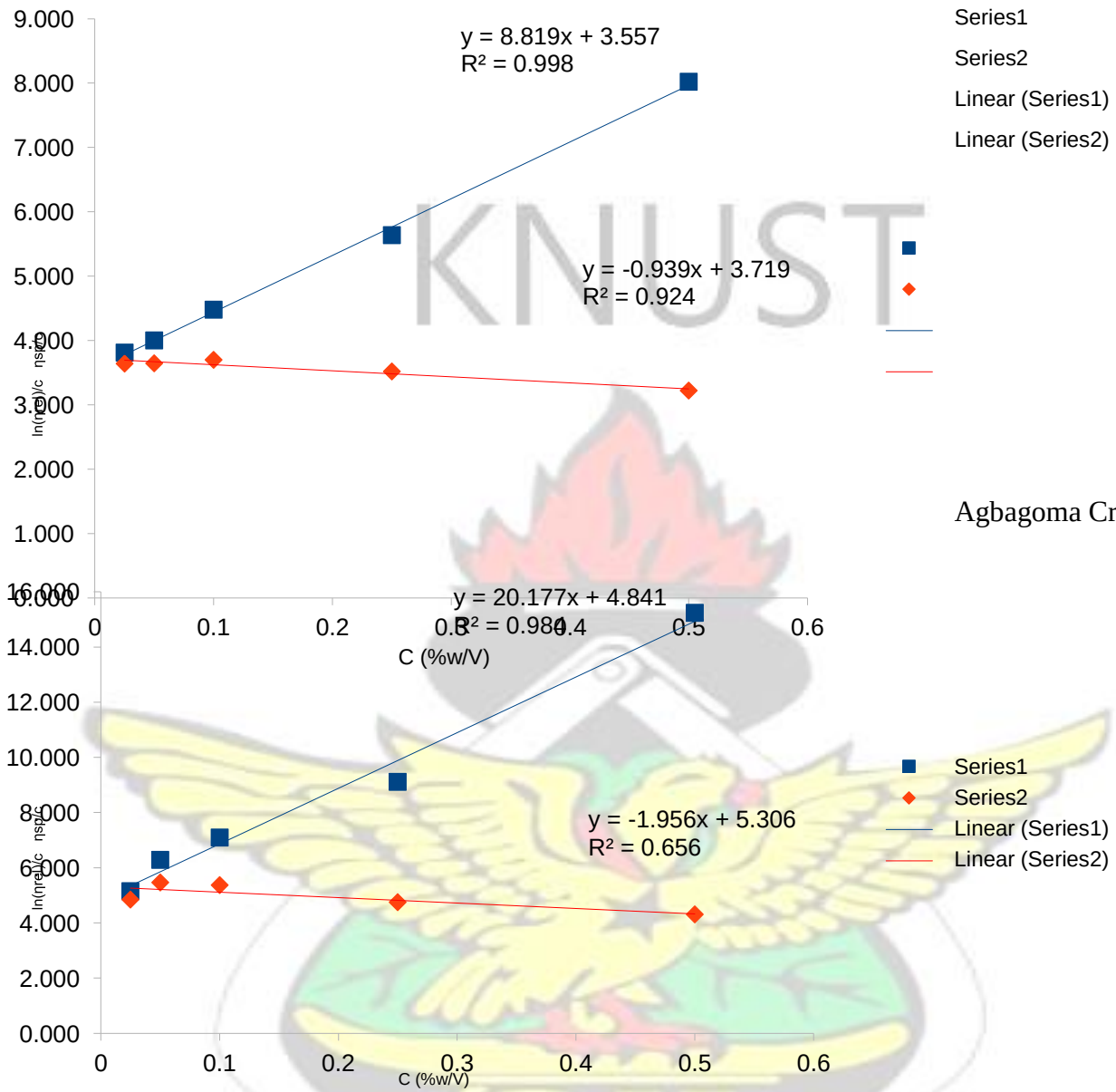
Penkruma Pure



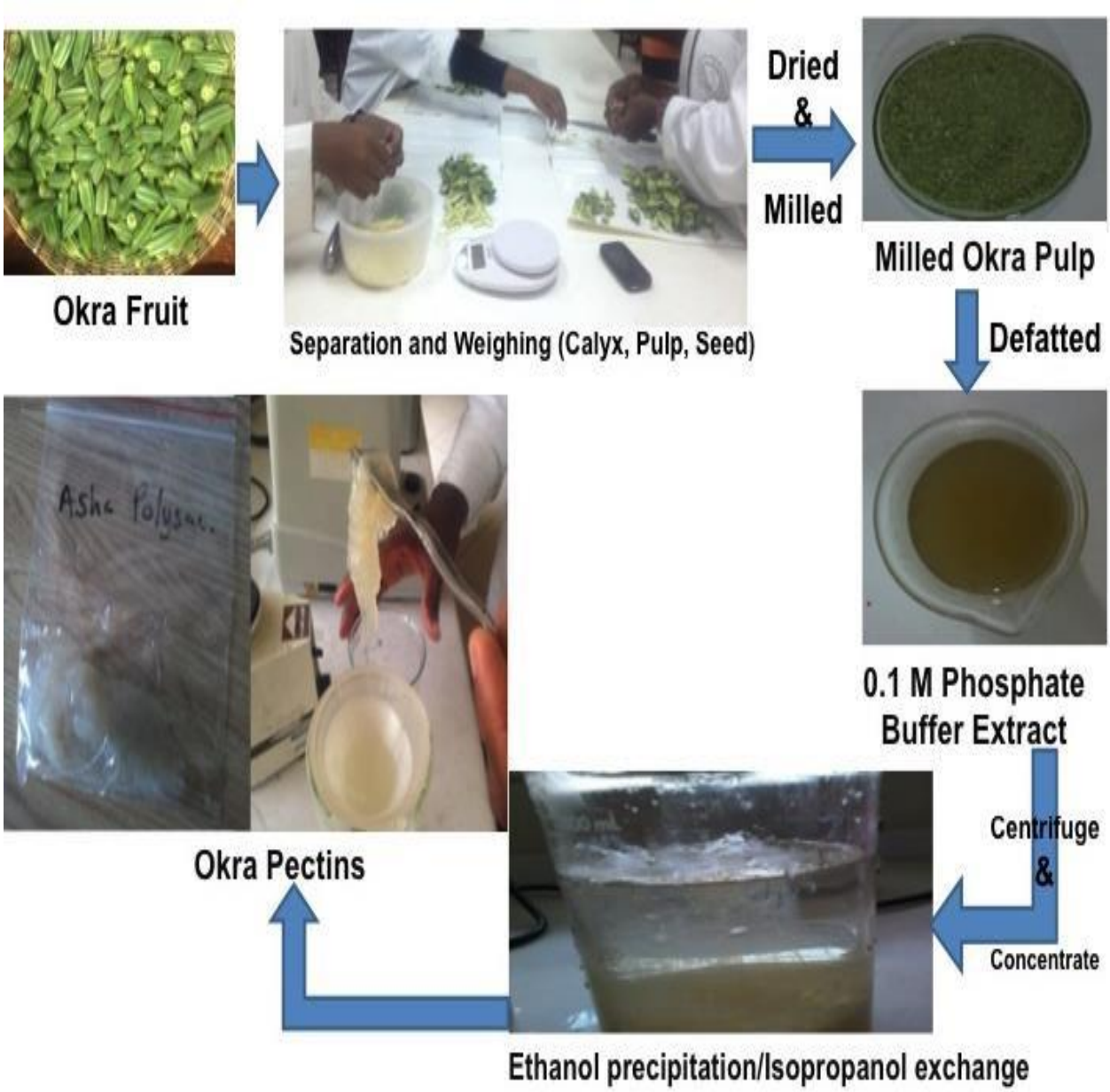
Penkrumah Crude



Agbagoma Pure



Appendix 5.2: Okra Pectin Isolation


Okra Fruit
Separation and Weighing (Calyx, Pulp, Seed)
Dried
&
Milled
Milled Okra Pulp
Defatted
0.1 M Phosphate
Buffer Extract
Centrifuge
&
Concentrate
Ethanol precipitation/Isopropanol exchange
Okra Pectins

Appendix 5.3

Appendix 5.4

Agbagoma okra genotype

Appendix 5.5

Asha okra genotype

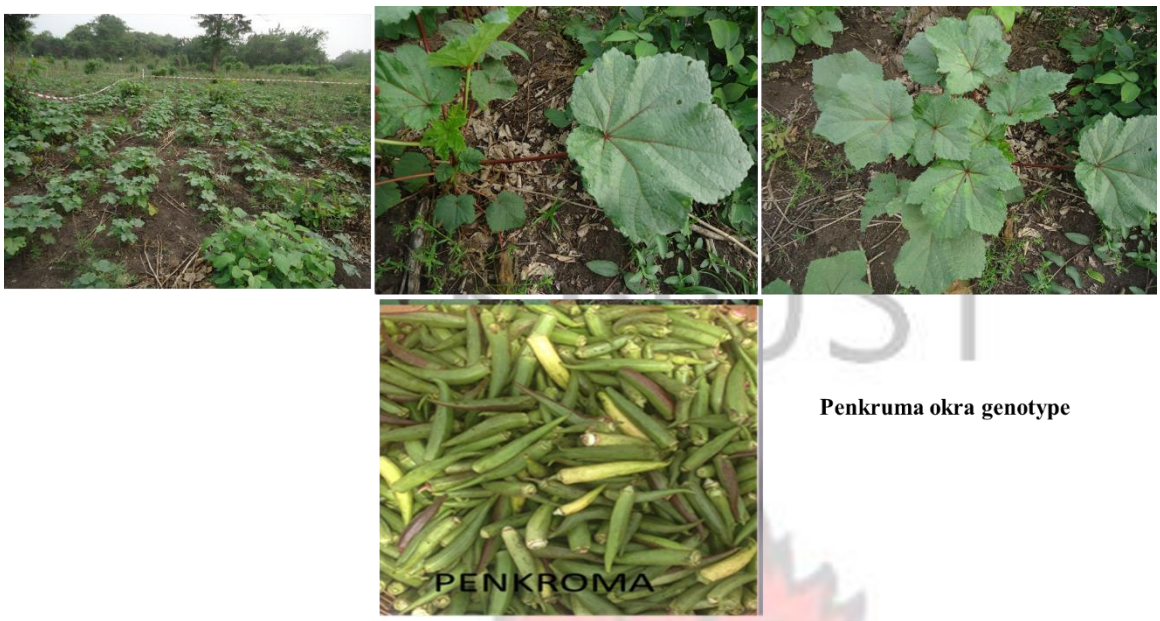
Appendix 5.6

Asontem okra genotype

Appendix 5.7

Balabi okra genotype

Appendix 5.8



Penkruma okra genotype

Appendix 5.9

Sengavi okra genotype