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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

POLYSACCHARIDE YIELD FROM OKRA GENOTYPES USING DIFFERENT DRYING METHODS

BY

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DECLARATION

I hereby declare that this thesis is the result of my own novel study with references to specific authors duly acknowledged and that it is neither in part nor whole been presented for another certificate in this university or elsewhere.

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DEDICATION

I dedicate this work to my family for their immense support through it all.

ACKNOWLEDGEMENT

Thanks to the Almighty God, for His grace and mercies through my entire life and the life of my family and friends so far.

In another measure my sincere appreciation goes to my supervisor, PhD. Jacob Agbenorhevi for his counseling, supervision, encouragement and numerous inputs during my progress with this work. I personally acknowledge all lecturers of the Department of Food Science and Technology who in their kind gestures and support have contributed to my entire training.

Thanks also to Mr. Nathaniel and Nana O.B Boakye, respectively of the Biochemistry and Food Science Laboratories who offered assistance during the various lab sessions in pursuit of the work. Last but not least, I want to thank all my friends and course mates for supporting me in various ways.

ABSTRACT

This study was undertaken to ascertain how the genotype of okra, method of drying affect polysaccharide yield. Three okra genotypes *Penkruma*, *Aisha*, and *Agbagboma Deep Green* were taken through oven, solar and freeze drying methods and fresh portions maintained prior to polysaccharide extraction.

Solar dried form of the *Aisha* genotype gave the highest yield of 15.50% followed by *Aisha freeze dry* 14.80%, *Penkruma solar dry* 14.60%, *Agbagboma freeze dry* 14.30%, *Aisha oven dry* 13.22%, *Penkruma freeze dry* 12.82%, *Penkruma oven dry* 10.90%, *Agbagboma oven dry* 10.50%, and *Agbagboma solar dry* 7.90%. Fresh forms of *Aisha*, *Penkruma* and *Agbagboma* genotypes are 9.90%, 9.57% and 10.15%, respectively. For all forms of drying, *Aisha* genotype gave the highest yield, followed by *Penkruma* and then *Agbagboma*.

The carbohydrate content ranged between 6.5% to 53.3% whiles the Protein content of the polysaccharides obtained from the various genotypes also ranged from 7.7% to 21.3% .In terms of protein, all samples proved to be significantly different at $p \leq 0.05$ whereas a significant difference existed at $p \leq 0.05$ amongst the samples with the exception of *Agbagoma Fresh, Agbagboma Solar, Penkruma Fresh* and *Penkruma Solar* in terms of carbohydrate composition.

The genotypes gave antioxidant activities in the range of 69.4% and 78.8% with no significant difference amongst the test samples except for *Aisha Oven* and *Agbagboma Fresh*. Total Phenolic Content ranged from 3.78 mg GAE/g to 10.36 mg GAE/g. There was a significant difference in the phenol contents of the entire samples at $p \leq 0.05$.

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CHAPTER ONE

1.0 INTRODUCTION

1.0 Background

The place of vegetables in the diet of people in our world today per growing awareness carried out has caused the popularity of many vegetable crops. Majority of these vegetables have crossed continents and to other places other than locations of their indigenous origin. Okra (*Abelmoschus esculentus*), is no exception to this. Like many other vegetables, its continual production has been recognized as a means to access important nutrients. According to Ngbede *et al.,* (2014) amino acids present in okra can be likened to Soybean and per its nutrient variation, okra has often been referred to as "a perfect villager's vegetable" (Holser and Bost, 2004; Sanjeet *et al.,* 2010).

Genotypes of Okra vary on the basis of plant height, number of branches, days to 50% flowering with days to first harvest, pod yield, number of pods per plant, pod weight and amongst others (Sharma and Prasad, 2010). In Ghana some common cultivars include *Torkor, Asontem, Saloni* (F_1) and *Indiana*. According to Oppong-Sekyere *et al*., (2012) okra (*Abelmoschus esculentus* L.), a member of the *Malvaceae* family can be found in nearly every market in Africa. The world okra production, as of 2007, was estimated at 4.8 million tons with India leading the production by 70% followed by Nigeria (15%), Pakistan (2%), Ghana (2%), Egypt (1.7%) and Iraq (1.7%) (Gulsen *et al*., 2007).

The form in which Okra is consumed sometimes gives room for processing. Amongst the processing techniques applied, drying is the most common one (Chan *et al*., 2009) Drying may be achieved by solar, Oven (hot air) and more recently freeze-drying techniques.

Various dried portions of okra come with numerous benefits. Beyond post harvest management, its application in various food systems cannot be ruled out. According to Farinde *et al.,* (2001) okra seeds when dry can be used to prepare vegetable curds, or roasted and ground to be used as coffee additive or substitute.

Aside the readily edible portion of okra crop is pectin, a multifunctional constituent of the cell walls. In plant cells, pectin consists of a complex set of polysaccharides that are present in most primary cell walls and particularly in non-woody parts of nearly all terrestrial plants. It is a high value functional food ingredient widely used as a gelling agent and as a stabilizer (Srivastava and Malviya, 2011). In the pharmaceutical circles, pectin is being used in the formulation of various dosage forms wherein it acts as a natural polymer for drug delivery, more or less a binding agent (Biswal *et al.,* 2014). In our world today, polysaccharides from okra are potentially a new source of natural polysaccharides, which can be used as substitutes in different food systems to serve as functional ingredients (thickeners and texture modifiers), by the food industry (Georgiadis *et al.,* 2011).

1.1 Statement of Problem

Recent studies such as "Emulsifying Properties of Extracted Okra Mucilage", "Polysaccharide-Free Nucleic Acids And Protein of Okra", "Antibacterial Properties Of Extract of Okra" and " Functional Properties of Okra Mucilage" have mainly focused on characterization of okra polysaccharides obtained with sequential extractions, starting with acidic hot buffers followed by chelating agents and dilute alkali buffers as well as investigating other conditions during the extraction (pH) process. However, the effect of drying techniques employed prior to pectin extraction has not been extensively studied as well as how a particular okra genotype can affect pectin yield. There is therefore dearth

of information on how different drying methods and the genotype of okra influence pectin yield of the crop.

1.2 Justification

This research would identify the appropriate drying method and okra genotype whose combined effect will be a resulting high pectin yield. This will further make available data for potential commercialization of pectin from okra source through processing of okra polysaccharides (mucilage). Increased commercial activities based on okra-pectin will go a long way to enhance local production thereby supporting agro-economic activities while reducing okra losses.

1.3 Objectives

- To determine the effects of drying methods and okra genotype on pectin yield

Specific Objectives

- To determine the effects of freeze drying, oven drying and solar drying on pectin yield of okra from different genomes
- To determine the antioxidant property of pectin yield of different okra genotype.

CHAPT ER TWO

2.0 LITERATURE REVIEW

2.1 Nutritional Composition of Okra

The Okra crop is of significant nutritional value. It contains a high percentage of water, averaging 85%, total fat of 0.5%, protein content of 4% and 5.4% carbohydrate. The carbohydrate is present as cellulose, starch in small quantity and sugar. It also contains non-cellulose, non-starch, polysaccharides (Fellows, 2000). Proteins play a particularly important role in human nutrition.

According to Kouassi *et al*., (2013), in the region of Yamoussoukro, two varieties of okra are the most cultivated. These are *Dioula* and *Baoule* varieties. Both varieties are rich in (Iron, Calcium, Copper and Zinc.) Beyond that they also contain significant levels of(magnesium, potassium, sodium and manganese) (Kouassi *et al*., 2013). Okra is used to promote a healthy life in pregnancy. An incredibly essential B vitamin for creating and maintaining new cells, foliate is a vital substance for optimum pregnancy. The vitamin aids in preventing birth defects just like spina bifida and enables the baby to develop completely. Vitamin C is additionally required for baby development. Okra is full of both foliate and vitamin C. The high quantity of foliate included in the okra is helpful for the fetus while pregnant. Foliate is a vital nutrient that increases the growth and development of the fetus' brain. The high quantity of folic acid within okra performs a huge role within the neural tube formation of the fetus through the fourth to the 12^{th} week of pregnancy (Zaharuddin *et al*., 2014).

It has many medicinal applications such as stabilising the blood sugar, binding excess cholesterol, replenishing sodium in the body and as anti-oxidants among others (Adeboye and Oputa, 1996; Junji, 2004). Studies have shown that high fiber present in

okra aids in the reduction of serum cholesterol thereby decreasing the risk of cardio related issues (Ngoc *et al*., 2008).

2.2 Okra Oil and Nutritional Composition

Okra seed oil is rich (60 to 70%) in unsaturated fatty acids (Savello *et al*., 1980; Rao, 1985). The proteins present in the seed have also been found to be rich in tryptophan (94 mg/g N) and also some amounts of sulfur-containing amino acid (189 mg/g N) (NAP, 2006). Okra seed protein with good protein efficiency ratio (PER) and net protein utilization (NPU) values is comparable to many cereals (except wheat) and its oil yield is comparable to most oil seed crops except oil palm and soybean (Rao, 1985). Moreover, okra seed oil has potential hypocholesterolemic effect (Rao *et al*., 1991).

2.3 Diversified Uses of Okra

Okra pods are rich in phenolic materials mostly of oligomeric catechins and flavonol derivatives, while the polyphenol profile of the epidermis is composed principally by hydroxycinnamic and quercetin derivatives (Arapitsas, 2008). The thick and slimy texture of okra water-extracts is attributed to its polysaccharide content and is of primary technological interest for various food applications (Whistler and BeMiller, 1993).

The dried seed is a nutritious material that can be used to prepare vegetable curds, or roasted and ground to be used as coffee additive or substitute (Farinde *et al.*, 2001). Tender green fruits are cooked in curry and soup, while the crop has adapted in some countries as leafy vegetable. Okra leaves may be cooked as the green.The leaves are also eaten raw in salads. Okra seeds may be roasted and ground to form a non-caffeinated substitute for coffee. In the U.S., Mexico and Japan, the young fruiting pods are the edible portion, while young leaves and mature seeds may be consumed in other countries (Duzyaman, and Vural, 2002). The use of okra is not only restricted to the area of food.

In the pharmaceutical industry, okra mucilage has been explored as a potential binder for the preparation of tablet formulation (Biswal *et al*., 2014). In the case of paper industry, the mature fruit, stem and roots are used for making ropes, fiber and are used as raw materials (Jideani and Adetula, 1993)

2.4 Okra, Pectin and Pectin Composition

Pectin, a complex mixture of polysaccharides occurring in the primary cell walls of terrestrial plants, is an important functional ingredient in many foods. It consists of a linear backbone of - (1-4)-D-galacturonic acid residues partially esterified with methanol, with periodic interruptions to L-rhamnose residues that make the backbone irregular and with some other neutral sugars present as side chains. The general makeup of the pectin content varies with the ripening of the fruit (Wilkins *et al.*, 2005).

Pectin is produced commercially in the form of white to light brown powder, mainly extracted from citrus fruits and is used in food as a gelling agent particularly in jams and jellies. It is also used in fillings, sweets, as a stabilizer in fruit juices and milk drinks and as a source of dietary fiber (Tobias *et al*., 2011). Okra contains large quantities of glycans, which are responsible for the viscosity of the aqueous suspension (Owoeye *et al.,* 1990). Industrially, okra mucilage is usually used to glace certain papers and also useful in confectionery among other uses (Markose and Peter 1990).

Several studies have reported novel pectin usages, like biodegradable water-soluble films, bulking agents, coating agents, chelators, emulsifiers and viscosity modifiers (Kanmani *et al*., 2014). Although pectin has long been associated with citrus fruits, it can be found in different varieties of fruits and vegetables. Thus, not only does pectin exist as a structural form of just citrus fruits but equally non-citrus fruits and vegetables in nature. According to Krishnamurthi and Giri (2003), the amount, structure and chemical

composition of the pectin differs between plants, within a plant over time and in different parts of a single plant.

Though Pectin occurs commonly in most of the plant tissues, the number of sources that may be used for commercial manufacture of pectin is limited. This is because the ability of pectin to form a gel depends on molecular size and the degree of esterification (DE) (Kanmani *et al*., 2014). Many investigators have studied the influence of different parameters on the pectin extraction from different sources.

2.5 Varieties of Okra

Abelmoschus esculentus (L.) commonly known as okra is believed to be native to tropical Africa and belongs to the Malvaceae family. It is classified among the semi salt tolerant vegetable crops (Mass and Hoffman 1977).

It is the only vegetable crop of economic importance in the Malvaceae family and cultivated throughout the tropics and subtropics (Kochhar 1986; Hammon and van Sloten 1989).

In Ghana it is the fourth most popular vegetable after tomatoes, capsicum (peppers) and garden eggs. It is mainly produced for local consumption with a few farmers now producing for the export market in all the ecological zones (Tweneboah, 1998). According to Sharma and Prassad (2010), the genetic divergence in Okra have widely been based on differences in the number of pods per plant, pod length, pod diameter, days to flowering and plant height. Most okra cultivars produce green pods, but a few varieties are yellow or dark in colour (Kumar, 2006).

In Ghana okra accessions that have been chiefly cultivated are *Asontem, Fetri , Dikaba, Aisha*, and *Nkrumahene* (Oppong-Sekyere, 2012).

2.6 Drying as a processing method

Drying is a heat and mass transfer process resulting in the removal of water moisture, by evaporation from a solid, semi-solid or liquid to end in a solid state. The drying technique is probably the oldest and the most important method of food preservation practiced by humans (Mujumdar, 1995).

Most of the time the main goal in drying farm produce is to reduce the moisture content to a level which allows safe storage for a period.

Under different processes, it simply becomes a pre-requisite to obtaining the desired product from an agricultural produce such as in the case of pectin extraction. During drying many changes take place; structural and physico-chemical modifications affect the final product quality, and the quality aspects involved in dry conversion in relation to the quality of fresh products and applied drying techniques (Wankhade *et al*., 2012). Thus different drying techniques are likely to yield dried products whose characteristics can be completely different from each other

All over Ivory Coast, okra represents 24% of the vegetables consumed fresh and 41% of vegetables consumed dried (Siemonsma, 1982). Indeed, to preserve the large-scale production (FAOSTAT, 2008), the producer and or consumer conducts its drying sliced then left untouched or powdered.

The drying can be done in different forms. This include solar drying, freeze-drying, and oven drying techniques. Freeze drying is the process of dehydrating frozen foods under a vacuum so that the moisture content changes directly from solid to a gaseous form without having to undergo the intermediate liquid state through sublimation and desorption. The process is used for drying and preserves the food product in a way that the dried product remains the same size and shape as the original frozen material and will

be found to have excellent stability and convenient reconstitution when placed in water (Alexandraki *et al*., 2013).

Most works including recent works (Eze and Akubor, 2012; Doymaz , 2011) conducted have centered on the relationship between drying techniques and nutrients, organoleptic properties as well as the optimization of the drying technique on okra pods but not on the pectin yield.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Sample Preparation

Okra samples used in the work were obtained from the *Techiman* and *Mpatatia* Markets of the Brong Ahafo and Ashanti Regions (Ghana), respectively. The vegetable were first thoroughly washed then the pods split opened along their horizontal axis with sharp sterilized knives. Seeds were carefully removed and sliced into 5 mm thickness. The head and extreme tail portions of the okra were cut off. The varieties involved in the preparation were Aisha, *Agbagboma* green and *Penkruma*. Each okra variety obtained was divided into four portions. A portion kept fresh, and three other portions to be dried by solar, oven, and freeze-dry techniques. The fresh portions were grated and milled using the *Preethi* blender then separately bagged.

3.2. Moisture Content Determination of Okra (AOAC, 2005)

Pre- dried moisture cans of known weight were labeled. A mass of 5 g of sample was accurately weighed into moisture cans. These were oven dried in a hot air oven (FS Tupola Plant- Wageningen) for 6 hours to attain a constant weight. Moisture cans with samples were then cooled in desiccators before weighing. Moisture content was calculated as;

- $\%\, Moisture = \frac{(Initial\ weight\ of\ can + Sample) - (Final\ weight\ of\ can + Sample)}{weight\ of\ sample\ taken} \times 100$

3.3.Drying of Okra Pods

The samples for each variety to be dried were solar dried, oven dried and freeze dried separately. *Aisha, Agbagboma* green and *Penkruma* samples used for solar drying were dried for a period of 8 days. For the Oven drying batch samples, drying was carried out

at 60 °C in a hot air oven (FS Tupola Plant- Wageningen) for 36 hours. Freeze drying was carried on the okra sample s in three phases but as a continuous processing method. Okra pods were first pre-frozen at -16 °C for 24 hours followed by secondary freezing at -46 °C to -58 °C for 24 hours and lastly drying at 24 to 27 °C for 12 hours. Samples were milled and kept in zip-lock bags prior to polysaccharide isolation.

3.4 Isolating Polysaccharide from Okra Pods

Well milled samples of each of the three varieties (*Aisha, Agbagboma green* and *Penkruma*) resulting from the oven, solar and freeze-drying techniques (100 g) was defatted with petroleum ether (1500 mL) using a shaker bath for 4 hours. This was followed by an initial aqueous extraction using 0.1 M phosphate buffer of volume 3000 mL with a pH of 6 heated to 80 °C for one hour. The resulting mixture was then centrifuged at 2500 rpm for 15 minutes using the MSE MISTRAL 300E (SG95/10/256 - UK Made) centrifuge to obtain the first supernatant with pellets as residue. The pellets obtained were taken through the aqueous extraction such that for every gram, 15 mL of the aqueous phosphate buffer solution was added. The supernatants were combined and solvent evaporated followed by ethanol extraction of the polysaccharide and finally washed with isopropanol. A graphical representation of the entire extraction process is seen in Figure 1.1.

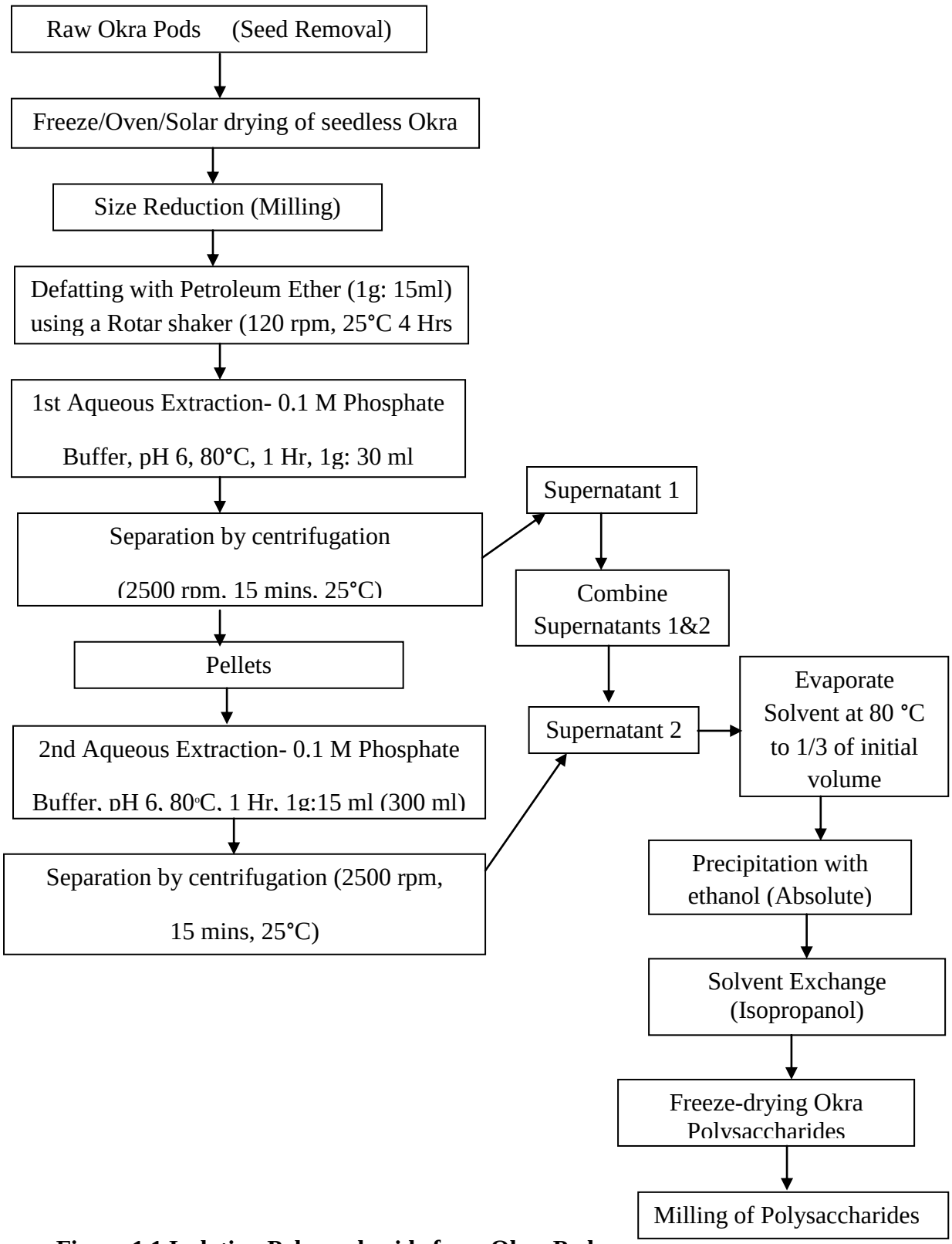

Figure 1.1 Isolating Polysaccharide from Okra Pods

3.5 Chemical Characterization

3.5.1 Total carbohydrates

Phenol- Sulphuric Acid Method (Gardner et al., 2000; Bailey, 2007)

Carbohydrates in the presence of strong acids and high temperatures undergo a series of reaction which lead to the formation of various furan derivatives (Brummer and Cui 2005). The furan derivatives condense with themselves and various phenol compounds such as phenol, resorcinol, orcinol, α-naphtol and napthoresorcinol to form coloured complexes (Nielsen 2003). These complexes are useful for carbohydrate determination since they absorb UV-VI light and the absorbance is proportional to the concentration of sugar in the sample. All classes of sugars including polysaccharides can be determined by this method. An absorbance at 490nm is observed for hexoses and 480nm for pentoses and uronic acids as using a UV-VI spectrophotometer.

Phenol, in a 5% solution is added to a glass test tube containing a clear sample solution. Concentrated Sulphuric acid is added directly to the surface of the liquid in the test tube. The mixture is thoroughly combined using a vortex mixer and then permitted to stand for a time of 30 minutes to allow for colour development. The solution absorbance was read at 490nm using nanodrop *ND 1000* spectrophotometer. A standard curve was prepared using glucose solution in a serial manner of concentrations 0.00, 20, 30, 40, and 50 mg/ ml and absorbance read at same wavelength after colour development (Refer to Appendix for standard curve).

3.5.2 Protein content determination

Lowry Protein Assay

Protein quantification using the Lowry method combines the Biuret reaction in which peptide bonds of proteins react with copper under alkaline conditions to produce Cu^{+}, and the Folin-Ciocalteau reaction in which the Folin-Ciocalteau phenol reagent (phosphomolybdic-phosphotungstic acid) is reduced to heteropolymolybdenum blue by copper catalysed oxidation of aromatic amino acids (Nielsen, 2003). The reactions result in a strong blue colour, which depends partly on the tyrosine and tryptophan content. It is this blue protein form that is detected in the assay using a spectrophotometer or microplate reader. Absorbance reading of the samples was done using nanodrop *ND 1000* spectrophotometer

3.5.3 Antioxidant Activity (Free Radical Scavenging Activity)

Determination of the Free Radical Scavenging Activity was carried out by the 1, 1-Diphenyl-2-picrylhydrazyl Free-Radical Scavenging Assay. Scavenging activities of the Okra extracts on the stable free radical DPPH were assayed using the modified Blois' method in which the bleaching rate of DPPH is monitored at a characteristic wavelength in presence of the sample. A volume of 0.1 mL of an aqueous dilution of the extracts was mixed with 0.5 mL of a 500 µM DPPH solution in absolute ethanol and 0.4 mL of a 0.1 M Tris-ClH buffer at pH 7.4. The mixture was kept for 20 min in darkness, and then the absorbance was read at 517 nm using Nanodrop (*ND 1000*) spectrophotometer in UV-Vis mode. The percentage of decrease of DPPH bleaching was calculated by measuring the absorbance of the sample and applying the following equation:

% of Inhibition = $[1 - (A_s/A_0)] \times 100$,

Where A_s is the absorbance of sample (i.e., extracts), and A_0 is the absorbance of the DPPH solution. Trolox solutions of different concentrations were used as standards for antioxidant activity (Refer to Appendix for standard curve).

Preparation of Trolox Solution

A 1.5 mM Trolox stock solution was prepared. Six serial dilutions of trolox solutions were prepared and absorbance measured.

Tube	1.5 mM Trolox Stock Solution (µL)	PBS (µL)	Final Concentration (µM)
1	333	667	1000
2	266	734	800
3	200	800	600
4	167	833	500
5	100	900	300
6	33	730	100

3.5.4 Total Phenol Content

Preparation of Solutions

Sodium Carbonate (20% (w/v) Na_2CO_3) Solution was prepared by dissolving 40g anhydrous sodium in 160 mL of distilled water to dissolve then topped up to 200 mL final volume.

Gallic acid stock solution: A mass of 1000 mg dry gallic acid was dissolved in 20 mL ethanol and further diluted to give a total volume of 1000 mL.

Standard Calibration Curve for Phenol Analysis

Volumes 0, 1, 2, 3, 5 and 10 ml of prepared gallic acid stock solution was placed into separate 100 mL volumetric flasks and diluted to volume with distilled water to give a standard gallic acid solution of 0, 50, 100, 150, 250 and 500 mg/L, respectively. A volume of 0.1 mL standard gallic acid was pipetted into a volumetric flask and 6.0 mL

distilled water added. 0.5 mL Folin Ciocalteu reagent (2N) was added, shaken and left for 5 minutes. Thereafter 1.5 mL of 20% sodium carbonate solution was added. The solution was topped with distilled water to the 10 mL mark. The resulting solution was incubated at 25° C for 2 hours (Refer to Appendix for standard curve).

Folin-Ciocalteu Method for Phenol Analysis

The Folin-Ciocalteu(FC) method is dependent on measurement of colour change from yellow to blue as a result of reduction of the tungstate-molebdate mixture in the FC reagent by phenols present in the solution being analyzed.

The absorbance readings for the incubated solutions were read at 750 nm using Nanodrop (*ND 1000*) spectrophotometer in UV-Vis mode. Absorbance readings were taken for each of the duplicate determinations for each sample. The results were expressed as concentration of gallic acid equivalent (GAE, mg/L) using equation of line of best fit obtained from the standard calibration curve. The final concentration was calculated as;

a. $C = \frac{c \times m}{v}$

Where

C = total content of phenolics of sample in gallic acid equivalent (GAE)/g

c = concentration of gallic acid established from the calibration curve

V= volume of extract (ml)

m = weight of raw sample used (g)

(Singleton and Rossi, 1965; Gardner *et al*., 2000; Bailey, 2007)

3.60 Experimental design and Statistical Analysis

Statistical analyses were carried out on a completely randomized design on duplicate per sample.

Data were subjected to analysis of variance and Duncan's multiple range tests was used for comparison of means and the significance level at $\rho \leq 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Varying Polysaccharide Yield of Different Okra Genotypes

The extraction protocols resulted in the isolation of polysaccharide of high yield (g pectin/100 g dry okra pods). This result is shown in Fig 2.0. The solar form of the processed *Aisha* okra gave the highest yield amongst the samples. Out of the drying techniques applied, the freeze-dried forms of the okra genotypes generally gave higher yields. This was followed by solar drying then lastly oven drying. It is observed that it is not only the drying technique that matters prior to the extraction but also the genotype. Considering the solar technique, the *Aisha* genotype gave the highest yield of 15.5 % followed by *Penkruma* 14.6 % and *Agbagboma* the least value of 7.9 %. Same is observed for both freeze drying and oven methods. *Aisha* freeze dried sample gave the highest yield whereas *Penkruma* and *Agbagboma* samples followed in decreasing order. Again same is seen of the oven drying method. *Aisha* produced the highest yield of 13.2 %, while *Penkruma* had a yield of 10.9 % and *Agbagboma* gave the least of 10.5 %. The *Aisha* genotype responded best to each one of the drying methods. This makes it a genotype of choice when considering the economic pectin value of the genotypes. In comparison, *Agbagboma*, did not perform well against the drying techniques employed. All these point out the influence of the genotype on the yield. Comparing the output of the fresh forms of the okra samples against the dried forms, it can be seen that drying enhanced the polysaccharide purification process as fresh dried samples only yielded a maximum of 10.15 %. The yields for the fresh samples are 9.9 %, 9.57 %, and 10.15 % respectively for *Aisha, Penkruma* and *Agbagboma* respectively. The data provided also show that both Aisha and *Penkruma* genotypes respond best to solar drying while

Agbagboma deep green responds best to the freeze drying method in terms of Pectin yield. Alba *et al*., (2014) achieved a polysaccharide yield of 13.3 % and 15.2 % at pH(s) of 2.0 and 6.0 when oven drying technique was applied to samples prior to polysaccharide extraction. But for *Agbagboma* oven sample, oven samples of the other two genotypes were close to and or higher than the values obtained by Alba *et al*., (2014). Solar samples of both Aisha and *Penkruma* samples were by far higher. *Aisha* solar, *Aisha* Freeze dry *Penkruma* solar and *Penkruma* freeze dry samples exhibited very high yields. These are 15.5 %, 14.8 % 14.6 % and 12.82 % respectively. These percentage yield values are comparable to the yields reported by Nelson *et al.*,(1976) for commercially used raw materials of apple pomace (15-20%) indicating how okra could be a very good source of commercial polysaccharide.

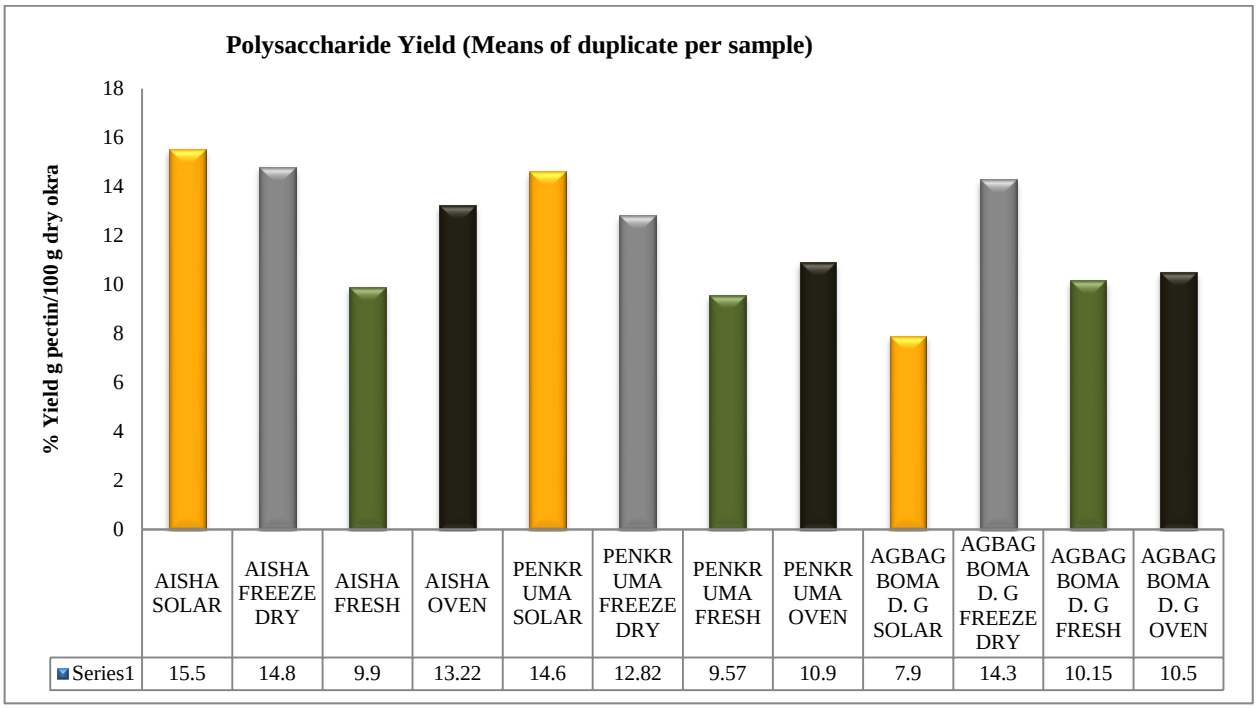

Fig 2.0: Polysaccharide Yield of Oven, Freeze and Solar Dried Forms of Different Okra Genotypes

4.1.1 Carbohydrate - Protein Content and Polysaccharide Purity

According to Alba *et al*., (2014) the carbohydrate protein balance in extracted polysaccharide is a key determining factor of the purity of the polysaccharide extract. Where the extraction process results in relatively high carbohydrate content and low protein concentrations, it is considered much purer than vice versa. In this work, the extraction protocols resulted in the isolation of pectin of average purity as evidenced by their generally low total carbohydrate content when compared to that of the proteins. From Table 1.0, *Aisha Solar* can be considered relatively purer than the others. *Aisha Solar* gave a carbohydrate content of 53.335/mg/mL and a protein content of 11.8050/mg/mL. Samples that closely followed were Aisha Oven and *Agbagboma* Deep Green Oven; 40.3550/mg/mL and 31.9250/mg/mL respectively for total carbohydrate while their protein contents measured 8.61/mg/mL and 12.285/mg/mL accordingly. In drying the type of drying technique employed as well as its associated conditions determine how some physicochemical and nutritional factors are affected (Eze and Akubor 2012). Though same conditions were meted out to all samples, the differences in their genotypic make-up resulted in the various variations observed (Table 1.0). There was a significant difference at $\rho \leq 0.05$ amongst the samples with the exception of *Agbagoma Fresh, Agbagboma Solar, Penkruma Fresh* and *Penkruma Solar*. In terms of protein, all samples proved to be significantly different at $\rho \leq 0.05$. Also, each drying technique is unique and has its own advantages and limitations (Wankhade *et al*., 2012). It is evident that the solar worked best to preserve protein and carbohydrate composition for the *Aisha* genotype followed by freeze dry technique while the oven worked best for all genotypes. Thus, where cost is a factor during commercialization, the solar and oven dried forms could potentially be considered.

Table 1.0: Total Carbohydrate and Protein Content of Different Okra Genotypes (Means of duplicate per sample)

Sample	Carbohydrate Content (%)	Protein Content (%)
AISHA SOLAR	53.3^{a}	11.8^{f}
AISHA OVEN	40.4^{b}	8.6^{h}
AGBAGBOMA D.G OVEN	31.9^{c}	12.3^{ef}
AISHA FREEZE DRY	21.8^{d}	12.7^{de}
PENKRUMA OVEN	18.1^{e}	14.5^{b}
AISHA FRESH	12.5^{f}	7.7^{h}
AGBAGBOMA FREEZE DRY	10.6^{g}	11.7^{f}
AGBAGBOMA FRESH	10.4^{gh}	13.1^{cd}
AGBAGBOMA SOLAR	10.2^{gh}	10.8^{g}
PENKRUMA FRESH	9.9^{h}	13.6^{c}
PENKRUMA SOLAR	9.7^{h}	21.3^{a}
PENKRUMA FREEZE DRY	6.5^{j}	12.5^{de}

NB: Means in the same column not followed by the same superscript (s) are significantly different from each other by Duncan's multiple range tests at the $p \leq 0.05$.

4.2 Antioxidant Activity of Extracted Okra Polysaccharide from Different Genotypes

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide, are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen. Although ROS at physiological concentrations may be required for normal cell function, excessive production of ROS can consequently induce different kinds of serious human diseases including atherosclerosis, rheumatoid arthritis,

muscular dystrophy, cataracts, diabetes, cancer, and some neurological disorders (Sharififar *et al.,* 2007; Kovatcheva *et al.,* 2001). It has been found that phenol compounds could serve as antioxidants against various diseases induced by ROS. In this study, high DPPH radical scavenging activity was observed by both the extract and Trolox in a concentration-dependent manner (Table 2.0). The inhibition or scavenging activity is seen to increase with increasing concentrations in both the standard (Prepared Trolox Concentrations) as well as the samples. Higher percentage Inhibition activities corresponded with high concentrations as estimated by Trolox Standard Curve (Appendix B). Polysaccharide from the different Okra genotypes exhibited very good radical scavenging activities. *Agbagboma Solar* gave the highest antioxidant activity of 78.80 % with an equivalent concentration of 0.539μM. The least activity was 69.38 % with an equivalent concentration of 0.474 μM by *Penkruma Freeze Dry*. Geng *et al*., (2014) in a similar work stated that, superior antioxidant activity can be observed where concentrations values are closer to 0.8 than when values go below 0.4. All concentration values fell within the 0.4 to 0.8 range. Again comparing the samples to that of Trolox, a concentration of 0.675 gave a scavenging activity of 82.35 %. Although high, all samples tested which were of concentrations from 0.474 to 0.539 exhibited activities that can be said to be high on the average. There were no significant difference amongst the test samples at $\rho \leq 0.05$ except for *Aisha Oven* and *Agbagboma Fresh*.

Table 2.0: Antioxidant Activity of Polysaccharide from Different Okra Genotypes (Means of duplicate per sample)

Sample	Antioxidant Activity (% Scavenging)	Concentration (µM)
AISHA SOLAR	78.7[a]	0.54
AISHA OVEN	73.4[bc]	0.50
AGBAGBOMA D.G OVEN	78.7[a]	0.54
AISHA FREEZE DRY	75.4[c]	0.52
PENKRUMA OVEN	78.0[a]	0.53
AISHA FRESH	78.0[a]	0.53
AGBAGOMA FREEZE DRY	75.4[c]	0.52
AGBAGOMA FRESH	76.6[b]	0.52
AGBAGOMA SOLAR	78.8[a]	0.54
PENKRUMA FRESH	77.4[a]	0.53
PENKRUMA SOLAR	78.7[a]	0.54
PENKRUMA FREEZE DRY	69.4[d]	0.47

NB: Means in the same column not followed by the same superscript (s) are significantly different from each other by Duncan's multiple range tests at the $p \leq 0.05$.

4.3: Total Phenol Content of Extracted Okra Polysaccharide from Different Genotypes

Many reported works have indicated that antioxidant activities of various food systems have had to be involved one way or the other with their phenol content (Cai *et al*., 2004; Apea-Bah *et al*., 2009). The Total Phenol Content of the ten okra genotypes is shown in Table 3.0. Work conducted by Khomsug *et al*., (2010) revealed that, the phenol content of *Abelmoschus esculentus* (pulp) was 10.75 mg GAE/g. In this related work, Fresh and Solar Samples of *Agbagboma* genotype gave 10.3600 and 10.2000 mg GAE/g respectively values very close to that. Also fresh and solar samples of the *Penkruma*

genotype in a similar pattern showed high phenols. Again in the case of *Aisha*, the solar dried sample scored the highest phenol content.

This indicates how well the solar method was able to preserve much of the phenols as against freeze drying and oven method. There was a significant difference in the phenol contents of the entire samples at $p \leq 0.05$.

Table 3.0:

Total Phenol Content of Different Okra Genotypes of Different Drying Methods

Sample	Total Phenol Content (mg GAE/g)
AISHA SOLAR	6.5^{a}
AISHA OVEN	4.7^{fg}
AGBAGBOMA D.G OVEN	6.3^{ab}
AISHA FREEZE DRY	3.8^{h}
PENKRUMA OVEN	5.2^{de}
AISHA FRESH	5.5^{cd}
AGBAGBOMA FREEZE DRY	4.4^{g}
AGBAGBOMA FRESH	10.4^{gh}
AGBAGBOMA SOLAR	10.2^{gh}
PENKRUMA FRESH	9.9^{h}
PENKRUMA SOLAR	9.7^{h}
PENKRUMA FREEZE DRY	6.5^{j}

NB: Means in the same column not followed by the same superscript (s) are significantly different from each other by Duncan's multiple range tests at the $p \leq 0.05$.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Aisha genotype of okra samples responded best to all the drying techniques employed and that makes it a genotype of choice in considering the economic pectin value of okra genotypes. In terms of drying method used prior to polysaccharides extraction, solar is best combined with both Aisha and Penkruma to give high polysaccharide yields while Agbagoma deep green responds best to freeze drying.

Low values of polysaccharide yields of fresh samples indicate that the right drying technique when adopted, improves the polysaccharide yield. The fact that the polysaccharide extracts are in range as proposed by Nelson *et al*.,(1976) indicates how useful okra could as a very good source of commercial polysaccharide. The polysaccharide yield of okra is dependent not just on the associated drying technique and conditions of pretreatment prior to extraction but also on the particular okra genotype. The very high phenol content and equivalent high antioxidant activities of derived polysaccharides indicate how useful they can be in various food systems in the fight against radicals which mostly occur through rancidity.

5.2 Recommendation

Investigations of the rheological properties of the extracted polysaccharides should be investigated to uncover its full potential in terms of utilization.

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APPENDICES

A. **Homogeneous Subsets**

Carbohydrate

	SAMPLE	N	Subset for alpha = 0.05								
			1	2	3	4	5	6	7	8	9
Duncan[a]	PENKRUMA FREEZE DRY	2	6.5								
	PENKRUMA SOLAR	2		9.7							
	PENKRUMA FRESH	2		9.9							
	AGBAGOMA DEEP GREEN SOLAR	2		10.2	10.2						
	AGBAGOMA DEEP GREEN FRESH	2		10.4	10.4						
	AGBAGOMA DEEP GREEN FREEZE DRY	2			10.6						
	AISHA FRESH	2				12.5					
	PENKRUMA OVEN	2					18.1				
	AISHA FREEZE DRY	2						21.8			
	AGBAGOMA DEEP GREEN OVEN	2							31.9		
	AISHA OVEN	2								40.4	
	AISHA SOLAR	2									53.3
	Sig.		1.000	.056	.238	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Protein

	SAMPLE	N	Subset for alpha = 0.05								
			1	2	3	4	5	6	7	8	9
Duncan[a]	AISHA FRESH	2	7.7								
	AISHA OVEN	2		8.6							
	AGBAGOMA DEEP GREEN SOLAR	2			10.8						
	AGBAGOMA DEEP GREEN FREEZE DRY	2				11.7					
	AISHA SOLAR	2				11.8					
	AGBAGOMA DEEP GREEN OVEN	2				12.3	12.3				
	PENKRUMA FREEZE DRY	2					12.4	12.5			
	AISHA FREEZE DRY	2					12.7	12.7			
	AGBAGOMA DEEP GREEN FRESH	2						13.1	13.1		
	PENKRUMA FRESH	2							13.6		
	PENKRUMA OVEN	2								14.5	
	PENKRUMA SOLAR	2									21.3
	Sig.		1.000	1.000	1.000	.077	.192	.051	.088	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Total Phenol

	SAMPLE	N	Subset for alpha = 0.05							
			1	2	3	4	5	6	7	8
Duncan[a]	AISHA FREEZE DRY	2	3.8							
	PENKRUMA FRESH	2	3.8							
	AGBAGOMA DEEP GREEN FREEZE DRY	2		4.4						
	AISHA OVEN	2		4.7	4.7					
	PENKRUMA FREEZE DRY	2		4.7	4.7	4.7				
	AGBAGOMA DEEP GREEN FRESH	2			5.0	5.0				
	PENKRUMA OVEN	2				5.1	5.2			
	AISHA FRESH	2					5.5	5.5		
	PENKRUMA SOLAR	2						5.8		
	AGBAGOMA DEEP GREEN SOLAR	2						6.0	6.0	
	AGBAGOMA DEEP GREEN OVEN	2							6.3	6.3
	AISHA SOLAR	2								6.5
	Sig.		1.000	.096	.154	.053	.136	.053	.079	.529

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

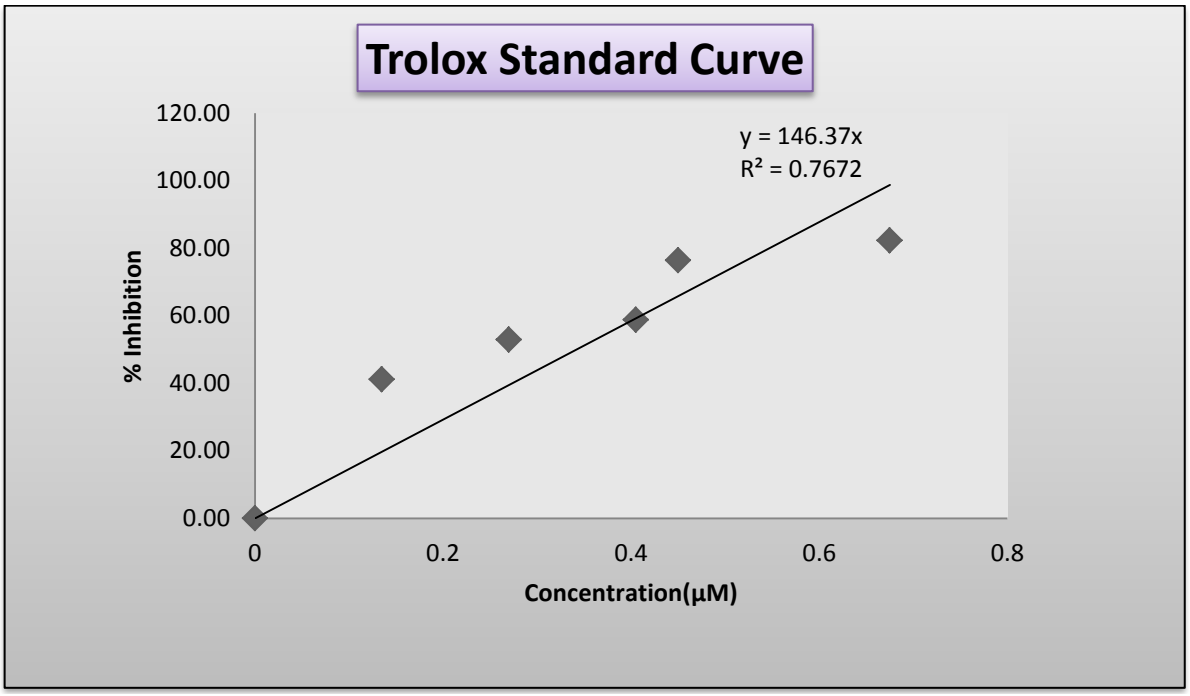
Antioxidant

	SAMPLE	N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	PENKRUMA FREEZE DRY	2	69.4			
	AISHA FREEZE DRY	2		75.4		
	AGBAGOMA DEEP GREEN FREEZE DRY	2		75.4		
	AISHA OVEN	2		73.4	76.6	
	AGBAGOMA DEEP GREEN FRESH	2			78.7	
	PENKRUMA SOLAR	2				77.4
	PENKRUMA FRESH	2				78.6
	AGBAGOMA DEEP GREEN OVEN	2				78.7
	AISHA SOLAR	2				78.0
	AISHA FRESH	2				78.0
	PENKRUMA OVEN	2				78.0
	AGBAGOMA DEEP GREEN SOLAR	2				78.8
	Sig.		1.000	.246	.114	.274

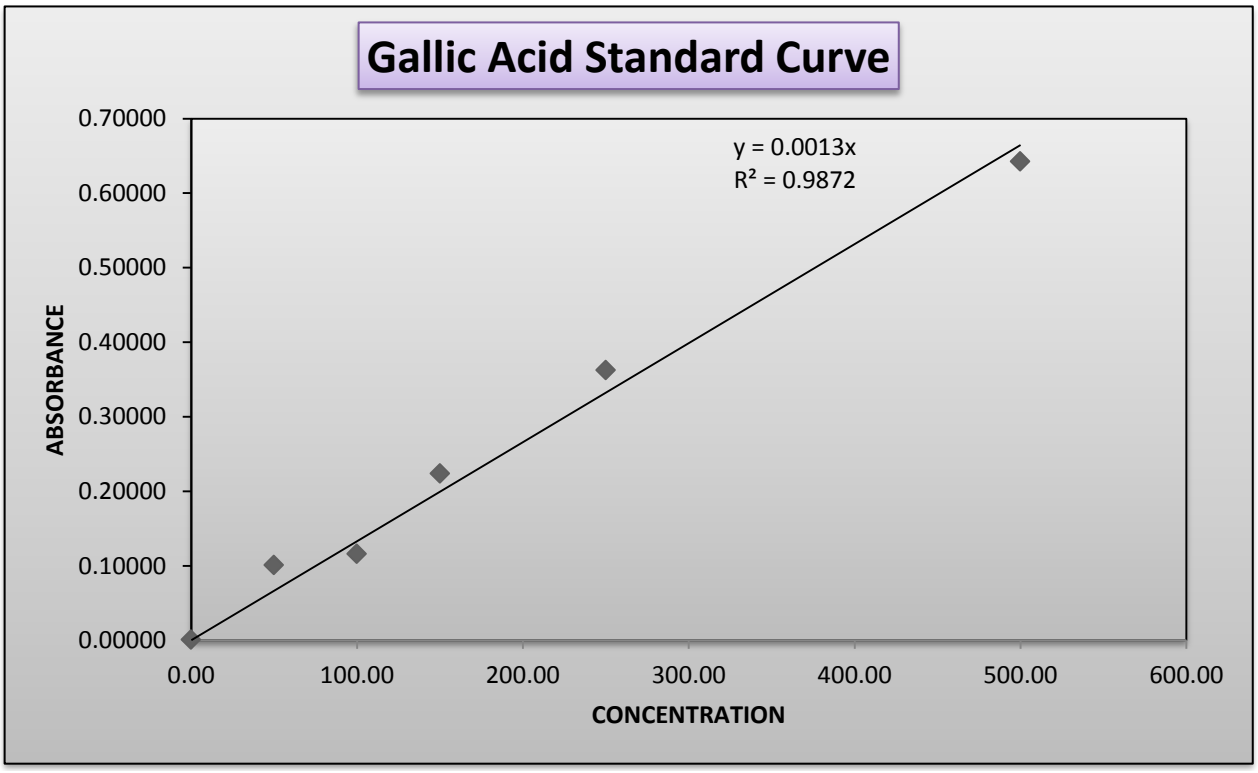
Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

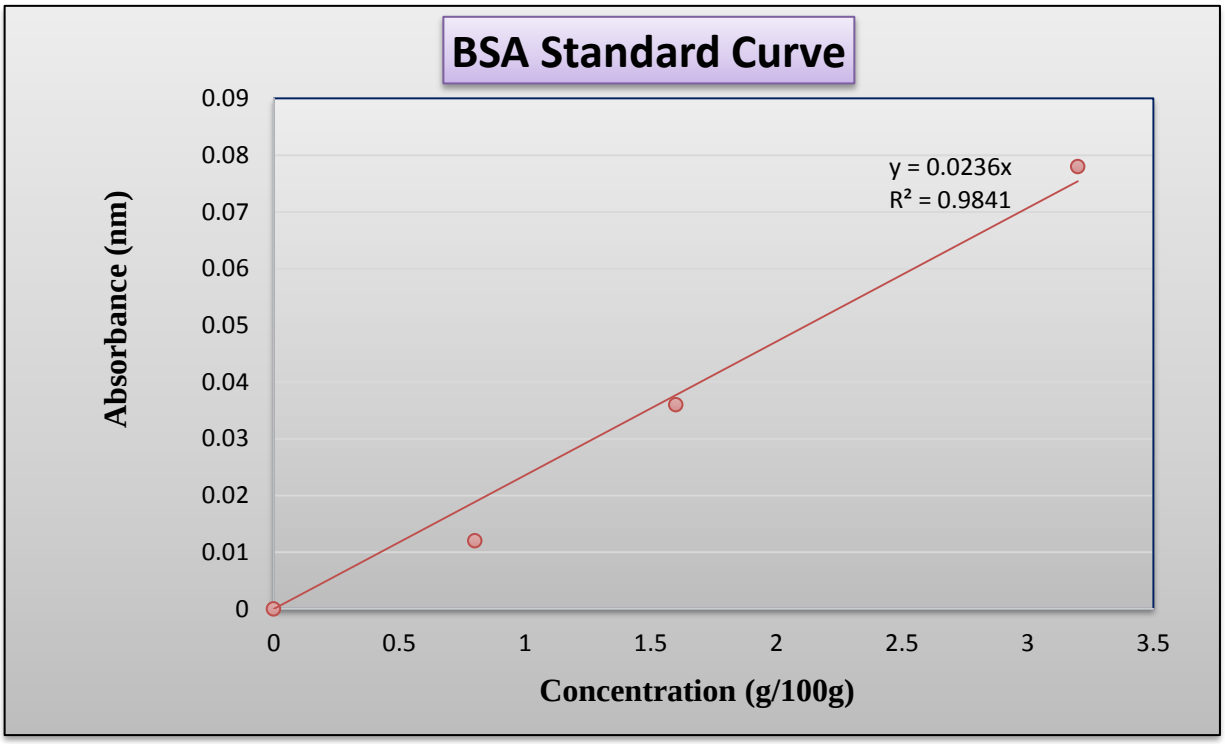
B.


Trolox Standard Curve
y = 146.37x
R² = 0.7672
% Inhibition
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
0.2
0.4
0.6
0.8
Concentration(µM)

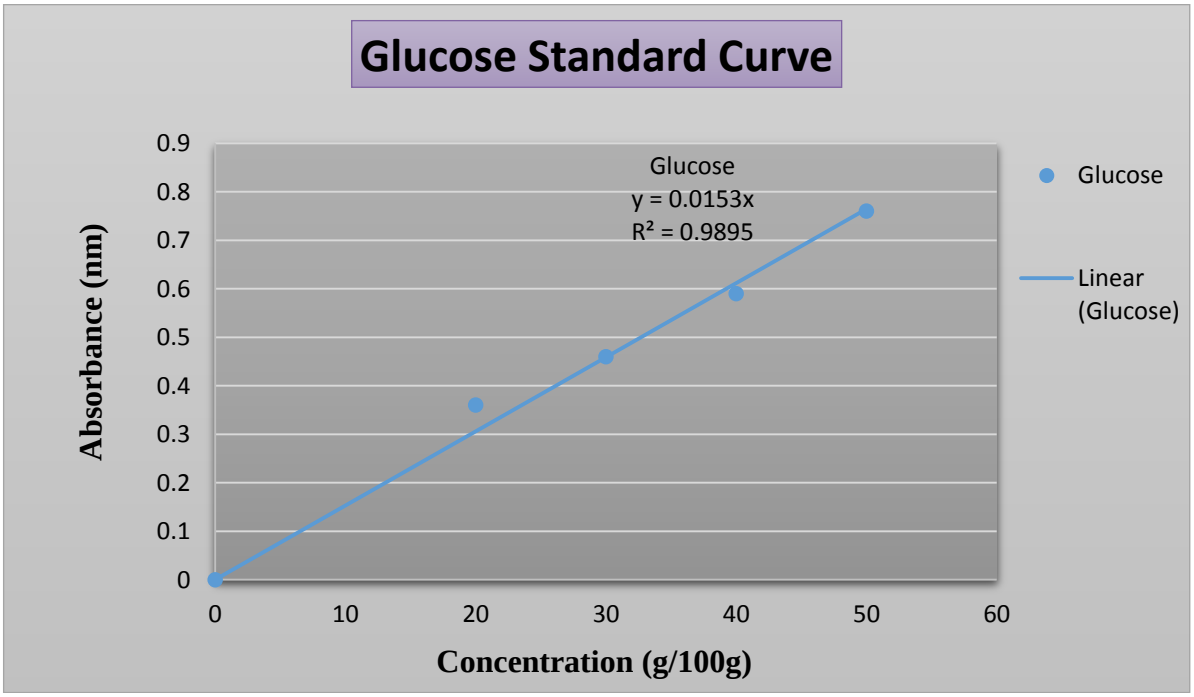
C.


Gallic Acid Standard Curve
y = 0.0013x
R² = 0.9872
ABSORBANCE
0.70000
0.60000
0.50000
0.40000
0.30000
0.20000
0.10000
0.00000
0.00
100.00
200.00
300.00
400.00
500.00
600.00
CONCENTRATION

D


BSA Standard Curve
Absorbance (nm)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
y = 0.0236x
R² = 0.9841
0
0.5
1
1.5
2
2.5
3
3.5
Concentration (g/100g)

E.


Glucose Standard Curve
Absorbance (nm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Glucose
y = 0.0153x
R² = 0.9895
0
10
20
30
40
50
60
Concentration (g/100g)
Glucose
Linear (Glucose)