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Physicochemical Characterization of Cassava (*Manihot esculenta*) and Potato (*Ipomea batatas*) Varieties from Southeast of Nigeria in Comparison with Agarose for use in the Separation of Deoxyribonucleic Acid

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Starch is an essential biomaterial and food product globally used for different purposes. This study determined the physicochemical composition of starch extracted from selected cassava (M.ExOboma I and M.ExOboma II) and sweet potato (I.ExOboma I and I.ExOboma II) varieties in comparison with agarose for use in the separation of deoxyribonucleic acid. Starch extraction was carried out using standard procedure. Granule morphology and amylose-amylopectin contents were determined using Polarized Light Microscopy and Dual Wavelength Iodine-Binding methods respectively. Relationships between starch properties were determined using correlation analysis. The result of the starch granule morphology revealed that there was no significant difference (p >

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0.05) in the pore sizes of M.ExOboma I (5.527 ± 1.9346 µm) and M.ExOboma II (5.650 ± 1.0472 µm). Similarly, I.ExOboma I (8.275 ± 1.5064 µm) and I.ExOboma II (6.075 ± 2.3838 µm) showed no significant difference ($p > 0.05$) in their pore sizes. Meanwhile, the pore size of agarose was reported to be within the range of 6 to 35µm. No significant difference ($p > 0.05$) was also observed in the ratios of amylose and amylopectin (Am:Ap) contents between the varieties of cassava (M.ExOboma I 25:75 and M.ExOboma II 24:77) and sweet potato (I.ExOboma I 26:72 and I.ExOboma II 27:75). No significant difference ($P > 0.05$) between the pH range values (7.7 to 7.8) of cassava and sweet potato with that of agarose (7.5). The study also revealed that there were significant differences ($p \leq 0.05$) in the clarity as well as the purity level of the starch samples compared to agarose. The results of this study are of great significance in predicting starch functionality and will form a basis for the improvement of the physicochemical attributes of starch for use in gel electrophoresis for the separation of biomolecules.

Keywords: Cassava starch; potato starch; amylase; amylopectin; granules morphology; Agarose.

1. INTRODUCTION

Starch is one of the most common and important polysaccharides in nature. It is a major economic and nutritional composition of many carbohydrate sources such as wheat, corn, rice, barley, sweet potato, and cassava [1]. Starch is widely used industrially as an emulsion stabilizer, water binder, thickener, and gelling (pasting) agent, it is, therefore, a very good ingredient for the manufacturing of various products [2].

Cassava and sweet potato starches are good sources of soft and transparent gel regardless of their low protein content [3]. Cassava can breed well in low rainfall areas and unfertile soil thus making it a good and readily available source of starch [4].

Starch comprises of two most essential components which are amylose and amylopectin molecules in molar ratios of 15 - 25% and 75% - 85% respectively Chen *et. al.,* [5]. The length of the α-glucan chains, amylose-amylopectin ratio, and branching degree of amylopectin describe the usefulness, size, and structure of starch granules in each plant species. Studies have revealed that the amount of amylose present in the granule significantly affects the functional and physicochemical properties of starch [6,7]. Starch films made from different amylose and amylopectin ratios are of diverse characteristics. Amylose (Am) in starch leads to tougher film whereas amylopectin generally produces lesser mechanical properties. The physical and chemical properties of starch films can be controlled by adjusting Am: Ap ratio [5]. Jiménez *et. al*. [8] stated that when starch constituents are in solution, their granules swell and the medium properties change from simple starch granules suspension to a starch paste thereby forming separate amylose and amylopectin phases because of thermodynamic immiscibility. According to Šubarić *et. al*. (2012), starch functionality is directly related to gelatinization and the properties of the paste.

This work was therefore carried out to assess the physicochemical composition of starch from Cassava (*Manihot esculenta*) and Potato (*Ipomea batatas*) varieties in comparison with agarose for use in the separation of Deoxyribonucleic acid.

Agarose is a polysaccharide extracted from red seaweed, *Gelidium* spp. It is a polymer made up of the repeating units of agarobiose which is a disaccharide consisting of one hundred and twenty thousand (120,000) alternating D-galactose and 3,6- anhydro-L-galactopyranose (L-galactose units) linked by α-(1→3) and β-(1→4) glycosidic bonds [9].

Agarose is the common and standard polysaccharide used in nucleic acid electrophoresis. However, the prohibitive cost of agarose has informed research into finding a more affordable and readily available alternative to agarose, particularly in resource-limited countries like Nigeria. This work was therefore carried out to assess the physicochemical composition of cassava and potato starches in comparison with agarose as an alternative for the separation of biomolecules.

2. MATERIALS AND METHODS

2.1 Collection of Cassava and Sweet Potato Tubers

A total of two varieties of fresh cassava and two varieties of sweet potato tubers were obtained from farms in Obomanagu Ukwuagba Ngbo,

Ohaukwu Local Government Area, Ebonyi State (southeast Nigeria). The samples were identified as M.Ex.Oboma I (Rubber Cassava), M.Ex.Oboma II (Opokopo Cassava), I.Ex.Oboma II (White Sweet potato) and I.Ex.Oboma I (Yellow Sweet potato).

2.2 Extraction of Starch

Starch extraction was carried out within 2 hours of harvesting based on the method of Brabet *et al*., [10] and Kamaljit *et. al*. [11]. Fresh tubers of each cultivar were washed, peeled, chopped into approximately 1 cm^3, and were ground in a high-speed blender for 5 minutes. The pulp was suspended in ten times its volume of water (2 L), stirred for 5 minutes, and filtered using a double-fold cheesecloth (0.05 mm). The filtrate was allowed to stand for 2 hours for the starch to settle and the top liquid was decanted and discarded. Clean water (2 L) was added to the sediment and the mixture was stirred again for 5 minutes. Filtration was repeated as before and the starch from the filtrate was allowed to settle. After decanting the top liquid, the sediment (starch) was dried at 55°C for 7 hours. The starch powders were used for the determination of the granule morphology, amylose and amylopectin contents. pH, purity, and clarity

2.3 Determination of Starch Granule Morphology

The granule morphology of the starches was determined according to a method of Fitt and Snyder, [12]. A drop of water was placed on one side of a standard microscope slide, then, 5 mg of the starch sample was transferred onto the water and mixed thoroughly to disperse the starch with the aid of a narrow-pointed spatula. A coverslip was placed over the suspension taking care to avoid entrapment of air bubbles and excess water was wicked off with a small piece of tissue paper held at the edge of the coverslip to obtain a thin film. The specimens were examined by looking at several fields at 150-300X magnification. Granule size range, granule shape, and appearance under polarized light were photographed and measured using a light microscope fitted with a calibrated eyepiece to calculate the average and range of the granule using a computer software application.

2.4 Determination of Amylose and Amylopectin Present in the Samples

Amylose and Amylopectin content of the starches were measured by the formation of complex according to a method by Hai and Mei, [13] as described briefly. Starch from each sample was mixed with water at a ratio of 2:1 (w/v) for 30 minutes with a stirrer and then placed at room temperature for 30 minutes. The slurry was centrifuged at 4,000 rpm for 3 minutes and the yellow gel-like layer on top of the white starch was carefully removed. The starch granule pellet was suspended in water.

2.4.1 Isolation of amylose

To isolate amylose, a 500 ml volume of starch granule slurry (2% w/v) was heated on a hot plate to 65°C with continuous magnetic stirring (78HW-1 Constant temperature magnetic stirrer, Search Tech) and then maintained at 65°C for 1 hour. The slurry was centrifuged at 3,000 rpm for 10 minutes and the precipitate was discarded. N-butanol was added to the supernatant at a ratio of 1:3 (v/v) (100 ml supernatant: 300 ml N-butanol). The solution was mixed and maintained at room temperature (25 °C) overnight. The supernatant was collected after centrifugation at 3,000 rpm for 1 minute and filtration and then mixed with n-butanol (200 ml) for 1 hour to improve their flow, gloss, and resistance to blushing. The precipitate was collected after centrifugation at 3,000 rpm and washed with 20 ml absolute ethanol four times and allowed to dry under the high-speed electric fan.

2.4.2 Isolation of amylopectin

The isolation of amylopectin was performed following the method described by Liu *et. al*., [14]. A 500 ml volume of slurry (2% w/v) was heated on a hot plate to 100 °C with continuous magnetic stirring for 1 hour. When the temperature of the slurry dropped to 55 °C, the pH was adjusted to 6.3 using phosphate buffer (40 g/l NaH_2PO_4, 10 g/l Na_2HPO_4). Then the slurry was boiled at 100 °C in a water bath for 1 hour with frequent shaking and heated on a hot plate at 100 °C with continuous magnetic stirring for 1 hour. N-butanol was dispensed to the slurry at a ratio of 1:3 (v/v) and mixed thoroughly with the aid of continuous magnetic stirring until the temperature dropped to 55 °C. The slurry was allowed to cool in a foam box overnight. The slurry was centrifuged at 8,700 rpm for 30 minutes. After centrifugation, the top layer of n-butanol and the precipitate, which contain amylose and other impurities, was discarded; the middle layer was a crude extract of amylopectin. In order to precipitate residues of amylose, n-butanol was added to the amylopectin crude

extract at a ratio of 1:3 (v/v) and the solution was mixed and centrifuged at 8,700 rpm for 30 minutes. To isolate amylopectin from this solution, excess methanol was added, mixed, and centrifuged at 8,700 rpm for 10 minutes. The precipitate was washed with absolute ethanol four (×4) times, allowed to dry under the high-speed electric fan, and ground using a mortar and pestle.

2.4.3 Absorption spectra and standard curves

100 mg of amylose or amylopectin was dispersed in 1 ml absolute ethanol, and 9 ml 1 M NaOH was dispensed into the solution. The solution was placed in a boiling water bath for 15 minutes with frequent shaking to obtain a clear solution; then the total volume was adjusted to 100 ml using distilled water to obtain the stock solution.

To generate absorption spectra, 4 ml amylose stock solution or 2.6 ml amylopectin stock solution was added to 30 ml distilled water, and the pH was adjusted to 3.6 using 0.5 M HCl. Then, 0.5 ml potassium iodide–iodine (I_2-KI) solution (2 g/l I_2, 20 g/l KI) was added, and the total volume was adjusted to 50 ml using distilled water. The solution was mixed and placed at room temperature for 30 minutes, after which they were scanned using a spectrophotometer (Spectrumlab 23A No. 23A08182) at 471, 600 and 610 nm wavelengths using control (distilled water and iodine). A standard curve was generated by plotting the concentration of amylose or amylopectin on the X-axis and the difference in absorbance on the Y-axis.

2.5 Determination of Starch Clarity and Impurity Level

Solutions (1%, w/w) of reconstituted starch were used for this study to determine the starch clarity. Measurements of light transmittance of starch solutions were carried out by the method of Craig et*. a1.* [15]. The agarose powder, cassava, and sweet potato starch solutions were separately measured into 250 ml conical flask capacity and microwaved at 90^{o}C for 50 minutes. The Flask was thoroughly shaken every 5 minutes. After cooling to room temperature, the transmittance at 610 nm was measured using a spectrophotometer (Spectrumlab 23A No. 23A08182). To determine the starch purity, the method described by Bhagya *et. al.,* [16] was adopted. Each starch extract (100 mg) was boiled in 30 ml of 80% ethanol for 10 minutes and the collected residue was dried at 70^{o}C for 4 hours. To digest the starch, 10 ml of 52% $HClO_4$ was added to the dried residue and placed in a water bath for 15 minutes at 28^{o}C, then made up to 25 ml with distilled water and filtered with Whatman No. 1. About 20 µl of the filtrate was made up to 1 ml with distilled water before adding 1 ml of 5% phenol and 5 ml 36N H_2SO_4. Pure glucose (20 - 100 µm) was taken as standard. The absorbance of the solution was read at 490 nm using a spectrophotometer. The amount of sugar present in each sample was calculated as total sugar multiplied by 0.9.

2.6 Determination of pH of Starch and Agarose

This was done using a pH meter (model HI 8424 with pH buffer 7). One and a half gram (1.5 g) of starch sample and agarose were separately dissolved in 10 ml of distilled water in a beaker, the mixture was stirred properly. The pH of the solution was read and recorded.

3. RESULTS

3.1 Cassava and Sweet potatoes Starch Granule Morphology

Fig. 1 shows the result of the granule morphology of cassava and sweet potato starch samples studied. The results revealed that the pore size of starch for M.ExOboma I and M.ExOboma II were 5.525 ± 1.9346 µm and 5.650 ± 1.0472 µm respectively. Similarly, I.ExOboma I I.ExOboma II pore sizes were 6.075 ± 2.3838 µm and 8.275 ± 1.5064 µm respectively (Table 1). The results revealed that I.ExOboma II showed the widest pore size (8.275 ± 1.5064 µm) when compared to all the other starch samples studied. Most starch granules studied were round or oval, but asymmetrical granules were also observed.

3.2 Amylose and Amylopectin contents of starch sources Determined based on the Dual Wavelength Iodine-Binding Method

The results of amylose and amylopectin contents in cassava and sweet potato starch showed that there was no significant difference ($p \geq 0.05$) in amylose content among each cassava and sweet potato variety. The ratio of amylose and amylopectin in M.Ex.Oboma I and M.ExOboma II were 25:75 and 24:77 respectively. Similarly, I.ExOboma I and I.ExOboma II have amylose and amylopectin in a ratio of 26:72 and 27:75 respectively as shown in Table 2.

Table 1. Cassava and sweet potatoes starch granule morphology

Common name	Starch pore size (µm)	Shape
M. ExOboma I	5.525 ± 1.9346[a]	Oval, round
M. ExOboma II	5.650 ± 1.0472[a]	Oval, asymmetrical
I. ExOboma I	6.075 ± 2.3838[b]	Oval, round
I. ExOboma II	8.275 ± 1.5064[b]	Round, asymmetrical

Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at P ≤ 0.05. Tukey's-HSD post hoc test

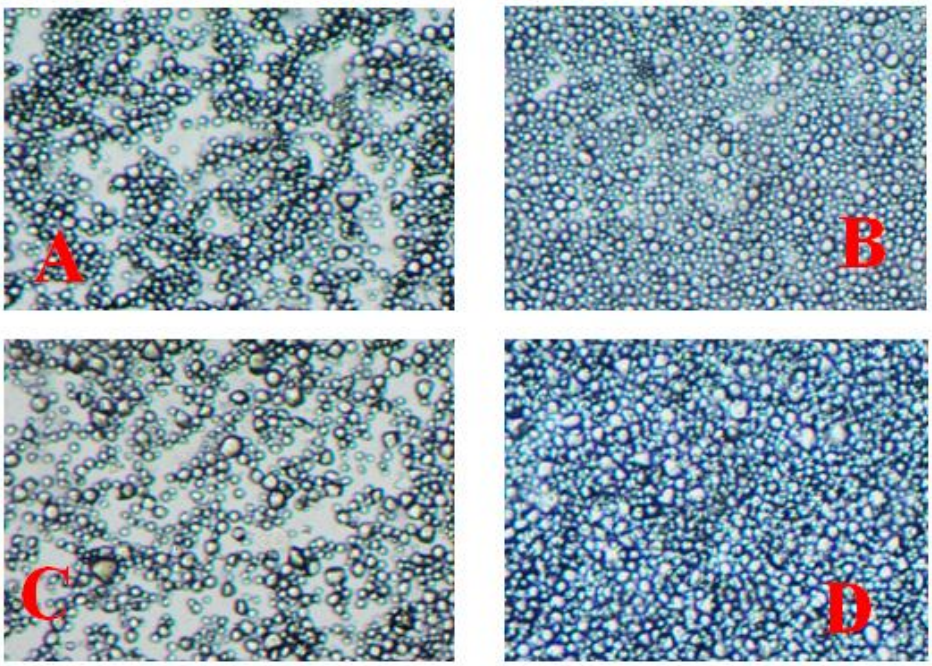

Fig. 1. High-resolution micrographs of portions of the starch granule surface showing (A) the M. ExOboma I granule surface (B) M. ExOboma II granule surface (C) I. ExOboma I granule surface (D) I. ExOboma II granule surface

Table 2. Amylose and Amylopectin contents of starch sources

Botanical	Amylose	(%) Mean ± S.D	Amylopectin (%)	Mean ± S.D
M.Ex.Oboma I	25	23.25 ± 1.708[a]	75	72.008 ± 3.162[a]
M.ExOboma II	24	21.75 ± 2.217[a]	77	72.50 ± 2.887[a]
I.ExOboma I	26	24.50 ± 1.291[a]	72	70.25 ± 1.708[a]
I.ExOboma II	27	25.50 ± 1.291[a]	75	73.25 ± 1.708[a]

Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at P ≤ 0.05. Tukey's-HSD Post Hoc test

Table 3. pH and Purity of the Samples (parts per million)

Samples	pH ± S.D	Purity Mean ± S.D (ml)
Agarose	7.51± 1.57[a]	0.0000 ± .00000[a]
M.ExOboma I	7.84 ± 0.09[a]	114.8333 ± 4.72264[c]
M.ExOboma II	7.76 ± 0.12[a]	40.0867 ± 1.11541[b]
I.ExOboma I	7.89 ± 0.30[a]	129.4000 ± .00000[e]
I.ExOboma II	7.71 ± 0.36[a]	123.3667 ± .05774[d]

Results are presented as mean ± SD. N = 3. Values with different superscripts down the column are significantly different from each other at P ≤ 0.05. Tukey's-HSD Post HOC Test

3.3 pH and Purity of the Samples (parts per million)

This study revealed that the pH values of cassava and sweet potato starch studied ranged from 7.7 to 7.8 while that of agarose was 7.5 as shown in Table 3. Hence, there was no significant difference (P ≥ 0.05) between the pH of the starch samples and agarose. The results as presented in Table 3 also showed that there was a significant difference (p ≤ 0.05) between the purity of the samples. M.ExOboma II cassava showed the least contamination level (40.0867 ± 1.11541 ml) in comparison with the other starch samples, but much higher than the agarose.

4. DISCUSSION

Over the years, agarose has been the only gel matrix used for the separation of nucleic acids. Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen bonding when heated in a buffer and allowed to cool [17]. Starch is a polysaccharide that is also held by hydrogen-bond. A study of the gel formation by starch revealed that starch granules swell, gelatinize and hydrate easily when heated in water (Pan *et. al*., 2018). However, the functional properties and applications of starch depend on the crop source (Pan *et. al*., 2018).

The separation of nucleic acids and proteins by gel electrophoresis method is not only dependent on the molecular size and charge of the biomolecules but also on the pore size of the matrix of agarose or starch source used. The results revealed starch granule distribution in the range of (5.525 ± 1.9346 µm) for M.ExOboma and (8.275 ± 1.5064 µm) for I.ExOboma II. Comparatively, Altland and Hackler, [18] reported the pore size of agarose to be within the range of 6 to 35µm. The values of starch pore sizes obtained from this work are also consistent with literature figures which have been reported to fall within the ranges of 2 to 42µm [19, 20].

Since starch granules are usually white, the occurrence of different colors of the starch extracts as seen in Fig. 1 may be a result of the presence of non-starch impurities such as polyphenols, ascorbic acids, and carotene which may have negative effects on the starch quality and the final functionality of starch [21]. The levels of impurity observed in starch were also significantly higher than that of the agarose as shown in Table 3, with M.ExObama II having the lowest impurity level when compared with all the other starch samples.

The results of amylose and amylopectin contents in cassava and sweet potato starch studied showed that there was no significant difference (p ≥ 0.05) in amylose content among each cassava and sweet potato variety as shown in Table 2. These values of amylose contents are in line with those reported in the literature of between 8.5 to 38% (Tian *et. al*., 1991) [19, 20]. Reports on the botanic source of the starch chain such as tapioca, sweet potato, sago, and corn stated that the mass of amylose and amylopectin are up to 20% and 80% respectively [22, 23]. On the contrary, agarose is a polymer made up of the repeating units of agarobiose which is a disaccharide consisting of one hundred and twenty thousand (120,000) alternating D-galactose and 3,6- anhydro-L-galactopyranose (L-galactose units) linked by α-(1→3) and β-(1→4) glycosidic bonds [9].

The pH values of cassava and sweet potato starch studied ranged from 7.7 to 7.9 as shown in Table 3. Hence, there was no significant difference (P ≥ 0.05) in the pH of the starch when compared with that of pure agarose which is 7.5. Starch pH has been speculated to have some effects on starch functionality. High pH starches have been found to have increased solubility. This is due to increased hydrophilic characteristics of the starch at these pH values [24,25].

5. CONCLUSION

This study has demonstrated that cassava and sweet potato starch possess pH range and pore size comparable to that of agarose and therefore suitable for the formation of gel that can be utilized in gel electrophoresis. It was also observed that there was a significant difference in the purity level of agarose compared to the starch gels. Moreover, essential chemical components such as amylose and amylopectin were observed. Extraction of starch is relatively easy because it does not require complicated purification processes, and this makes it attractive as an alternative for agarose in the separation of biomolecules. The results of this study are of great significance in predicting starch functionality and will form a basis for improvement of the physicochemical attributes of starch for use in gel electrophoresis for the separation of biomolecules and when applied in the food industry and other scientific settings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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