KWAME NKRUMAH UNIVERSITY OF SCIENCE TECHNOLOGY

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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

INVESTIGATION OF OKRA PECTIN AT DIFFERENT HARVEST MATURITY

A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, COLLEGE OF SCIENCE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MSc FOOD SCIENCE AND TECHNOLOGY

BY

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DECLARATION

I hereby declare that except for references to works of other researchers which have been duly acknowledge, this work 'characterization of pectin from okra at different harvest maturity' is my own original research and neither part or whole has been present elsewhere for the award of a degree.

27th February 2018

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ABSTRACT

Okra (*Abelmoschus esculentus*) is a staple vegetable in Ghana and several African countries but underutilized. The mucilage or pectin content is currently of interest for various food and nonfood applications. The objective of this work was investigate the yield and intrinsic viscosity of okra pectin extract as influenced by fertilizer application and maturity of the fruit pods. Isolation of okra pectin was done by an aqueous extraction at pH 6.0 from the pods of 2 different okra genotypes at three harvest maturity and two different weight of NPK applications (6g and 9g).

The intrinsic viscosity [η] of the okra pectin solutions was determined at 20^{o}C with a Ubbelohde capillary viscometer and calculations made according to the Huggins equation. Pectin yield ranged between 8-24% depending on genotype, fertilizer treatment and maturity of the fruits. There were significant differences ($p < 0.05$) in the pectin yield between the okra genotypes as well as among the stages of maturity of the okra fruits. Interaction between okra varieties and the stages of maturity of fruits was also significant for pectin yield. The pectin yield of AGRA genotype was higher than that of AGBAGOMA. However, the intrinsic viscosity of pectin of AGBAGOMA was higher than that of AGRA. Highest pectin yield was observed for intermediate matured fruits, followed by that of immature fruits and lowest was observed for overgrown fruits. The intrinsic viscosity values however was highest for immature fruits as compared to the intermediate and overgrown okra fruits. Pectin yield and intrinsic viscosity increased significantly with fertilizer application ($p < 0.05$). The present findings show that harvesting at intermediate maturity would be most appropriate if high pectin yield is desired. A 9g NPK treatment suggest the opportunity to increase yield and intrinsic viscosity of okra pectin. The study showed that it is important to identify the most appropriate stage of development to harvest the okra fruit for maximize pectin yield. The results of this study suggest that intermediate fruit maturity would be most appropriate to harvest okra fruits to increase pectin yield.

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CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Vegetables such as okra, which is of Africa origin, are essential food constituent due its nutritious benefits to humans (Oppong-Sekyere *et al.,* 2012). It originated from Ethiopia and it is cultivated in many places especially humid and subtropical countries (Eze and Akubor, 2012). It is either cultivated as cash crop or as household crop. It has different names with different spellings from different geographical locations. In India it is called bhindi, krajiabkheaw in Thailand, quiabo in Angola, quimbombo in Cuba, mbinda in Sweden, okura in japan and ass gumbo in French speaking countries. Middle East people call it bamia, gumbo in Southern part of USA and with the queens country been known as lady's finger. In Ghana, it is known by the Akans as Nkruma, Fetri by the Ewes.

The okra is scientifically known as *Abelmoschus esculentus* (L.) Moench belongs to the family of Malvaceae. Okra is found in two different states at different times. It is fresh in the wet period and desiccated in desiccated period rainfall. It is characterized by minuscule seeds and adhesive in texture when fresh. It develops to altitude of 2.5 m and conveys yellow mallow-type flowers which

ripens as greenish fruit (Sengkhamparn, 2009). Okra is a slightly curved shaped vegetable with seeds. Due to its high moisture content, it is must be preserved else perished (Eze&Akubor, 2012). Okra consist of numerous nutrients with varied percentages as reported by different researchers. This is attributed to the variances in the extraction techniques and uses of diverse varieties of okra. The calories of fresh okra contain essential nutrients such as vitamin c and foliate. Benchasri (2012) gave explicit composition of okra pod; it has water content of 88.6 g, energy being 144.00 kJ (36 kcal), protein of 2.10 g, carbohydrate of 8.20 g, fat of 0.20 g, fiber of 1.70 g, Ca of 84.00 mg, P of 90.00 mg, Fe of 1.20 mg, β-carotene of 185.00 μg, riboflavin of 0.08 mg, thiamine of 0.04 mg, niacin of 0.60 mg and ascorbic acid of 47.00 mg. The moiture content in okra is reduced when dried which results in, ash and minerals (Zn, Ca, Mg and Fe) growing in dry matter content of protein in the okra (Eze andAkubor, 2012). The production of okra has being on the increase with India been the highest producers. In Africa, West African (WA) countries account for three-quarters of okra production on the continent though its productivity is low compared to other regions (FAO, 2008). In West Africa, Nigeria is the leading producer of okra followed by Cote d'Ivoire and Ghana.

Pectin is a component of okra and it is usually part of the cell wall. Pectin exists as macromolecule with several different monosaccharaides being well arranged (Vincken *et al.*, 2003). It has a range of use in many foodstuffs. It is use as crystallizing and solidifying agent. Pectin can be obtain by several methods and this reveals different compositions. Pectin characteristic, yield, property such as viscousity being characterized by diverse reasons ranging from sort, origin, level and dissemination of methyl esters and acetyl groups (Sengkhamparn, 2009) and it is against this background the study seeks to find out how different harvest maturity influence the yield of okra pectin.

1.2 Problem statement

Okra is widely found in almost all Ghanaian markets and it is widely consumed by the people due to its nutritional value. The market value of okra decreases annualy due to limited use in soup/stew preparation. There is inadequate knowledge on practices to produce varieties of okra and maximize production for specific technological applications, this has led to a situation where Ghanaian farmers are at a disadvantaged position. As a result of this farmers have lots of difficulty in seed storage, soil fertility management, controlling pest and diseases and also as soon as the quantities for food are achieved value for okra drops to very low levels as there is only very limited processing by drying. Pectin is derived from the pods of the okra plants, the maturity at which the okra pods are harvested affects pectin yield since there is lignification of the cell walls of the okra pod. Okra pods should be picked while they are tender and immature. During maturation pods become tough due to thickening of fiber bundles present in the pericarp region (Salunkhe *et al.*, 1984). Tough pods have less market value as well as their culinary value since they are not suitable for curry and soup preparation (Chutichudet *et al.*, 2007).

1.3 Objectives

The objective of this study is to characterize okra pectin at three harvest maturity stages.

Specifically, the study seeks to:

- Determine okra pectin yield as a function of maturity
- Determine the intrinsic viscosity of okra pectin varying in maturity.

1.4 Justification

The findings on the effect of maturity on the yield of okra pectin will suggest the suitable maturity stage to achieve high pectin yield. It would also provide useful fundamental information on the

intrinsic viscosity of pectin sample as influenced by maturity. Ultimately, this project is expected to contribute significantly towards the effort to improve the economic value of okra.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Okra production

Okra (*Abelmoschuse sculentus L.*) is a member of Malvaceae family. It originated from Ethiopia and Sudan, the north-eastern African countries. It is primogenital grown crop in the world with a wide range of distribution in Africa to Asia, southern Europe and America. It is a humid and frost crop and requires low temperature, water logging and drought conditions. The weather and soil requirements are specifics to the country to which they belong. The several names of okra are characterized by different spellings from different geographical locations. It is known in India as bhindi, krajiabkheaw in Thailand, quiabo in Angola, quimbombo in Cuba, mbinda in Sweden, okura in japan and ass gumbo in French speaking countries. Middle east people call it bamia, gumbo in Southern part of USA and with the queens country been known as lady's finger (Ndunguru and Rajabu 2004). It dwells well in humid and subtropical areas (India, Middle East, Southern USA and West Africa) The world okra production is estimated at 7 million MT with India leading the production by 70%, followed by Nigeria (15%), Sudan (2%), Iraq(2%), Cote d'ivoire (1.7%) and other countries including Ghana which produces about 60,000 MT (FAOSTAT, 2012). Of okra produced in Africa, West and Central Africa region accounts for more than 75% of total okra produced (FAOSTAT, 2012). In Ghana, Brong Ahafo, Ashanti, Northern, Volta, Greater Accra and Central regions are the bulk producers of okra (in terms of tonnage) (NARP, 1993).

Many species of okra have been identified with *A. moschatus*, *A. manihot* and *A. esculentus* commonly cultivated (Figure 1). Okra has potential applications in the food and phamarceutical industries due to the antioxidant activity, fibre, polysaccharides, nourishing and serviceable properties of okra pods (Arapitsas, 2008; Lengsfeld *et al*., 2004; Adelakun *et al*., 2009, Sengkhamparn *et al*., 2010; Ghori *et al., 2015*).

Figure 1: Some typical okra plants and fruits/pods

2.2 Nutritional and Physiochemical composition of okra

Okra is a freshly consuming food substance in the world with the seed higher in nutrition than the pod. It is believed to be the significance crop in the Malvaceae family due to its popularity. Okra seed is rich in protein (Ndangui *et al*., 2010) (amino acid content been similar to that of soyabean) and oil and contains other substances making it a substituent for coffee. It is mostly consumed as green in numerous ways due to its high nutritional content for human nutrition. It is a highly rich in oil, protein, unsaturated fatty acids such as linoleic acid, which is essential for human nutrition.

The crude fibre content obtained from fruit and stem is useful in the paper industry. Okra contains edible oil which is quite high when extracted from its seed and the oil has a pleasant taste and odour, and is high in unsaturated fats such as oleic acid and linoleic acid. The oil content of the seed is quite high at about 40% (Tripathi and Warrier, 2011). The components of the fibre are 67.5 % a-cellulose, 15.4 % hemicelluloses, 7.1 % lignin, 3.4 % pectic matter, 3.9 % fatty and waxy matter and 2.7 % aqueous extract. It is clear that the main constituents of OBF are a-cellulose, hemicelluloses and lignin and the rest are minor in proportion. Therefore, the structure of a-cellulose, hemicelluloses and lignin and the mode of combinations that exist in between themselves are dominating the structure of OBF (Kumar *et al.*, 2013).

2.3 Ecosystem and Season cultivation and growth of Okra

Propagation of okra is by seeds and has duration of five to six months. It is generally a yearlycultivated plant and it characterized by a stem of robust, erect, and variable in branching and varying from 0.5 to 4.0 meters in height. Okra Leaves are alternate and usually palmate of five lobed and the flower is both axillary and solitary. Okra plants are characterized by indeterminate growth. Flowering is unremitting but vastly reliant on biotic and abiotic stress. First flowering starts one to two months after sowing. The fruit produced is a pill and grows quickly after flowering. Changes in fruit length, height and diameter occur during 4th to 6th day after pollination making fruit ready to be plucked for consumption. The okra pods are harvested when immature and high in mucilage, but before becoming highly fibrous (Tripathi and Warrier, 2011). Okra requires a moderate rainfall of 80-100 cm well distributed to produce its young edible fruits over a relatively long period. An average temperature of 20°C to 30°C is considered optimum for growing, flowering and fruiting. Flower initiation and flowering are hardly affected by day length in popular subtropical cultivars. Most tropical cultivars show quantitative shortday responses, but qualitative responses also occur. The shortest critical day length reported is

12.30 hours. Okra plants continue to flower and to fruit for an indefinite time, depending upon the variety, the season and soil moisture and fertility. In fact the regular harvesting stimulates continued fruiting, so much that it may be necessary to harvest every day in climates where growth is especially vigorous. A vegetable yield of 10 t/ha can be considered a good harvest, but yields of over 40 t/ha can be realized under optimal conditions. Yields are usually low (2–4 t/ha) as a result of non-intensive growing methods (Kumar *et al*., 2013). Seed yields are in the range of 500–1000 Kg/ha.

2.4 Features of Okra pectin

Pectin is found in cell wall of okra. Pectin exists as macromolecule with several different monosaccharaides been well arranged (Vincken et al., 2003). Okra pectin's are acidic in nature with random coil polysaccharides composed of galactose, rhamnose and galacturonic acid (Alba, 2015). The repeating unit was reported to be α-(1-2)-rhamnose and α-(1-4)-galacturonic acid residues including disaccharide side chains and they form viscous solutions that exhibit pseudoplasticbehavior (Kontogiorgos et al., 2012). Protein and acetyl contents make pectin from okra differ from other pectin's of other plants and this make pectin derived from okra can be used as an stabilizer (Sengkhamparn et al., 2009). Pectin characteristic, yield, property such as viscous influenced by many different factors ranging from type, origin and the level and dissemination of methyl esters and acetyl groups (Sengkhamparn, 2009). Pectin is made up of different monosaccharide's been arranged in a number of structural elements forming the building blocks of the pectin network. The pectin spine can be classified into three classes based on the elements present; homogalacturonan, substituted galacturonan and rhamnogalacturonan.Homogalacturonan (HG) is composed of a backbone of α-(1, 4)-linked-D-galacturonosyl residues in which a variable part of the galacturonic acid is methyl esterified. The degree of methyl esterification (DM) in HG causes pectin to be grouped into two main types (high

DM pectin (more than 50%) and low DM pectin). Functionality is highly influenced by the level of DM. Parts of HG may be cross-linked and be involved in forming a three dimensional network, a pectin gel, which is important in controlling the porosity and mechanical properties of the cell wall and contributing to the maintenance of intercellular adhesion. The gelling property of pectin's may be influenced by many different factors such as type and origin of the pectin and the level and distribution of methyl esters and acetyl groups. The methyl esters can be distributed either randomly or block-wise over the HG segment, which strongly effect the calcium binding of pectin as will be discussed later. Higher solubility of pectin polysaccharides is attributed to the heterogeneous structure (HG, RG-I, RG-II), the presence of hydrophilic carboxyl (degree of methyl esterification) and hydroxyl groups that interact with water mainly through hydrogen bonding. Other factors affecting pectin solubility kinetics include pH of solution, DM, branching of side-chains, solution concentration, presence of counter ions and molecular weight (Sengkhamparn, 2009).

2.5 Extraction of Okra Pectin

Several methods are available for the extraction of pectin. The techniques include the isolation of pectin with enzymes (e.g., polymethylgalacturonases, polygalacturonases, polygalacturonatelyases), electromagnetic induction heating, and microwave- and ultrasound-assisted isolation (Bagherian *et al*., 2011). The more conventional methods of pectin cell wall extraction are cold and/or hot aqueous, buffers, use of chelating agents (e.g., potassium-oxalate), diluted acids (e.g., HCl) or diluted sodium hydroxide solutions. Although the various alternative extraction methods have been recently proposed, the isolation of pectin until now is mainly performed using hot acid (nitric acid, sulfuric acid and hydrochloric acid) treatment in combination with high temperatures between 70 - 90°C. The pH varies between 1.5 and 2.5 and the time of extraction depends on raw material (e.g., efficiency of protopectin release), desired chemical

composition of pectin and manufacturer's individual needs. Moreover, the chemical composition of isolated pectin also varies with respect to the extractant used. It has been shown that pectin isolated from various plant sources (e.g., leek, pineapple, sugar beet, cucumber, lemon, fennel) appear to be rich in HG when isolated with mild agents (e.g., water or K-oxalate) and become considerably richer in RG-I when extracted by stronger agents (e.g., HCI, NaOH). Following the hot extraction step, the precipitation of pectin from extraction liquor is performed with organic solvent (e.g., methanol, ethanol or isopropanol). Therefore, the pectin extract obtained by commercial acid extraction is composed of those polymer molecules that are soluble at a certain pH and time-temperature regime. However, those harsh acidic conditions of pectin extraction particularly during longer times could contribute to the depolymerisation of pectin. The isolation of pectin substances from cell walls is pH sensitive and could be also performed in the presence of basic extraction medium. The modifications of pH of extraction result in pectin with a different degree of methyl-esterification (DM) and therefore, functional properties. Extractions performed at high pH typically result in isolation of pectin with low DM due to the saponification of the ester groups. In contrast, acidic extractions yield pectin of high DM.

2.6 Factors affecting pectin yield

Previous work on isolation of pectin reported the remarkable effect of extraction time, temperature, pH, type of acid, number of extraction cycles, the ratio of water to raw material and volumes of organic solvent on the yield and chemical composition of pectin from various plant sources, such as apple and peach pomace, mango peel, okra pods and passion fruit (Kumar &Chauhan, 2010).

Extraction time is when the extraction process as well as the selectivity of the fluid is efficiently influence, thus yield increases significantly as extraction time rises. A lengthier extraction time result in favourable yield and polysaccharide production as the time allows for disclosure of the

okra crude polysaccharide to the medium and allows them to be dissolved and drawn-out out of the raw materials. (Samavati, 2013).

2.6.1 Extraction temperature

It increases the solvent ability to solubilize the compounds and reduce the viscosity of the liquid solvent which allows for better penetration of the solvent into the solid matrix. It has been reported that extraction at elevated temperatures resulted in faster and easier mass transfer of water soluble polysaccharide from the cell wall into extract (Tabatabaee and Mirhosseini, 2012).

2.6.2pH

Study shows that when pectin's are extracted at pH of 3.0, they have similar compositional characteristics to water- soluble pectin's b but gives a low yield 'pH values below 3.0 for extraction gives higher yields and also gives pectin's that are rich in rhamnogalacturonnans (Levigne et ai.2002). Extraction of pectin from okra at different pH values were examine for the effects of the extraction conditions on the molecular and compositional characteristics of the pectin (Alka 2015; Alba *et al.* 2015). Pectin's were extracted at pH 2 and 6. The difference in extraction conditions resulted in isolates with distinct molecular characteristics.

2.7 Functions of pectin

Pectin functions as gelling agent in various food products (Laurent and Boulenguer, 2003).Gel formation is as a result of numerous polysaccharides. This occurs as a result of joining together of several polymers through bond formation. These bond formation can either be covalent or non-covalent (Walstra, 2003). The viscosity of a polysaccharide solution depends on many factors such as molecular mass, hydrodynamic volume, stiffness and charge of the molecule (Williams and Phillips, 2000). The charged polymers generally have a higher viscosity than nonionic polymers at similar mass and chemical structure due to the intermolecular charge repulsion (Williams and Phillips, 2000). Although pectin carries, free carboxyl groups on the backbone and it behaves as a

polyelectrolyte. The viscosity of pectin solutions depend on chemical and physical characteristic of the pectin, on the ionic strength of the solution and on the presence of sugar. The junction zones of hydrocolloid gels are normally formed via physical interaction such as hydrogen bonding, hydrophobic association and cat ion-mediated cross-linking (Williams and Phillips, 2000). Previous studies have shown that the gel properties of pectin gels strongly depend on molecular properties of the polymer. The gelation properties of pectin are influenced by the molecular weight of the pectin, the length of the pectin side chains, the level and distribution of methyl esterification and the level of acetylation. For example, Schmelter *et al*. (2002) suggested that pectin with shorter side chains gave better gelation properties than pectin with longer side chains. Moreover, the pattern of esterification, block-wise or random, has a great impact on gel characteristics (Willats et al., 2001) the enzymatic removal of acetyl groups as present in sugar beet pectin led to an improved gelation and a much stiffer gel (Oosterveld *et al*., 2000).

2.8 Application of okra polysaccharide

2.8.1 Uses in food

Many uses of okra polysaccharide have been reported. Rheological properties of its polysaccharide makes it suitable for application in the food industry as viscosity enhancer, thickener, gelling agents and modifiers in some food emulsions including dressing and among others. Okra polysaccharide as stabilizer prevent ingredient from separating and also increases the viscosity. They also bind to water to improve texture (Hussien *et al.*, 2011). They studied how feasible how feasible it will be to use polysaccharides from okra to improve yogurt consistency. Okra polysaccharides have also been reported to have been used to clarify sugar cane juice in India (Kumar *et al.*, 2010)

Pharmaceutical uses

The demand for mucilage and natural gum which serve as emulsifiers, stabilizers, gelling agents, suspending agents, binders film formers and disinter grants are increasing as they are more preferred instead of synthetic or semi-synesthetic ones in drug delivery because of their low cost and availability, they are also nontoxic and are biodegradable and therefore echo friendly (Sangwan *et al*. 2011).

2.9 Viscosity

The measurement of resistance of a fluid to flow is viscosity. It is the ratio shear stress being applied to the resulting strain rate. There are existence several methods for exist characterizing the solution viscosity. When η_o is the viscosity of the pure solvent η is the viscosity of a solution using that solvent and *c* is the concentration (expressed in g/dl), most common solution viscosity expressions are express follows:

Relative viscosity, $$\eta_r = \frac{\eta}{\eta_o} \quad \text{(Eq.1)}$$

Specific viscosity, $$\eta_{sp} = \frac{\eta - \eta_o}{\eta_o} = \eta_r - 1 \quad \text{(Eq.2)}$$

Inherent viscosity, $$\eta_i = \frac{In\,\eta_r}{c} \quad \text{(Eq.3)}$$

Intrinsic viscosity$[\eta]$ = $$\frac{In\,\eta_{sp}}{c} \quad \text{(Eq.4)}$$

Specific viscosity states the rise in viscosity due to the polymer existence in the solution. Regulating*'s* to concentration gives η_{sp}/c which express the ability of a polymer to cause the

solution viscosity to rise. The extrapolated value of η_{sp}/c at zero concentration is known as the intrinsic viscosity, [η] (Figure 2).

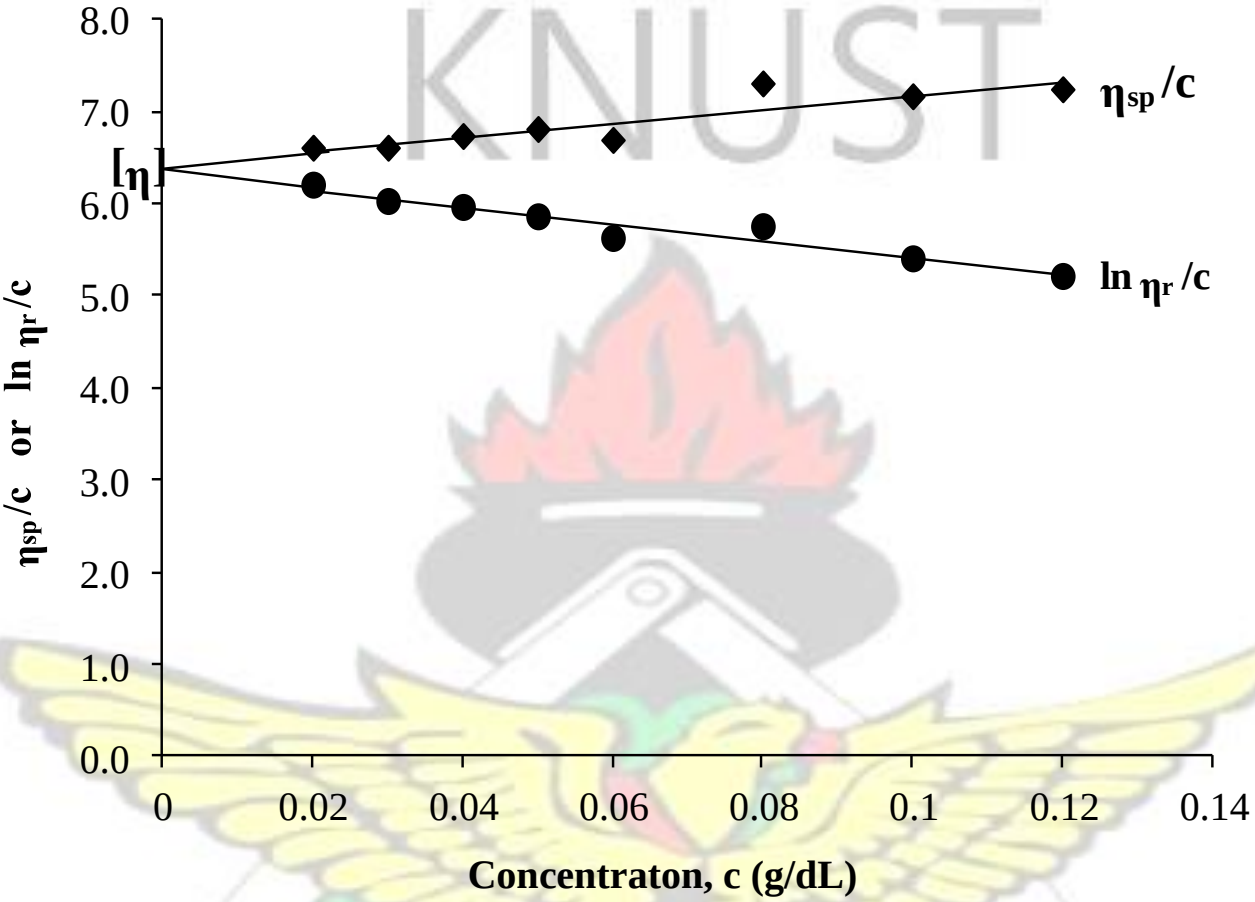

Figure 2: Plots of η_{sp}/c and ln η_r/c as a function of concentration (Adapted from Agbenorhevi, 2011).

Like η_{sp}, lnη_r is zero for pure solvent and rises with increasing concentration, thus lnη_r also states the incremental viscosity due to the presence of the polymer in the solution. Normalizing lnη_r to concentration or lnη_r/c gives the key viscosity. In the boundary of zero concentration, η_i same as η_{sp}/c when is being g extrapolated which then turn out to be equal to the intrinsic viscosity. Thus [η] can be found be extrapolating either η_i or η_{sp}/c to zero concentration. Units of [η] are inverse concentration and typically stated as dL/g. [η] is determined through the use of the Ubbelohde

capillary viscometer the simplest and most useful approach s greatest when is being selected such that the flow time being greater than 100 seconds (Rao, 2007).

2.10 Capillary viscometer

Time taken for a volume of dilute polymer solution to flow through a thin capillary is compared to the time for a solvent flow. The flow time is proportionate to the viscosity, and inversely proportional to the density.

$$t_{solvent} = \frac{\eta_{solvent}}{\rho_{solvent}} \quad \text{(Eq. 5)}$$

$$t_{solution} = \frac{\eta_{solution}}{\rho_{solution}} \quad \text{(Eq.6)}$$

Since r*elative viscosity* is defined as the ratio $\eta_{solution} / \eta_{solvent}$ and for most polymer solutions at the concentrations of interest, $\rho_{solution} / \rho_{solvent} \approx 1$, therefore, approximately the relative viscosity is a simple time ratio:

$$\eta_r = t_{solution} / t_{solvent} \quad \text{(Eq.7)}$$

Specific viscosity is also defined as the fractional change in viscosity upon addition of polymer:

$$\eta_{sp} = \frac{\eta_{solution} - \eta_{solvent}}{\eta_{solvent}} = \frac{t_{solution} - t_{solvent}}{t_{solvent}} = \eta_r - 1 \quad \text{(Eq. 8)}$$

Therefore, relative viscosity and specific viscosity can be calculated by comparing the time it takes solution to the flow time of the pure solvent at several different concentrations and extrapolating to zero concentration to determine intrinsic viscosity, [η].

The extrapolation of experimental viscometric data (at a constant temperature, e.g. 20^{o}C) to zero concentration can be done according to the Huggins equation:

$\eta_{sp}/c = [\eta] + k'[\eta]^2c$ (Eq.9)

or the Kraemer's equation:

In $\eta_r/c = [\eta] + k''[\eta]^2c$ (Eq.10) $k' = k'' + 0.5$ (Eq.11)

Where k' is the Huggins' constant and k'' is the Kraemer's constant.

The viscosity of a polymer depends on the relations between the immediate polymeric chain and the solvent, and also molecular weight (Mn) as well. The existence of a polymer in solution is expressed as the specific viscosity (η_{sp}) for an increase in fractional of the viscosity whereas the occupied volume by the individual polymer molecules in isolation is being characterized by concentration conferring to the Huggins equation (Eq. 9) (Agbenorhevi, 2011).

CHAPTER THREE

3. METERIALS AND METHODS

3.1 Okra Samples

Agbagoma okra genotypes was obtained from Akrofo in the Volta Region where as AGRA genotype was cultivated at the Soil Sciences experimental field, KNUST from October 2016 to January 2017. The land was well prepared for the cultivation and harvest of the okra by slashing, ploughing and harrowing to make it a fine tilt for a successful experiment. Sowing was done by direct seeding Two seeds per hill at a field rate. fourteen days after germination, Seedlings were

thinned to one plant per stand. Standard agronomic practices including thinning, weed control, watering through to it developmental stages. Compound fertilizer in the form NPK were applied to the plants 30 days after sowing. Plants were also sprayed against insects, pests and diseases using Pyrical 480EC at a rate of 20 ml/15 L of water. Weeding was done with a hoe at 2 and 4 weeks after emergence and at early flowering respectively and when necessary.

3.2 Extraction of okra pectin

The okra pods were harvested at different maturity stages (five days after flowering, six to ten days, eleven to eighteen days) as immature, intermediate, and overgrown respectively. The okra pods were cut and the seeds removed. The separated okra pods were dried, milled to powder and then stored in zip-lock bags in a freezer until ready for extraction. Okra pectin was isolated using extraction method at pH 6.0 according to previous extraction protocol (Alba *et al.*, 2015;). The dried okra powder (20g) was defatted with petroleum ether (1g:10 ml) by placing the okra-ether mixture on a rotar shaker (120 rpm, 25^{o}C) for 4 h. The defatted okra powder was subjected to aqueous extraction with 0.1M phosphate buffer (1g powder: 30ml buffer solution), pH 6 at 80 ^{o}C for 1 h. After extraction, the soluble polymer was separated from the insoluble residue by centrifugation (3000 rpm for 10 min at 25 ^{o}C). The solubilized pectin in the supernatant was concentrated by evaporation at 80 ^{o}C and then precipitated with 96 % (v/v) aqueous ethanol at 40 ^{o}C for 1h (1:2). Extraction with aqueous alcohol is to remove proteins and some polar compounds. Pectin substances were precipitated with the ethanol. The extraction with ethanol was followed with washing using isopropanol and then oven dried at .180 ^{o}C for 15 minutes

3.3 Yield of okra pectin

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

$$Yield(\%) = \frac{mass\ of\ oven\ dry\ pectin}{mass\ of\ dried\ okra\ powder} \times 100$$

3.4 Intrinsic Viscosity

Okra pectin of 0.5g and 1g respectively in 100ml of distilled water were stirred overnight to ensure complete solubilisation. Intrinsic viscosities [η] of okra pectin were measured using an Ubbelohde capillary viscometer (PSL Rheotek OB. C 80705) at 20 ± 0.1^0C. Intrinsic viscosities each of okra pectin were obtained by extrapolation to to the Huggins equation: $\underline{\eta_{sp}/C} = \eta + k_H[\eta]^2C$

The extrapolations of the experimental viscometric data to zero where $\eta_{sp} = \eta_{rel} - 1$ and $\eta_{rel} = t/t_0$ where t is the average flow time of the solutions at each concentration, t_o is the flow time for water, kH, is the Huggins constant, and c is the biopolymer concentration (g dL^{-1}). All measurements were carried out on freshly prepared samples.

3.5 Statistical analysis

Data, taken from the okra plants. Averages, range, standard deviation and coefficient of variation were computed for the measurement data. Quantitative data was subjected to analysis of variance (Anova) using spss. Means were seperated by least significant difference at 5% Pearson's correlation analyses beween pairs of quantitative parameters were also performed using SPSS version 20s with reference to yield (%) parameters.

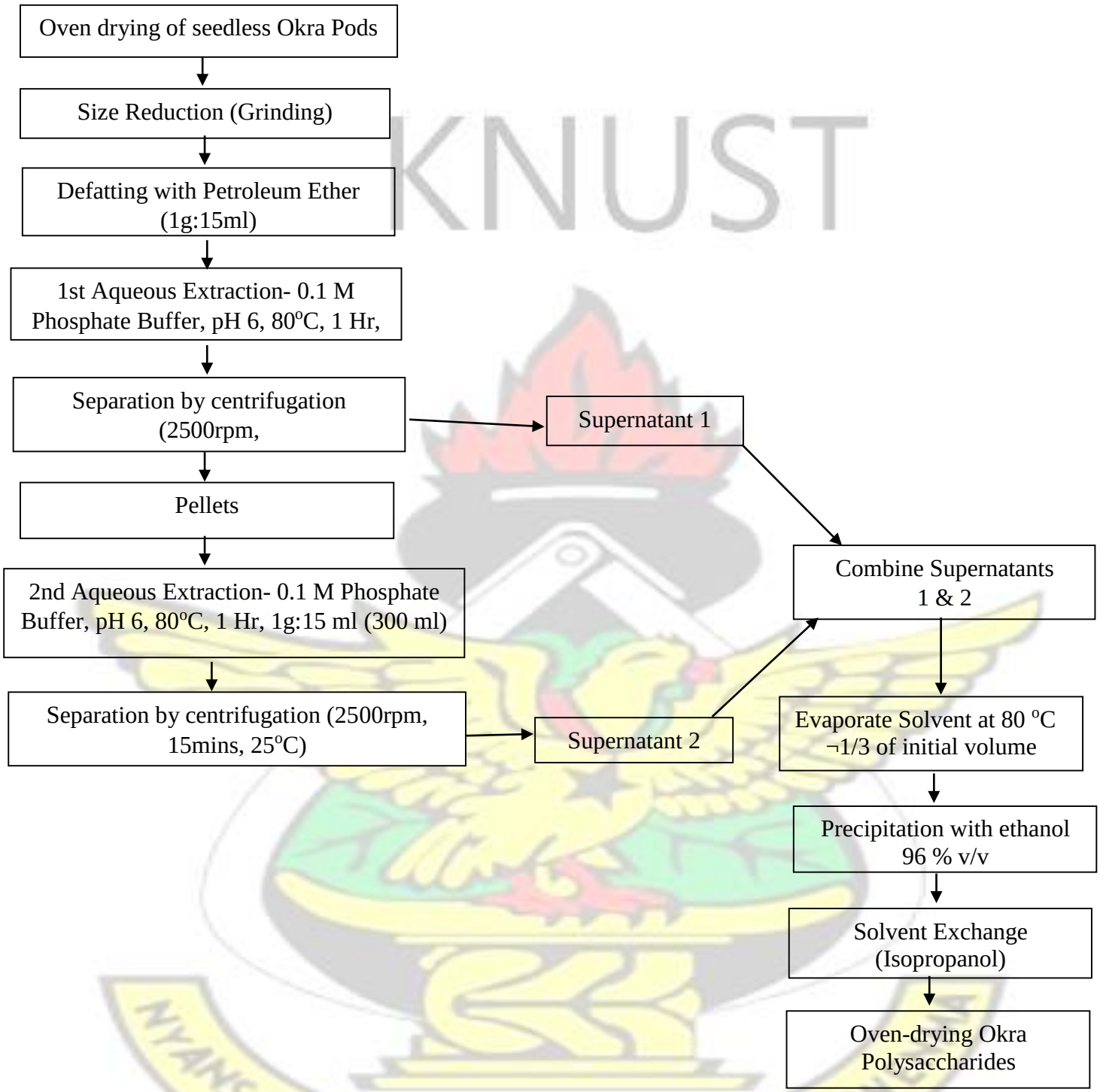

Figure 3: Flow diagram of pectin extraction.

CHAPTER FOUR

4. RESULTS AND DISCUSSIONS

4.1 Okra pectin yield

Table 1 and 2 show pectin yield and intrinsic viscosity of two okra genotypes and three stages of maturity of the okra fruits. The pectin yield of AGRA was higher than that of AGBAGOMA. However, the viscosity of pectin of AGBAGOMA was higher than that of AGRA (Table 1).

Table 1: Pectin yield and intrinsic viscosity[η] of AGRA and AGBAGOMA Okra Genotypes

Okra Genotype	Pectin yield (%)	[η] (dL/g).
AGRA	16.28 ± 1.95^{a}	4.9 ± 0.29^{a}
AGBAGOMA	12.68 ± 0.62^{b}	9.3 ± 0.96^{b}

The values are means ± SD. $^{a-b}$Values with different superscript in a column are significantly different ($p < 0.05$).

The highest pectin yield was observed for intermediate matured fruits (17.65%), followed by that of immature fruits (16.82 %) and lowest (8.97 %) was observed for overgrown fruits. The intrinsic viscosity value (**[η]**) was 11.9, 4.5 and 5.0 dL/g for immature, intermediate and overgrown stages of okra fruit maturity, respectively (Table 2). Intrinsic viscosity values of the present work are either similar to those reported by Kpodo *et al. (2017),* Alba, et al. (2015) and Ndjouenkeu, et al. (1996) or higher than those obtained using the sequential extraction methods (Kontogiorgos, et al., 2012) exemplifying the influence of isolation protocol on the molecular structure of the samples.

Table 2: Pectin yield and intrinsic viscosity([η]) of both okra genotype at different levels of maturity

Maturity level	Pectin yield (%)	[η] (dL/g).

Immature	16.82±1.95[a]	11.9±0.29 [a]
Intermediate	17.65±3.33[a]	4.5±0.96 [b]
Overgrown	8.97±0.69[b]	5.0±0.82 [b]

The values are means ± SD. [a-b]Values with different superscript in a column are significantly different ($p < 0.05$).

The results indicate that okra at pectin yield increased from the early maturity or immature stage to intermediate and then decreased when overgrown. Previous study have shown that okra mucilage content increased from index 1(light green coloured with soft texture) to index 2 (light green coloured but its texture is hard) and then gradually decreased from the fruit tissues at maturity index 3 (green whitish or green yellowish with hard texture and tip not easily broken) (Sreeshma and Nair, 2013; Noorlaila *et al*. 2015). The increasing pectin content of okra from the early maturity to the middle age could be attributed to growth and development of the okra itself.

The mucilage in okra contributes to moisture balance of the fruit and prevent it from drying out (Sreeshma and Nair, 2013). However, the declining in mucilage content as okra matures is possibly due to degradation process and lignification of the cell walls. It could also be due to drying out as the fruit matures. Thus the muscilage or pectinous matrix of cell layers undergoes degradation process as it enters senescence period (Western *et al., 2*000; Sreeshma and Nair 2013; Noorlaila *et al*. 2015).

There were significant differences ($p < 0.05$) between the okra genotypes with respect to pectin yield and intrinsic viscosity. Interaction between okra genotype and the stages of maturity of fruits was observed to be significant for pectin yield. This suggest that amount of pectin yield in okra depends on both genotype and the level of maturity of the fruits.

Table 3 shows the analysis of variance for intrinsic viscosity of pectin between two okra genotypes and three stages of maturity of the okra fruits. There were significant differences ($p > 0.05$) between the two okra varieties for viscosity of pectin. Also, differences among stages of maturity of okra was observed to be highly significant ($p<0.001$). Interaction Between genotype and stages of maturity was also significant.

Table 3: Variation and interaction of okra varieties and fruit maturity levels for pectin yield

Source of variation	df	Mean square
Replication	2	20.23
Okra variety	1	58.30**
Maturity level	2	137.58***
Okra genotype x Maturity level	2	62.26**
Error	10	6.22
Total	17	

Significant at *p <0.05, **p<0.01, *** p < 0.001

Table 4: Variation and interaction of okra varieties and fruit maturity levels for intrinsic viscosity([η]).

Source of variation	df	Mean square
Replication	2	111.0

Genotype	1	87.1**
Maturity level	2	102.7**
Genotype x Maturity level	2	252.2**
Error	10	131.7
Total	17	

Significant at *p <0.05, **p<0.01, *** p < 0.001

There were variations among okra varieties and fruit maturity levels for intrinsic viscosity. Interaction among genotype and maturity level of okra was observed to be significant

4.2 Intrinsic viscosity of okra pectins

Table 5 shows effects of the rate of NPK fertilizer application on pectin yield and viscosity in okra. There were significant differences between the levels of NPK rates for pectin yeild. At the rate of 6g NPK, pectin yield was 8.20% while at that of 9gNPK was 24.0%. Intrinsic viscosity of 4.98 (dL/g). and 5.74 [η] (dL/g) were estimated for 6g NPK and 9gNPK, respectively.

Table 5: Effects of fertilizer rate on pectin yield and intrinsic viscosity ([η]).

Fertilizer rate	Pectin yield (%)	[η] (dL/g).
6g NPK	8.20± 3.79	4.98±0.08

9g NPK	24.00±5.23	5.74±0.20
LSD (5%)	9.59	0.46
S.E.	9.13	0.44

Similarly, Kpodo *et al.* (2017) also observed differences among six okra genotypes for pectin yield (11.4-14.6%). Their results further show that individual okra genotypes provide pectin with different structural properties that could potentially provide a new source of functional pectin for the food and pharmaceutical industries. Pectin are complex carbohydrate molecules; they are used mainly as gelling agents in the food industry. There is a need to explore many sources to be used as pectin. Berardini *et al.* (2005) were of similar opinion and they explored mango peels to be utilized as a source of pectin and polyphenols.

The significant differences in pectin among the three stages of okra fruit maturity suggest that pectin content or yield vary with the stage of development or maturity of the okra fruit. It is therefore important to identify the most appropriate stage of development to harvest the okra fruit for maximize pectin yield. The results of this study suggest that intermediate fruit maturity would be most appropriate to harvest okra fruits to increase pectin yield. Lin et al., (1975) also reported that pectin fractions vary with stages of maturity of sunflower.

The significant interaction between okra varieties and the stages of maturity of fruits for pectin yield suggest that the amount of pectin in okra depends on the type of variety used and the level of maturity of the okra fruits. It is therefore important to select appropriate stage of development of fruits of each variety in order to maximize pectin yield. This support findings of Sahari et al (2003)

who also reported significant varietal and age of maturity of flower head effect on yield and quality of sunflower pectin.

The significant differences between the okra varieties for viscosity indicates that viscosity of okra vary among varieties of okra. Also the significant differences among stages of maturity of okra fruits for viscosity suggest that viscosity is a variable in relation to stage of maturity of okra fruits. The significant interaction between okra varieties or genotypes and maturity stage of okra fruits indicates that viscosity of okra fruits depend on combinations of okra varieties and maturity stage of fruits.

The significant differences between the rates of NPK fertilizer application for pectin yield suggest that pectin yield varies with the rate of NPK application in okra. This provides the opportunity to identify the appropriate rate of NPK fertilizer to apply to increase pectin yield of okra. In this study, the 9g NPK produced the highest pectin yield of 24% as compared with the 6g NPK. Also the viscosity of pectin of okra was higher with the 9 gram of NPK as compared with the 6 gram of NPK application.

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Pectin yield varied depending on the okra genotype. A highly significant difference ($p<0.001$) was observed among the stages of maturity of the okra fruits for pectin yield. Interaction between the okra genotype and stages of maturity of okra fruits was significant for pectin yield. Also there were significant differences ($p>0.05$) between the okra varieties for viscosity of pectin suggesting similar molecular weight. Differences among stages of maturity of okra fruit for viscosity of pectin was not significant ($p>0.05$). Interaction between okra varieties and stages of maturity of fruits for

viscosity of pectin was not significant ($p>0.05$). A 9g NPK treatment resulted in okra with higher pectin yield and higher intrinsic viscosity.

5.2 Recommendation

- Further studies on other okra genotypes to investigate okra pectin yield and viscosity as influence by maturity of the fruit.
- Characterization of structural, molecular and rheological behaviour properties of pectin extract at different maturity.

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